Sascha A. Jovanovic
Dennis R. Hunt
George W. Bernard
Hubertus Spiekermann
Russell Nishimura
John M. Wozney
Ulf M. E. Wikesjö

Long-term functional loading of dental implants in rhBMP-2 induced bone

A histologic study in the canine ridge augmentation model

Authors' affiliations
Sascha A. Jovanovic, Dennis R. Hunt, George W. Bernard, Division of Oral Biology & Medicine, UCLA School of Dentistry, Los Angeles, CA, USA
Hubertus Spiekermann, Department of Prosthodontics, University of Aachen, Aachen, Germany
Russell Nishimura, Division of Advanced Prosthodontics, UCLA School of Dentistry, Los Angeles, CA, USA
John M. Wozney, Musculoskeletal Sciences, Bone Biology and Applications, Genetics Institute/ Wyeth-Ayerst Research, Cambridge, MA, USA
Ulf M. E. Wikesjö, Periodontology and Oral Biology, Laboratory for Applied Periodontal and Craniofacial Regeneration, Department of Periodontology, Temple University School of Dentistry, Philadelphia, PA, USA

Correspondence to:
Sascha A. Jovanovic
Division of Oral Biology & Medicine
UCLA School of Dentistry
Los Angeles
CA 90049
USA
Tel.: 1 310 825 7809
Fax: 1 310 825 5016
e-mail: sascha@jovanoviconline.com

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Abstract: Osseointegration [direct bone–implant contact (BIC)] is a primary goal following installation of endosseous dental implants. Such bone contact provides stability for the dental implant over time. The objective of this study was to evaluate bone formation and BIC at long-term, functionally loaded, endosseous dental implants placed into bone induced by recombinant human bone morphogenetic protein-2 (rhBMP-2) in an absorbable collagen sponge (ACS) carrier. Mandibular, saddle-type, alveolar ridge defects ($\sim$15 $\times$ 10 $\times$ 10 mm), two per jaw quadrant, were surgically induced in each of six young adult American fox hounds. The defects were immediately implanted with rhBMP-2/ACS. Two defects per animal additionally received a nonresorbable expanded polytetrafluoroethylene (ePTFE) membrane or a bioresorbable polyglycolide fiber membrane. Healing was allowed to progress for 3 months, when the ePTFE membrane was removed, and machined, threaded, titanium dental implants were installed into the rhBMP-2/ACS induced bone and into the adjacent resident bone. At 4 months of osseointegration, the implants were exposed to receive abutments and prosthetic treatment (two- or three-unit bridges). Some implants were removed for histologic analysis. The remainder of implants were exposed to functional loading for 12 months at which time the animals were killed for histometric analysis. One animal died prematurely due to kidney failure unrelated to the experimental protocol and was not included in the analysis. The 12-month block sections from a second animal were lost in the histological processing. Four sites receiving rhBMP-2/ACS and ePTFE or resorbable membranes experienced wound failure and membrane exposure, and subsequently exhibited limited bone formation. Defects without wound failure filled to contour with the adjacent alveolar bone. The newly formed bone exhibited features of the resident bone with a re-established cortex; however, it commonly included radiolucent areas that resolved over time. Dental implants block biopsied at 4 months exhibited limited, if any, crestal resorption, whereas those exposed to functional loading for 12 months exhibited some crestal resorption. Implants biopsied at 4 months exhibited a mean ($\pm$ SD) BIC of 40.6 $\pm$ 8.2% in rhBMP-2/ACS induced bone vs. 52.7 $\pm$ 11.4% in resident bone. Dental implants exposed to 12 months of functional loading exhibited a mean BIC of 51.7 $\pm$ 7.1% in rhBMP-2/ACS induced bone vs. 74.7 $\pm$ 7.0% in resident bone. There were no significant differences between dental implants placed into rhBMP-2/ACS induced bone and resident bone for any parameter at any observation interval. In conclusion, rhBMP-2/ACS-induced bone allows installation, osseointegration, and long-term functional loading of machined, threaded, titanium dental implants in dogs.

Endosseous dental implants have been used to support single-tooth, partial and complete arch dental reconstruction, and to support maxillofacial reconstruction. Osseointegration [direct bone-implant contact (BIC)] is a primary goal following dental implant placement. Such bone contact provides stability for the dental implant over time. Current dental implant technology achieves osseointegration after an initial healing period of several months during which nonloaded implant fixtures interact with and anchor to the alveolar bone (Brånemark et al. 1985).

Bone morphogenetic proteins (BMPs) have been shown to induce bone formation in a variety of skeletal sites including the alveolar processes (Lindholm et al. 1996; Wikesjö et al. 2001). Urist (1994) made critical contributions to BMP research through observations of cartilage and bone formation at decalcified bone matrix implants in muscle pouches in rabbits and rats. He showed that protein extracts sequestered in bone were responsible for the new bone formation. A paramount advancement in BMP research was the identification of a group of bone-inductive proteins from bovine bone (Wang et al. 1988). Wozney et al. (1988) cloned the first recombinant BMPs (BMP-1 through BMP-4) and identified their biochemical and biological characteristics, and amino acid sequences. The isolation and characterization of additional BMPs (BMP-5 through BMP-8) soon followed. Recombinant technology has allowed evaluation of BMP biological activities and several BMPs have been shown to induce bone in the rat ectopic implant model (Sampath et al. 1992; Gitelman et al. 1994). Subsequent studies have verified this potential of BMPs in large animal skeletal defects (Toriumi et al. 1991; Gerhart et al. 1993).

Preclinical studies have evaluated rhBMP-2 for augmentation of the alveolar process and placement of dental implants (Wikesjö et al. 2001). rhBMP-2 constructs have been used to augment alveolar sites prior to dental implant installation (Hanisch et al. 1997; Sigurdsson et al. 2001). rhBMP-2 in an absorbable collagen sponge (ACS) carrier has been used as an inlay (Cochran et al. 1999) and as an onlay (Sigurdsson et al. 1997) concomitant with dental implant placement. rhBMP-2/ACS has also been used for subantral augmentation prior to placement and osseointegration of dental implants (Hanisch et al. 1997). Without exception, the studies show that rhBMP-2 significantly supports alveolar augmentation. Dental implants placed into the newly formed bone, or placed concomitant with a rhBMP-2 construct, become osseointegrated.

Jovanovic et al. (Jovanovic, unpublished observations) and Hunt et al. (2001) have evaluated augmentation of large, mandibular, full thickness, alveolar ridge, saddle-type defects in the dog. They showed that surgical implantation of rhBMP-2/ACS, and rhBMP-2 in a hyaluronan carrier, completely resolved these defects whereas surgical controls resolved to a significantly lesser degree (Bernard et al. 2000). However, the rhBMP-2 induced bone was not functionally challenged by dental implants. Previous studies all represent short-term evaluations of rhBMP-2 induced bone in the dental–alveolar complex without functionally loaded dental implants (Wikesjö et al. 2001). The objective of this study was to evaluate bone formation and long-term osseointegration in the alveolar ridge, saddle-type defect model following implantation of rhBMP-2/ACS and subsequent placement and functional loading of dental implants.

Material and methods

Study design

Mandibular, saddle-type, alveolar ridge defects, two per jaw quadrant, were surgically prepared in each of six young adult American fox hounds. The defects were immediately implanted with rhBMP-2/ACS. Two defects per animal additionally received a nonresorbable expanded polytetrafluoroethylene (ePTFE) membrane or a bioresorbable polyglycolide fiber membrane. Healing was allowed to progress for 3 months, when the ePTFE membrane was removed and machined, screw-type, titanium dental implants were installed into the rhBMP-2 induced bone and into the adjacent resident bone. At 4 months of osseointegration, the implants were exposed to receive abutments and prosthetic reconstruction. Some implants were removed for histologic evaluation. Implants bearing prosthesis were exposed to functional loading for 12 months, at which time the animals were killed for histometric analysis (Table 1).

Animals

Six male American fox hounds, 18–36 months old, approximate weight 25 kg, were used. Animal selection and management, surgical protocol, and preparation of the alveolar ridge defects followed routines approved by the Institutional Animal Care and Use Committee, University of California, Los Angeles, CA.

Surgical protocol

For the surgical procedures, the animals were anesthetized with sodium thiopental (20–25 mg/kg intravenously) and maintained on gas anesthesia (1.5% halothane/O_2 to effect). Infiltration anesthesia (lidocaine 2% with epinephrine 1 : 100 000) was used at the surgical sites. A long-acting opioid (buprenorphine HCl; 0.015 mg/kg subcutaneously every 12 h for 5 days) was used for post-surgery pain control. A broad-spectrum antibiotic (cefazolin sodium; 25 mg/kg intramuscularly daily for 5 days) was used for post-surgery infection control. The animal's oral cavity was daily flushed with a 0.12% chlorhexidine solution for 14 days following each surgical procedure. The animals were fed a canned soft-consistency dog food diet until insertion of prosthetic devices, to reduce the potential of mechanical interference with healing.

Surgical extractions

To prepare for the alveolar ridge defects, left and right mandibular premolars and first molars were surgically extracted. The maxillary fourth premolars were also extracted to alleviate potential trauma to the mandibular sites during healing. Buccal and lingual mucoperiosteal flaps were then re-adapted and sutured using resorbable interrupted sutures. The resulting alveolar ridges were allowed to heal for 3 months.

Defect induction

Bilateral, alveolar ridge, saddle-type defects were created in the left and right edentulous posterior mandible for a total of four defects in each of the six animals (Fig. 1). Briefly, following crestal incisions and elevation of buccal and lingual mucoperiosteal flaps, two bone blocks (10 mm deep, 15 mm long), 10 mm apart, encompassing the width of the mandible (approximate width: 10 mm) were removed from each jaw quadrant. The bone blocks were outlined utilizing a power surgical hand-piece with a reciprocating blade (Stryker Corpor-

Table 1. **Surgical timeline**

Month	Procedure
– 3	Surgical extractions for preparation of mandibular edentulous alveolar ridges
0	Surgical induction and rhBMP-2 reconstruction of alveolar ridge defects
3	Dental implant installation
7	Dental implant abutment installation; implant biopsies
7	Insertion of bridges; functional loading
19	Sacrifice for histometric analysis of bone formation and bone–implant contact

ation, Kalamazoo, MI) under irrigation with sterile water. Following the vertical and buccal horizontal bone cuts, a chisel was utilized to remove the bone blocks.

RhBMP-2/ACS preparation

A vial of rhBMP-2 (Genetics Institute/Wyeth-Ayerst Research, Andover, MA), lyophilized formulation, was reconstituted with 1.0 ml of sterile water for injection to give a 4.0 mg/ml solution in buffer. For the 0.23 mg/ml rhBMP-2 stock dilution a 1-cc syringe with a 22G needle attached was used to withdraw 0.30 ml of the rhBMP-2 solution reconstituted to 4.0 mg/ml (liquid concentration). The rhBMP-2 solution was injected into a vial containing 5.0 ml of buffer and swirled to mix. The outer package of a sterile 75 × 100 mm ACS (Integra Life Sciences, Plainsboro, NJ) was then removed and the inner sterile tray with the ACS device placed on a sterile field. Using sterile scissors, 25 × 50 mm strips were cut from the 75 × 100 mm ACS. Next, using a 3-cc syringe with a 22G needle attached, 1.3 ml of the 0.23 mg/ml rhBMP-2 stock dilution was withdrawn and uniformly dispensed over the entire surface of a 25 × 50 mm ACS. The soak-loaded ACS, folded twice in length and rolled to fit the defect, was implanted following a 15-min incubation interval at room temperature.

Wound management

The alveolar ridge defects were immediately implanted with rhBMP-2/ACS (Figs 1 and 2). The dimensions of the rhBMP-2 construct approximated 20 × 10 × 8 mm (length × height × width). Two defects in each animal additionally received an ePTFE membrane (GTAM; Gore-Tex Regenerative material, submerged version; W.L. Gore & Associates, Inc., Flagstaff, AZ) or a bioresorbable polyglycolide fiber membrane with an embedded DL-polylactic–polyglycolic acid cell-occlusive layer (Resolut; W.L. Gore & Associates, Inc.; Table 2).

Primary, tension-free wound closure was accomplished by advancing the mucoperiosteal flaps using periosteal releasing incisions and suturing with interrupted and mattress ePTFE sutures (Gore-Tex® Suture CV5, W.L. Gore & Associates, Inc.). The sutures were removed at approximately 10 days post-surgery.

The alveolar defect sites were re-entered at 3 months following defect induction and reconstruction. The GTAM membranes were removed and machined, screw-type, titanium dental implants (Brånemark fixture 3.75 × 10 mm; Nobel Biocare AB, Göteborg, Sweden) were installed. Additional dental implants were installed into the adjacent resident bone (Figs 1 and 2, Table 3).

Prosthetic treatment

Following a 4-month healing interval, the implants were exposed to receive abutments and prosthetic treatment (two- or three-unit bridges) (Figs 1 and 2). Some implants were removed for histologic evaluation of bone–implant contact (Table 3). The animals were fed a normal hard-pellet dog food diet after insertion of the bridges.

Clinical and radiographic procedures

Photographs were taken at defect induction and reconstruction, at surgical re-entry and dental implant installation, at abutment installation and prosthetic reconstruction, and at sacrifice (following 12 months of functional loading). Observations of experimental sites regarding mucosal health, wound closure, edema, and purulence, were made at these observation intervals and recorded.

Radiographic registrations were performed at defect induction and reconstruction, monthly following defect reconstruction and dental implant installation, and at 3-month intervals following healing abutment installation and functional loading. A fixed target-film distance was used with the film placed lingual to the alveolar crest.

The reconstructed implants were exposed to masticatory function for approximately 12 months, at which time the animals were killed for histometric analysis. The animals were anesthetized and killed by an intravenous injection of concentrated sodium pentobarbital (Eutha-6, Western Medical Supply, Inc., Arcadia, CA; 5–10 ml).

Histologic processing

Dental implant biopsies obtained at abutment installation were immersed in 4% neutral buffered formalin, dehydrated in a graded ethanol series, cleared in xylene, infiltrated and embedded in methylmethacrylate, and sectioned on a saw microtome following routine protocols (Donath 1988). These undecalcified specimens were sectioned in a mesial-distal plane because of implant removal trauma to buccal-lingual tissues. At sacrifice, block sections of the experimental sites were collected and processed as described above. These undecalcified specimens were sectioned in a buccal-lingual plane as intended by protocol.

Analysis

The histometric analysis was performed by one blinded examiner using a microcomputer-based image analysis system (Image-Pro Plus™, Media Cybernetics, Silver Spring, MD) and included the following parameters.

Bone height: distance from the apex of the dental implant to the coronal extension of newly formed bone along the implant when the implant was totally immersed in new bone, or from the level of the resident bone along the implant surface as determined using polarized light microscopy.

Crestal resorption: distance from the top surface of the dental implant and the most coronal extension of newly formed bone along the implant.

Coronal bone–implant contact: distance from the coronal extension of newly formed bone along the implant to the first bone contact.

Bone–implant contact (BIC/osseointegration): percentage bone–implant contact between the newly formed bone and the dental implant surface along the five most coronal threads immersed in bone measured along the distal or lingual implant surface of implants biopsied following osseointegration for 4 months or exposure to functional loading for 12 months, respectively.

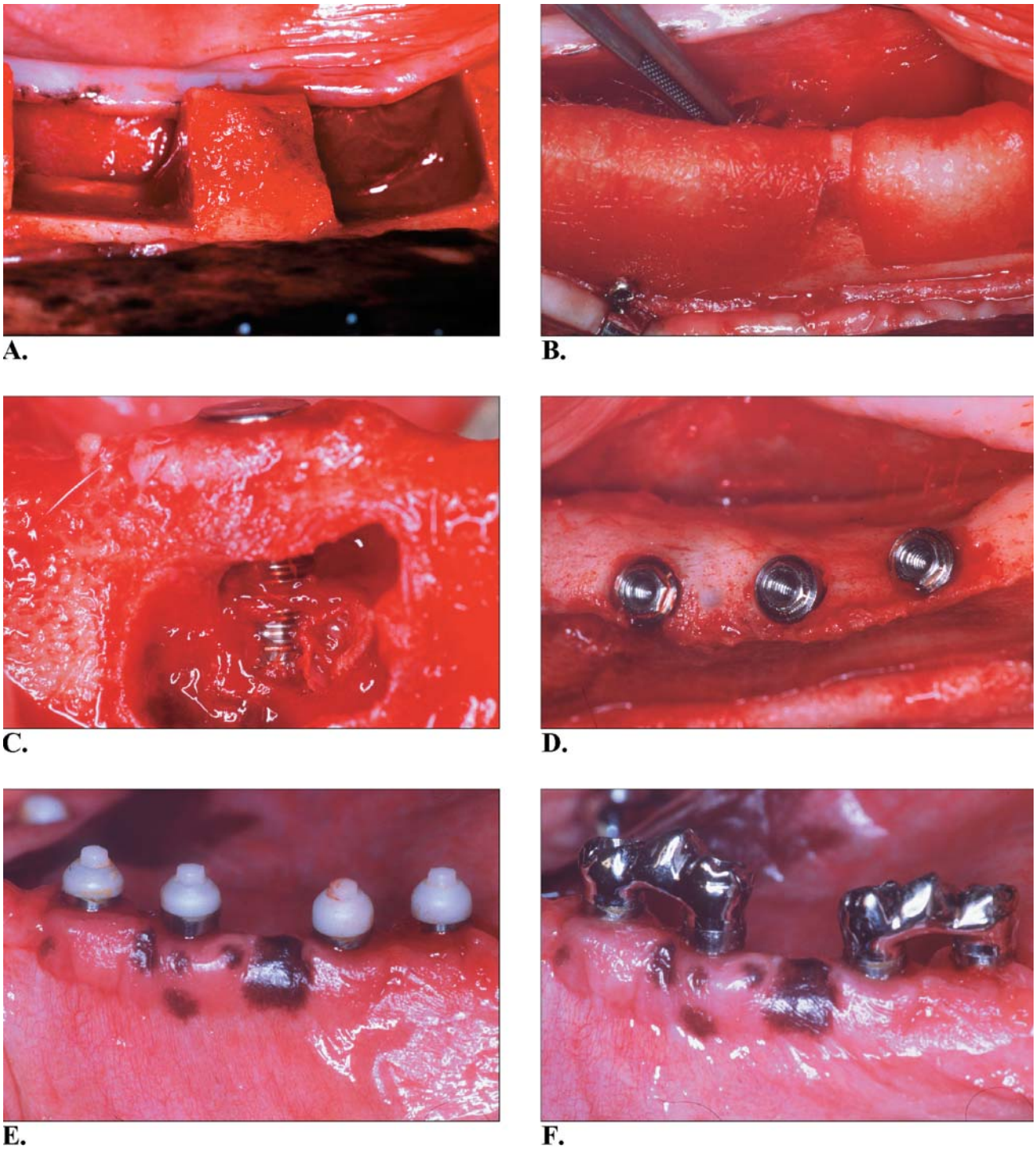

Fig. 1. Alveolar ridge defects (A) implanted with rhBMP-2/ACS with (left) or without a Resolut barrier membrane (B); lateral (C) and coronal (D) view of alveolar ridge defect site with cavernous bone defect at dental implant installation; defect sites following placement of healing abutments (E) and prosthetic reconstruction (F).

Table 2. **Surgical randomization**

Animal	Right posterior	Right anterior	Left anterior	Left posterior
216	rhBMP-2/ACS/Resolut	rhBMP-2/ACS	rhBMP-2/ACS/GTAM	rhBMP-2/ACS
217	rhBMP-2/ACS	rhBMP-2/ACS/GTAM	rhBMP-2/ACS	rhBMP-2/ACS/Resolut
218	rhBMP-2/ACS	rhBMP-2/ACS/Resolut	rhBMP-2/ACS	rhBMP-2/ACS/GTAM
219	rhBMP-2/ACS/GTAM	rhBMP-2/ACS	rhBMP-2/ACS	rhBMP-2/ACS/Resolut
220	rhBMP-2/ACS/Resolut	rhBMP-2/ACS	rhBMP-2/ACS/Resolut	rhBMP-2/ACS
221	rhBMP-2/ACS/GTAM	rhBMP-2/ACS	rhBMP-2/ACS/GTAM	rhBMP-2/ACS

Summary statistics (means ± SD) based on animal means for the experimental conditions were calculated using selected sections. Differences between experimental conditions were analyzed using appropriate nonparametric tests.

Table 3. **Distribution of dental implants placed into rhBMP-2 induced and resident bone (RM and LM)**

Animal	Right posterior	RM	Right anterior	Left anterior	LM	Left posterior
216	2	0	2	0	1	1
217	2	0	0	2 L	1B	2B
218	2	1B	2	2	1	0
219	0	1	1, 1B	2	1	2
220*	2	0	2	2	1	2
221	1**, 1B	1**	2**	2**	1B	2**

B, implants biopsied at 4 months of osseointegration.
L, implants lost as a result of infection.
*Animal died as a result of kidney failure.
**Block specimens from 12 months of functional loading lost in histologic processing.

Table 4. **Treatment and healing characteristics following surgical implantation of rhBMP-2/ACS**

Animal	Right posterior	Right anterior	Left anterior	Left posterior
216	rhBMP-2/ACS/Resolut normal	rhBMP-2/ACS bone void	rhBMP-2/ACS/GTAM bone void	rhBMP-2/ACS bone void
217	rhBMP-2/ACS normal	rhBMP-2/ACS/GTAM wound failure	rhBMP-2/ACS bone void	rhBMP-2/ACS/Resolut normal
218	rhBMP-2/ACS normal	rhBMP-2/ACS/Resolut bone void	rhBMP-2/ACS normal	rhBMP-2/ACS/GTAM bone void
219	rhBMP-2/ACS/GTAM wound failure	rhBMP-2/ACS bone void	rhBMP-2/ACS bone void	rhBMP-2/ACS/Resolut bone void
220	rhBMP-2/ACS/Resolut wound failure	rhBMP-2/ACS normal	rhBMP-2/ACS/Resolut wound failure	rhBMP-2/ACS normal
221	rhBMP-2/ACS/GTAM bone void	rhBMP-2/ACS bone void	rhBMP-2/ACS/GTAM bone void	rhBMP-2/ACS bone void

Table 5. **Dental implant–bone contact at 4 months of osseointegration in rhBMP-2/ACS induced bone**

Animal	Bone height (mm)		Mesial	Crestal resorption (mm)		Coronal bone contact (mm)	
	Mesial	Distal		Distal	Mesial	Distal	BIC (%)
217	9.5	9.7	0	0	0.9	0.6	34.6
219	9.8	9.8	0	0	0.9	2.0	49.9
221	9.4	9.4	0	0	0.3	1.2	37.3
Mean ± SD	9.6 ± 0.2*	9.6 ± 0.2*	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.3	1.3 ± 0.7	40.6 ± 8.2

*100% newly formed bone.

Table 6. **Dental implant–bone contact at 4 months of osseointegration in resident bone**

Animal	Bone height (mm)		Mesial	Crestal resorption (mm)		Coronal bone contact (mm)	
	Mesial	Distal		Distal	Mesial	Distal	BIC (%)
217	9.6	9.6	0.4	0.3	1.2	0.8	43.7
218	8.9	9.0	0.8	0.7	0.8	0.6	49.0
221	8.8	9.7	0.9	0.0	0.2	1.8	65.5
Mean ± SD	9.1 ± 0.4	9.5 ± 0.4	0.7 ± 0.5	0.3 ± 0.3	0.7 ± 0.5	1.1 ± 0.6	52.7 ± 11.4

Results

Clinical observations

Clinical observations are shown in Fig. 1 and are summarized in Tables 3 and 4. Defect induction, surgical implantation of rhBMP-2/ACS, and subsequent placement of GTAM and post-surgery membranes followed established routines. Primary wound closure was accomplished for all sites. The defect sites commonly exhibited post-surgery swelling, often assuming a bluish hue. The localized swelling and/or altered mucosal complexion remained for several weeks. Two sites receiving rhBMP-2/ACS/GTAM and two sites receiving rhBMP-2/ACS/Resolut exhibited wound failure and membrane exposure.

Three months following defect induction and reconstruction, a total of 39 titanium dental implants were placed into the rhBMP-2 augmented sites. Another nine dental implants were placed into resident bone between the augmented sites (controls). Some sites, having experienced wound failure or exhibiting extensive cavernous voids within the newly formed bone, were deemed unsuitable for dental implant installation. Still others received dental implants notwithstanding similar shortcomings (Figs 1 and 2). Eight dental implants (three controls) were removed in block sections at 4 months following installation. Two dental implants were lost as a result of infection (animal 217). The remaining dental implants received healing abutments. A total of 15 bridges (13 two-unit and two three-unit bridges) were placed onto these implants and were subsequently exposed to functional loading for 12 months, at which time the animals were killed for histologic analysis. One animal died at 4 months following defect induction and reconstruction as a result of kidney failure unrelated to the experimental protocol (animal 220). Block sections including dental implants exposed to 12 months of functional loading for another animal (animal 221) were lost in the histological processing. Thus, four dental implants placed into rhBMP-2/ACS induced bone were evaluated following 4 months of osseointegration, and 18 implants following 12 months of functional loading. The corresponding numbers of implants placed into the adjacent resident bone were three and four, respectively.

Radiographic observations

Radiographic observations are shown in Fig. 2 and summarized in Table 4. New bone formation was observed in the rhBMP-2/ACS augmented sites; however, oval-shaped radiolucent voids within the newly formed bone were observed in several sites from the 1-month observation interval. Bone voids were noted in seven of 12 sites receiving rhBMP-2/ACS, in four of six sites receiving rhBMP-2/ACS/GTAM (two remaining sites exhibited wound failure), and in two of six sites receiving rhBMP-2/ACS/Resolut (two remaining sites exhibited wound failure). Thus bone formation appeared normal in seven of 24 sites. Bone voids were observed in 13 sites.

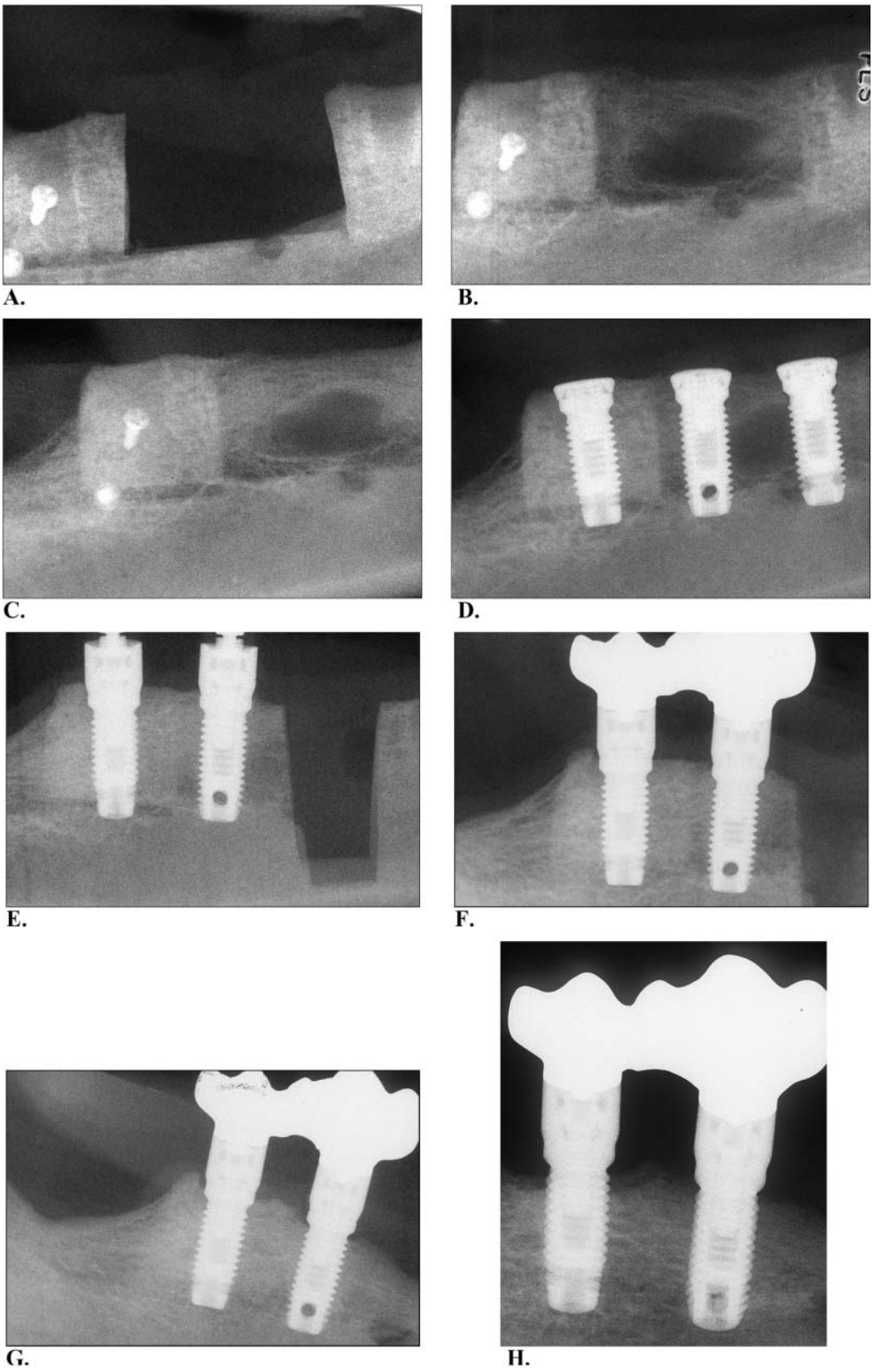

Fig. 2. Representative radiographs of defect site implanted with rhBMP-2/ACS (animal 219). The defect immediately following implantation of rhBMP-2/ACS (A) and at 2 months post-surgery (B) is shown. Note the radiolucent area centrally in the defect site (B). The radiolucent area appears to have regressed at dental implant installation 3 months post-surgery (C). Two dental implants were placed into the defect site and one implant into the adjacent resident bone (D). Following 4 months of osseointegration some dental implants placed into rhBMP-2/ACS induced bone (E) or resident bone were biopsied for histometric evaluation. The remaining implants are prosthetically reconstructed (F). Limited crestal resorption of peri-implant alveolar bone may be observed following 7 (G) and 12 months (H) of functional loading.

Four sites exhibited wound failure, membrane exposure and limited bone formation. The bone voids gradually filled with bone also in contact with dental implants placed into such sites (see Fig. 4 below). All dental implant sites, whether in rhBMP-2/ACS induced bone or in resident bone, exhibited limited crestal resorption over the 16-month observation interval.

Histologic observations

Dental implants biopsied at 4 months appeared osseointegrated and exhibited limited, if any, crestal resorption. The newly formed rhBMP-2/ACS induced bone exhibited normal trabeculation and cortex formation. There were no apparent differences between dental implants placed into rhBMP-2/ACS induced bone and dental implants placed into resident bone (Tables 5 and 6; Graphs 1 and 3).

Representative photomicrographs of dental implants exposed to 12 months of functional loading are shown in Figs 3, 4 and 5. The histologic sections provided evidence of normal cortical and trabecular lamellar bone. Dental implants exhibited limited, if any, crestal resorption at their oral aspect, which best can be characterized as a thick bony ledge with a re-established cortex (Graph 2 and Table 7). In contrast, the buccal aspect of the implants exhibited considerably thinner bony housing, often with crestal resorption exposing one or several threads to the immediate connective tissue (Graph 2 and Table 7). Apical migration of the peri-implant mucosal epithelium was not observed. The dental implants appeared osseointegrated as evidenced by bone contacts along the entire implant surface (Graph 3). There was no difference between dental implants placed into sites augmented by rhBMP-2/ACS and those placed into resident bone (Tables 7 and 8). Residual ACS matrix could not be demonstrated for any observation interval. None of the specimens provided evidence of residual bone voids.

Histometric observations

Individual and mean (± SD) histometric observations are shown in Tables 5–8. Tables 5 and 6 show BICs at dental implants placed into rhBMP-2/ACS induced bone or resident bone following 4 months of osseointegration. Specimens from three animals in each group were available for analysis. Dental implants placed into rhBMP-2/ACS induced bone exhibited similar conditions as those placed into resident bone. The implants were almost totally immersed in newly formed bone with limited, if any, crestal resorption. The dental implants exhibited similar contact patterns in rhBMP-2/ACS induced and resident bone. There were no statistically significant differences between the groups.

Tables 7 and 8 show BICs at dental implants placed into rhBMP-2/ACS induced bone or resident bone following 12 months of functional loading. Specimens from four and three animals, respectively, were available for analysis. Dental implants placed into rhBMP-2/ACS induced bone and resident bone exhibited similar conditions. The lingual aspect of the implants was almost totally immersed in bone with limited crestal resorption. In contrast, the buccal aspect almost always exhibited some crestal resorption. BICs following 12 months of functional loading were similar in rhBMP-2/ACS induced and resident bone and comparable to that observed following 4 months of osseointegration. There were no statistically significant differences between the groups.

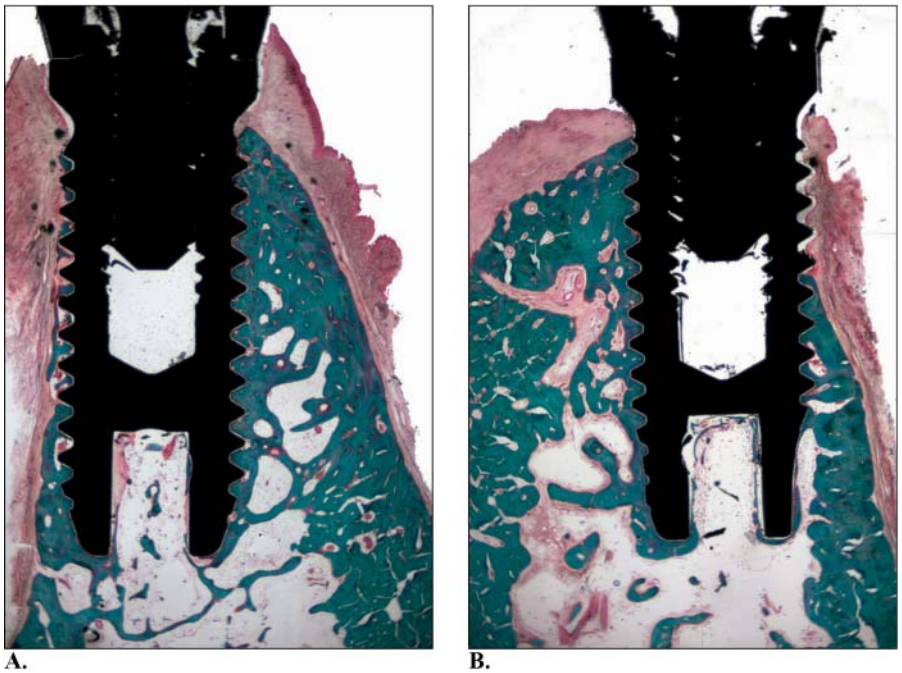

Fig. 3. Representative photomicrographs (animal 216) of dental implants placed into a defect site implanted with rhBMP-2/ACS (A) or into the adjacent resident bone (B). There were no remarkable differences in bone formation and BIC between the implant sites.

Discussion

The objective of this study was to evaluate bone formation and BIC at dental implants placed into rhBMP-2/ACS induced bone following 12 months of functional loading. Saddle-type, alveolar ridge defects, four per animal, were surgically induced in the mandible in six dogs planted with rhBMP-2/ACS with or without occlusive barrier membranes and were allowed to heal for 3 months before dental implant installation. Previous work (Jovanovic et al. 2001) with the same model demonstrated that a control group of collagen alone exhibited minimal bone regeneration comparable to a negative control. Therefore, this treatment was excluded from the study protocol. The implants were osseointegrated for 4 months before prosthetic reconstruction and functional loading. Healing was complicated by wound failure and membrane exposure in four defects. Defects without wound failure filled to the contour of the alveolar crest. The newly formed trabecular bone exhibited features of the adjacent resident bone with a re-established cortexformed bone comprised ovoid radiolucent voids that resolved over time. Dental implant biopsies collected following 4 months of osseointegration exhibited limitedthose exposed to functional

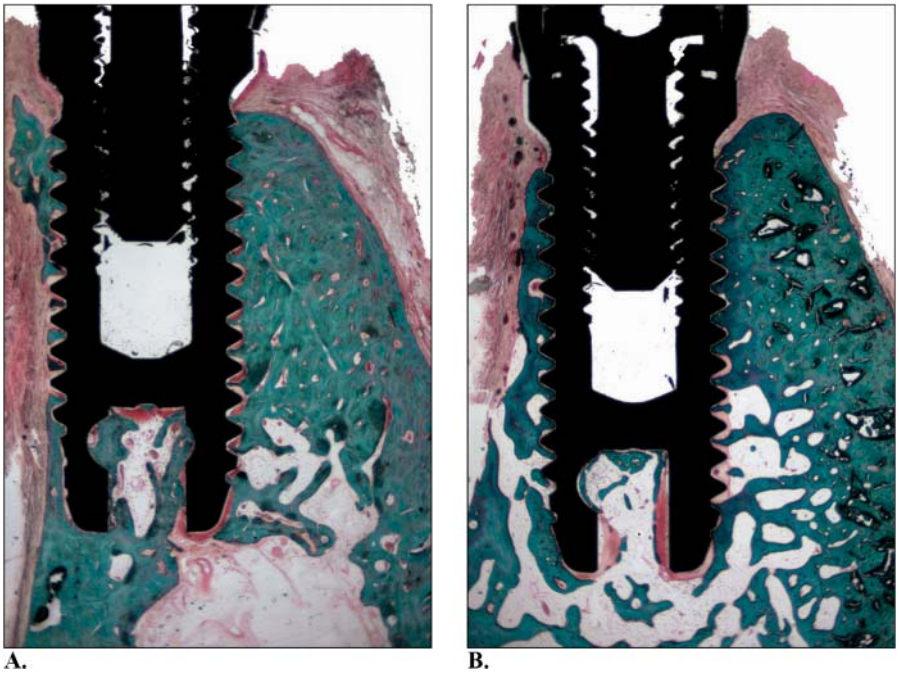

Fig. 4. Representative photomicrographs (animal 218) of dental implants placed into a defect site implanted with rhBMP-2/ACS in conjunction with a GTAM membrane (A) or into the adjacent resident bone (B). There were no remarkable differences in bone formation and BIC between the implant sites.

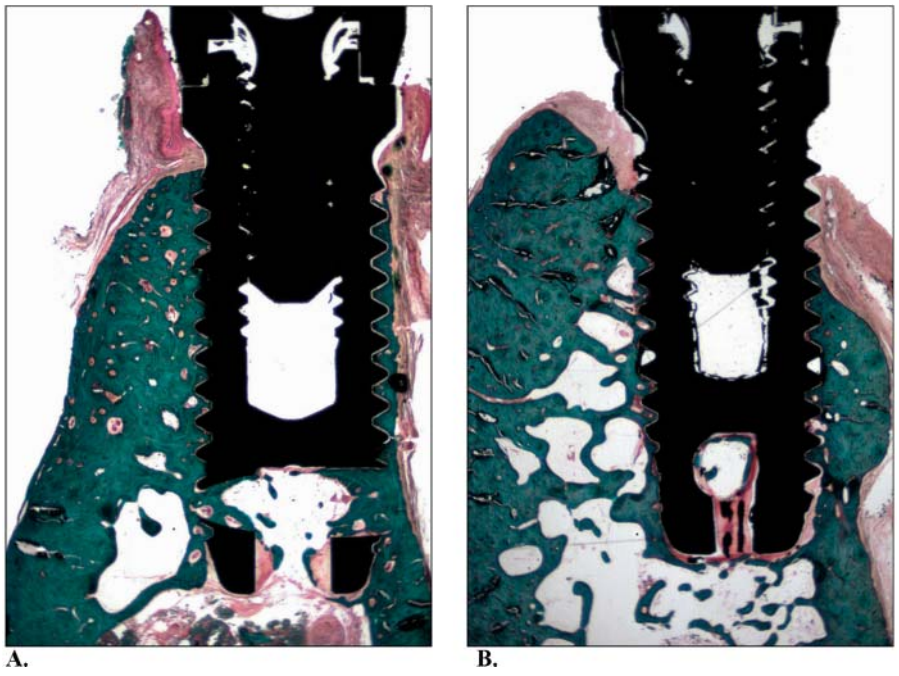

Fig. 5. Representative photomicrographs (animal 219) of dental implants placed into a defect site implanted with rhBMP-2/ACS (A) or into the adjacent resident bone (B). There were no remarkable differences in bone formation and BIC between the implant sites.

loading for 12 months exhibited some crestal resorption, in particular in their buccal aspect. The rhBMP-2/ACS induced bone compared favorably with the resident bone for all parameters evaluated at each time point.

Observations of localized post-surgery swelling and altered bluish complexion of the alveolar mucosa have been made in several previous studies following implantation of rhBMP-2/ACS in dogs (Wikesjö et al. 1999; Jovanovic et al. 2001; Selvig et al. 2001). The mucosal lesions appear closely associated with the oval-shaped radiolucent bone voids observed radiographically. In this study, these bone voids were observed clinically during dental implant installation. The bone voids, solitaire or contiguous, central or peripheral, large or of limited dimensions, have tentatively been diagnosed as seromas. Bone voids were observed in 13 of 24 defects in which the rhBMP-2/ACS/GTAM combination induced voids, which did not allow implant placement. Previous studies in this model have reported bone voids in 11 of 14 defects (Jovanovic et al. 2001) and in two of four defects (Hunt et al. 2001) augmented by rhBMP-2. Such bone voids have also been observed in supra-alveolar defects in dogs (Sigurdsson et al. 1997, 2001; Bernard et al. 2000; Selvig et al. 2001). In the present study, dental implants were installed into sites containing moderate bone voids; these voids resolved over time. Similar observations have been made in previous studies in the presence and absence of dental implants (Sigurdsson et al. 2001; Jovanovic et al. 2001). Apparently, implants placed into sites including moderate bone voids become osseointegrated without further compromise. The genesis of the bone voids is unknown. They may be species and/or site dependent, and they may be generated by concentration gradients of rhBMP-2 within the site. Regardless, bone voids have not been reported in evaluations of rhBMP-2/ACS induced bone in clinical studies (Boyne et al. 1997; Howell et al. 1997).

Histologic evaluation was based on buccal-lingual sections of all 12-month loaded implants. The 4-month biopsies were sectioned in a mesio-distal direction because of buccal-lingual tissue loss at the biopsy retrieval. All histologically evaluated dental implants were osseointegrated, whether installed into rhBMP-2/ACS induced or into native resident bone. Similar BIC values were observed in rhBMP-2 induced bone at 4 months of osseointegration (~ 41%) and at 12 months of functional loading (~ 52%). Corresponding values for dental implants placed into resident bone approximated 53 and 75%. The limited number of animals representing each

Table 7. **Dental implant–bone contact at 12 months of functional loading in rhBMP-2/ACS induced bone**

Animal	Bone height (mm)		Mesial	Crestal resorption (mm)		Coronal bone contact (mm)	
	Mesial	Distal		Distal	Mesial	Distal	BIC (%)
216	8.0	8.6	1.8	1.2	0.6	0.3	60.6
217	7.3	7.2	2.6	2.5	0.5	0.6	54.2
218	7.4	8.2	2.3	1.6	1.8	1.2	49.5
219	7.0	7.8	2.8	2.1	3.7	1.3	46.2
Mean ± SD	7.5 ± 0.4*	8.0 ± 0.6†	2.4 ± 0.5	1.8 ± 0.6	1.6 ± 1.5	0.9 ± 0.5	51.7 ± 7.1

*98% newly formed bone.
†96% newly formed bone.

Table 8.. **Dental implant–bone contact at 12 months of functional loading in resident bone**

Animal	Bone height (mm)		Mesial	Crestal resorption (mm)		Coronal bone contact (mm)	
	Mesial	Distal		Distal	Mesial	Distal	BIC (%)
216	5.4	8.3	4.5	1.6	0.6	0.5	81.3
218	8.0	9.1	1.8	0.7	0.1	1.0	75.4
219	6.3	8.6	3.8	1.1	0.0	1.1	67.3
Mean ± SD	6.6 ± 1.3	8.7 ± 0.4	3.3 ± 1.4	1.1 ± 0.4	0.2 ± 0.3	0.9 ± 0.3	74.7 ± 7.0

observation makes comparisons between BIC values precarious. Nevertheless, it may be concluded that rhBMP-2/ACS induced bone provided a foundation for long-term osseointegration of dental implants subject to functional loading. Several recent studies have evaluated BIC in rhBMP-2 augmented alveolar bone and in native alveolar bone in canine and nonhuman primate models (Jovanovic et al. 1993, 1995; Caplanis et al. 1997; Hanisch et al. 1997; Sigurdsson et al. 1997, 2001; Boyne et al. 1999; Cochran et al. 1999). Other preclinical studies have evaluated BIC following functional loading in sites augmented by guided bone regeneration (Buser et al. 1995, Simion et al. 2001), resorbable hydroxyapatite (Quinones et al. 1997), or distraction osteogenesis (Block et al. 1998). Comparisons of BIC values between studies should be made with caution because of variations in method of analysis, plane of analysis, sites of analysis, observation interval, and other factors that may make comparisons merely speculative. Regardless, the observations herein suggest that rhBMP-2-based technology appears an attractive and realistic alternative for augmentation of alveolar bone and subsequent osseointegration and functional loading of dental implants.

The dental implants exhibited radiographic and histologic evidence of crestal resorption at 12 months of functional loading. Crestal resorption appeared increased at the buccal compared to the lingual aspect of dental implants placed into rhBMP-2/ACS induced bone and into the native resident bone. It cannot be verified whether crestal resorption at the buccal and lingual sites was established already at 4 months of osseointegration as the block biopsies from that observation interval were sectioned in a mesial-distal plane. It is suggested from the histologic specimens, however, that the implants were placed in a buccal position (this assumption has been verified by the surgeon). Thus differences in crestal resorption between buccal and lingual implant surfaces likely relate to discrepancies in bony housing and subsequent remodeling, but not to functional loading. Limited crestal resorption following functional loading has also been observed at titanium plasma-sprayed and hydroxyapatite coated dental implants in mandibular sites in dogs (Corso et al. 1999), and at titanium plasma-sprayed dental implants in rhBMP-2 augmented alveolar ridges in dogs (Sigurdsson et al. 2001). Thus the crestal resorption may not necessarily be unique to the particular dental implant technology used in this study. Moreover, limited crestal resorption appears a routine observation in clinical practice (Brånemark et al. 1985).

Four defects exhibited wound failure, membrane exposure and subsequently limited bone formation. Two of the defects had received rhBMP-2/ACS and the occlusive nonresorbable ePTFE membrane. Two defects had received rhBMP-2/ACS and the occlusive bioresorbable polyglycolide fiber membrane. Radiographic bone fill in these defects was limited to that commonly observed in surgical controls in this defect model (Hunt et al. 2001; Jovanovic et al. 2001). Similar observations of wound failure and outcomes following implantation of rhBMP-2/ACS and occlusive barrier membranes have been reported (Jovanovic et al. 2001). Three of 10 defects implanted with rhBMP-2/ACS and an ePTFE membrane experienced wound failure, membrane exposure and subsequent limited bone fill. Four of six defects receiving the ePTFE membrane without rhBMP-2 experienced wound failure, membrane exposure and impaired bone regeneration. In contrast, all defects receiving rhBMP-2/ACS without occlusive barrier membranes in this study, and in previous studies by Hunt et al. (2001) and Jovanovic et al. (2001) healed without wound failure. These defects filled with bone to the contour of the immediately adjacent alveolar crest. Other previous studies have shown that bone formation in craniofacial defects is significantly impeded when rhBMP-2 has been combined with occlusive barrier membranes (Zellin & Linde 1997; Cochran et al. 1999). Collectively the evidence suggests that tissue occlusive membranes do not provide additional value to the rhBMP-2 technology. In contrast, the evidence suggests that tissue occlusive membranes may complicate wound healing in alveolar sites as they may impair the bone inducing potential of rhBMP-2.

Conclusion

rhBMP-2/ACS induced bone allows installation, osseointegration, and long-term functional loading of machined, threaded, titanium dental implants.

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Résumé

L'ostéoïntégration est le but principal poursuivi lors du placement des implants dentaires. Un tel contact osseux apporte une stabilité des implants. L'objectif de cette étude a été d'évaluer la formation osseuse et le contact direct os-implant (BIC) à long terme, lors de la charge d'implants dentaires osseux placés dans l'os induit par la protéine-2 morphogénique osseuse humaine recombinée (rhBMP-2) dans une éponge collagène absorbable (ACS). Des lésions du rebord alvéolaire du type selle au niveau mandibulaire (±15×10×10 mm), deux par quadrant, ont été produites chirurgicalement chez chacun des six jeunes chiens adultes. Les lésions ont été immédiatement implantées avec du rhBMP-2/ACS. Deux lésions par animal ont reçu une membrane en téflon ou une membrane biorésorbable en fibre polyglycolide. La guérison s'est effectuée pendant trois mois avec la membrane en téflon avant que celle-ci ne soit retirée et que des implants dentaires en titane fileté et usiné ne soient placés dans l'os induit rhBMP-2/ACS et l'os résiduel adjacent. Après quatre mois d'ostéoïntégration, les implants ont été exposés afin de recevoir les piliers et le traitement prothétique (des bridges de deux à trois unités). Quelques implants ont été enlevés pour l'étude histologique. Les autres implants ont été exposés à la charge fonctionnelle durant douze mois après lesquels les animaux ont été tués pour l'analyse histométrique. Un animal est mort prématurément dû à un problème rénal sans relation avec le protocole expérimental et n'a donc pas été inclus dans l'étude. Les coupes obtenues après douze mois d'un autre animal ont été perdues lors des manipulations histologiques. Quatre sites ayant reçu du rhBMP-2/ACS et des membranes en téflon ou résorbables ont accusé une mauvaise guérison, une exposition de la membrane et n'ont montré qu'une formation osseuse limitée. Les lésions sans problèmes de guérison ont présenté un remplissage osseux. L'os néoformé montrait des similitudes avec l'os normal ayant un nouveau cortex; cependant, des zones plus claires étaient présentes mais disparaîssaient avec le temps. Les biopsies de quatre mois montraient une résorption crestale limitée tandis que celles ayant eu une charge durant douze mois indiquaient quelques résorptions de la crête. Les implants prélevés à quatre mois signalaient une moyenne BIC de 40,6±8,2% dans l'os induit par rhBMP-2/ACS vs 52,7±11,4% dans l'os normal. Les implants dentaires exposés à douze mois de mise en fonction indiquaient un BIC moyen de 51,7±7,1% dans l'os induit par rhBMP-2/ACS vs 74,7±7,0% dans l'os normal. Il n'y avait aucune différence significative entre les implants dentaires placés dans l'os induit par rhBMP-2/ACS et l'os normal pour aucun des paramètres à aucun intervalle d'observation. L'os induit par rhBMP-2/ACS permet l'installation, l'ostéoïntégration et la charge fonctionnelle à long terme d'implants dentaires en titane fileté et usiné chez le chien.

Zusammenfassung

Hintergrund: Die Osseointegration (direkter Knochen-Implantat-Kontakt/BIC) stellt das primäre Ziel nach Plazierung von enossalen Implantaten dar. Ein solcher Knochenkontakt gewährleistet die Stabilität des Implantats im Laufe der Zeit. Das Ziel dieser Studie war, die Knochenbildung und BIC bei langzeitbelasteten enossalen dentalen Implantaten, welche in induzierten Knochen eingesetzt worden waren, zu untersuchen. Die Induzierung des Knochens erflogte mit rekombinantem menschlichem knochenmorphogenetischem Protein-2 (rhBMP-2) in einem schwammförmigen Träger aus resorbierbarem Kollagen (ACS).
Methode: Im Unterkiefer von sechs jungen amerikanischen Fuchshunden wurden im Alveolarkamm pro Quadrant zwei sattelförmige Defekte chirurgisch präpariert. Die Defekte wurden mit rhBMP-2/ACS versorgt. Zwei Defekte pro Tier erhielten zusätzlich eine nichtresorbierbare expandierte Politetrafluoroethylenmembran (ePTFE) oder eine bioresorbierbare poliglycolide Fasermembran. Die Heilung dauerte 3 Monate. Die ePTFE-Membran wurde entfernt und es wurden maschinell bearbeitete schraubenförmige dentale Titanimplantate in den rhBMP-2/ACS induzierten Knochen und in den angrenzenden ortsständigen Knochen eingesetzt. Nach 4 Monaten wurden die Implantate freigelegt, die Aufbauteile wurden eingesetzt und prothetisch versorgt (Brücken mit 2 oder 3 Einheiten). Einige Implantate wurden für die histologische Untersuchung entfernt. Die verbliebenen Implantate wurden für 12 Monate einer funktionellen Belastung ausgesetzt. Danach wurden die Tiere für die histometrische Analyse geopfert.
Resultate: Ein Tier verstarb vorzeitig wegen Nierenversagen ohne Zusammenhang mit dem experimentellen Protokoll und wurde nicht in die Auswertung miteinbezogen. Die 12-Monate Blockschnitte eines zweiten Tieres gingen während der histologischen Aufarbeitung verloren. Vier Stellen, welche rhBMP-2/ACS und eine ePTFE oder resorbierbare Membran erhalten hatten, zeigten eine gestörte Wundheilung mit Membranexposition und in der Folge eine eingeschränkte Knochenbildung. Defekte ohne gestörte Wundheilung füllten sich bis zur Kontur des umgebenden Alveolarknochens auf. Der neugebildete Knochen zeigte Eigenschaften des ortsständigen Knochens mit einer neugebildeten Kompakta. Jedoch konnten radioluzente Areale gesehen werden, welche sich mit der Zeit auffüllten. Die dentalen Implantate in den Blockbiopsien nach 4 Monaten zeigten, wenn überhaupt, nur geringe Knochenresorption, während die Implantate mit 12 Monaten funktioneller Belastung eine gewisse krestale Resorption aufwiesen. Die Implantate der Biopsien nach 4 Monaten zeigten einen mittleren (±SD) BIC von 40.6±8.2% im rhBMP-2/ACS induzierten Knochen gegenüber 52.7±11.4% im ortsständigen Knochen. Die Implantate nach 12 Monaten funktioneller Belastung zeigten einen mittleren BIC von 51.7±7.1% im rhBMP-2/ACS induzierten Knochen gegenüber 74.7±7.0% im ortsständigen Knochen. Es bestanden für keinen der Parameter und für kein Beobachtungsintervall signifikante Unterschiede zwischen den Implantaten im rhBMP-2/ACS induzierten und im ortsständigen Knochen.
Schlussfolgerung: rhBMP-2/ACS induzierter Knochen erlaubt das Einsetzen, die Osseointegration und die Langzeitbelastung von maschinell bearbeiteten, schraubenförmigen dentalen Implantaten bei Hunden.

Resumen

Antecedentes: La osteointegración (contacto directo hueso-implante/BIC) es la meta primaria tras la instalación de implantes dentales endoóseos. Tal contacto óseo proporciona estabilidad al implante dental a lo largo del tiempo. El objetivo de este estudio fue evaluar la formación ósea y el BIC en implantes dentales endoóseos a largo plazo, cargados funcionalmente, colocados en hueso inducido por proteína-2 ósea humana morfogenética (rhBMP-2) en un vehículo de esponja de colágeno reabsorbible (ACS).
Métodos: Se indujeron quirúrgicamente defectos mandibulares de la cresta alveolar (~15×10×10 mm), en silla de montar, dos por cada cuadrante mandibular, en seis Fox Hounds americanos jóvenes adultos. Los defectos se implantaron inmediatamente con rhBMP-2/ACS. Dos defectos por animal recibieron una membrana no reabsorbible de politetrafluoretileno expandido (ePTFE) o una membrana reabsorbible de fibras de poliglicol. Se permitió la progresión de la cicatrización. Se permitió la progresión de la cicatrización durante 3 meses cuando se retiró la membrana de ePTFE y se instalaron implantes dentales de titanio roscados y torneados en el hueso inducido por rhBMP-2/ACS y en el hueso residente adyacente. Los implantes se expusieron a los 4 meses de osteointegración, para recibir los pilares y el tratamiento protético (puentes de 2 o 3 unidades). Algunos implantes se retiraron para análisis histológico. Los implantes restantes se expusieron a carga funcional durante 12 meses tras los cuales los animales se sacrificaron para análisis histométrico.
Resultados: Un animal murió prematuramente debido a fallo renal sin relación con el protocolo experimental y no se incluyó en el análisis. Las secciones de los 12 meses de un segundo animal se perdieron en el procesamiento histológico. Cuatro sitios que recibieron rhBMP-2/ACS y membranas de ePTFE o reabsorbibles experimentaron fallos en la herida exposición de la membrana y subsecuentemente exhibió una limitada formación ósea. Los defectos sin fallos en la herida se rellenaron hasta contornear el hueso alveolar adyacente. El hueso neoformado exhibió características del hueso residente con un cortex reestablecido, de todos modos, incluyó comúnmente áreas radiolúcidas que se reabsorbieron con el tiempo. Los bloques de implantes dentales que se biopsiaron a los 4 meses exhibieron una limitada, si alguna, reabsorción crestal. Los implantes biopsiados a los 4 meses exhibieron un BIC medio (±SD) de 40.6±8.2% en hueso inducido por rhBMP-2/ACS frente a 52.7±11.4% en hueso residente. Los implantes dentales sometidos a carga funcional durante 12 meses exhibieron un BIC medio de 51.7±11.4% en hueso inducido por rhBMP-2/ACS frente a 74.7±7.0% en hueso residente. No hubieron diferencias significativas entre los implantes dentales colocados en hueso inducido por rhBMP-2/ACS y en hueso residente para ningún parámetro en ningún intervalo de observación.
Conclusión: El hueso inducido por rhBMP-2/ACS permite la instalación, osteointegración, y carga funcional a largo plazo de implantes dentales de titanio roscados torneados en perros.

要旨
背景：骨性統合（骨とインプラントの直接接触／ＢＩＣ）は、骨内歯牙インプラント埋入の初期目標である。このような骨の接触により、経時的な歯牙インプラントの安定性が得られる。本研究では吸収性コラーゲン・スポンジ（ＡＣＳ）担体中のヒト組換え骨形成タンパク－２（rhBMP-2）によって誘導された骨内に埋入した歯牙インプラントに、長期間の機能的荷重をかけた後、骨形成とＢＩＣを評価した。
方法：アメリカン・フォックス・ハウンド犬の若い成獣６匹の各々において、片顎につき２箇所の下顎鞍状顎堤欠損（～１５ｘ１０ｘ１０ｍｍ）を外科的に形成した。欠損部には即時にｒｈＢＭＰ

－２／ＡＣＳを埋入した。各動物につき２箇所の欠損部にさらに非吸収性の拡張ポリ４フッ化エチレン（ePTFE)製メンブレンあるいは生体吸収性のポリグリコライド線維メンブレンを貼付した。３ヶ月の治癒期間をおき、ePTFE メンブレンを除去し、機械加工面のネジ式チタン・インプラントをｒｈＢＭＰ－２／ＡＣＳが誘導した骨及び隣接する既存骨に埋入した。骨性統合４ヶ月後、インプラントを露出して、アバットメントの連結と補綴治療（２または３ユニットのブリッジ）を行った。何本かのインプラントを抜去して組織学的分析を行った。残りのインプラントは機能的荷重を１２ヶ月かけた後に露出し、動物を安楽死させ、組織形態測定法による分析を行った。

結果：動物１匹は実験プロトコールと関係のない腎不全のため早期に死亡したので、分析には含まれなかった。別の１匹から１２ヶ月後に採取したブロック生検の切片は組織学的処理中に失われた。ｒｈＢＭＰ－２／ＡＣＳと ePTFE あるいは吸収性メンブレンを使用した４部位では創傷の治癒に失敗し、メンブレンの露出、さらに骨形成の不全がおきた。創傷治癒に失敗しなかった欠損部には新生骨ができ、隣接する歯槽骨と接触していた。新生骨は既存骨の特徴を表しており、皮質骨が再生していたが、一般には経時的にレントゲン透過部分を伴うようになった。４ヶ月後の歯牙インプラントのブロック生検では、歯槽頂の吸収は限局されていたが、機能的荷重を１２ヶ月かけた後には、ある程度の歯槽頂の吸収が認められた。４ヶ月後のインプラン生検標本は、ｒｈＢＭＰ－２／ＡＣＳに誘導された骨においては平均（±ＳＤ）４０．６±８．２％のＢＩＣを示し、既存骨においては、５２．７±１１．４％であった。１２ヶ月間機能的荷重をかけた後のインプラントでは、ｒｈＢＭＰ－２／ＡＣＳが誘導した骨においては平均５１．７±７．１％、既存骨では７４．７±７．０％のＢＩＣを示した。ｒｈＢＭＰ－２／ＡＣＳ誘導骨と既存骨に埋入したインプラント間には、どの観察時点でもどのパラメーターについても有意差はなかった。

結論：ｒｈＢＭＰ－２／ＡＣＳが誘導した骨は、犬において研磨加工したネジ式チタン製インプラントの埋入、骨性統合と長期の機能的荷重が可能である。

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