Osseointegration of Dental Implants Complexed with rhBMP-2: A Comparative Histomorphometric and Radiographic Evaluation

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Purpose: *To evaluate the effect of rhBMP-2 on implant osseointegration using histomorphometric and radiographic imaging analyses and determine the diagnostic accuracy of periapical radiographs regarding clinical bone-implant contact levels.* ***Materials and Methods:*** *Hollow-cylinder implants were filled with an absorbable collagen sponge soaked with recombinant human bone morphogenetic protein-2 (rhBMP-2) or left empty and implanted in the mandibles of dogs. Animals were followed for 2, 4, 8, or 12 weeks. At the end of each time interval, the animals were sacrificed and specimens were collected for histomorphometric and radiographic evaluation of the bone-implant contact levels.* ***Results:*** *Both groups exhibited the same mean histologic bone-implant contact on the outer surface of the implant, except for the 4-week group. The radiographic evaluation of bone-implant contact overestimated the actual osseointegration levels by at least 30%, a significant amount.* ***Discussion:*** *The osteoinductive and regenerative potential of rhBMP-2 is of clinical benefit in cases where bone augmentation is indicated and improved levels of osseointegration are expected. Radiographic evaluation has been the most widely employed technique in clinical practice for assessing bone levels around dental implants and comparing changes over time. However, there is a limit to the diagnostic accuracy of conventional radiographs when compared to the data obtained by histologic analysis.* ***Conclusion:*** *Application of rhBMP-2 within the confined boundaries of the hollow chamber of the implant had a limited effect on the osseointegration level along its outer surface, perhaps because of physically restricted diffusion. Radiographic evaluation resulted in the overestimation of bone-implant contact, and poor correlation with the histomorphometric data was found.* INT J ORAL MAXILLOFAC IMPLANTS 2004;19:667–678

Key words: *bone-implant contact, bone morphogenetic proteins, dental implants, histomorphometry, osseointegration, radiography*

During the process of implant osseointegration, a well-coordinated sequence of cellular and molecular events occurs that promotes wound healing and eventual functional relationship between living bone and the implant body.[1,2] Histologic studies have contributed significantly to the current knowledge about the process of osseointegration. The literature is replete with reports on histomorphometric analyses attempting to evaluate the bone-implant interface quantitatively and qualitatively.[3–5] As important and necessary as these studies appear to be for the ultrastructural evaluation of the interfacial zone, they offer little help for the clinical judgment of successful osseointegration. Implant stability is of paramount importance for clinical success as proposed by Albrektsson and associates,[6] and for this reason additional noninvasive methods to assess it objectively have been developed.[7–10]

Radiographic evaluation has been the most widely employed technique for monitoring bone levels around dental implants and comparing changes over time. With endosseous implants, 2

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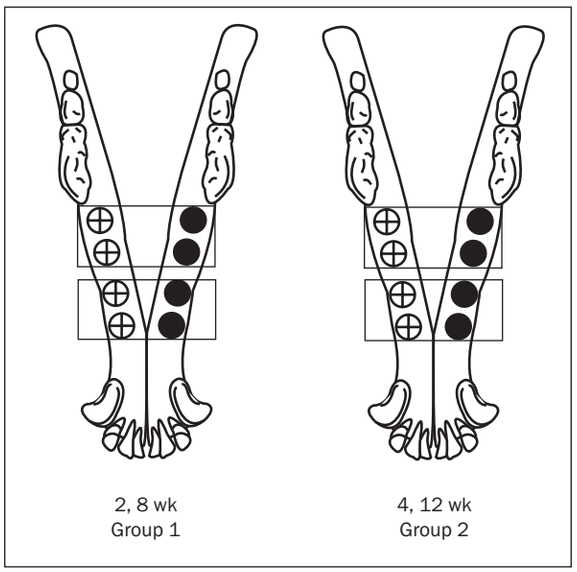

Fig 1 Schematic representation of implant arrangement in each group of dogs. Position (anterior vs posterior) and mandible side (right vs left) were alternately assigned for each observation time and dog.

types of radiographic assessments have been conventionally performed to evaluate the bone-implant response: *(1)* the distance between the crestal bone and the implant shoulder has been measured[11] and *(2)* qualitative bone changes have been measured based on density profiles and analyzed with sophisticated software programs.[12] However, although clinical symptoms are often associated with radiographic findings, there is a limit to the diagnostic accuracy of conventional radiographs that is inherent to their resolution capacity and influencing factors.

Sunden and coworkers[13] evaluated accuracy and precision in radiographic diagnosis of clinical instability and concluded that radiography is of little value in diagnosing implant instability. In a recent study, Sewerin and colleagues[14] concluded that radiography seems to be an unreliable method for diagnosing peri-implant spaces. This is in agreement with Adell and associates,[15] who stated that radiographs could not always provide diagnostic information related to osseointegration, even when computer-assisted analysis was used. Nevertheless, in daily clinical practice, conventional periapical radiography is commonly used to determine implant osseointegration.

The purpose of the present study was to evaluate the effect of rhBMP-2 on implant osseointegration using histomorphometric and radiographic imaging analyses and to determine the diagnostic accuracy of periapical radiographs in the clinical assessment of bone-implant contact levels.

MATERIALS AND METHODS

Animal Model

Twelve adult male American foxhounds weighing between 25 and 30 kg were used in the study, according to a protocol approved by the Institutional Animal Care and Use Committee at Baylor College of Dentistry. The animals were quarantined for an acclimation period of 10 days. At the beginning of the first phase of the protocol, each dog was weighed and was given a collar with a number on it for identification purposes during the study. The dogs were divided into 2 groups. Group 1 consisted of 6 dogs followed for 2 and 8 weeks, and group 2 consisted of 6 dogs followed for 4 and 12 weeks (Fig 1). The study was performed in 3 phases. In the first phase, the mandibular premolars of all 12 dogs were bilaterally extracted. In the second phase, hollow-cylinder implants were placed for 1 of the observation times in each group. In the third phase, the rest of the implants scheduled for the remaining observation times in each group were placed surgically.

SURGICAL PROCEDURES

Initial anesthesia was induced with intramuscular injections of 20 mg/kg ketamine hydrochloride (HCl) (Ketaset; Wyeth/Fort Dodge Animal Health, Fort Dodge, IA) and 2 mg/kg xylazine (Ben Venue Laboratories, Bedford, OH). After intubation, general anesthesia was maintained with a mixture of 2% halothane and O_2 at a rate of 1 L/min. The general anesthetic was delivered and monitored under the supervision of an experienced animal technician. Local anesthesia with 2 to 4 mL of 2% lidocaine HCl with 1:100,000 epinephrine was administered at the surgical site, and a full-thickness mucoperiosteal flap was reflected to expose the anatomic crowns of the teeth. All mandibular premolars were sectioned to the root furcation level using high-speed carbide burs under constant saline irrigation. Each segment was then individually removed with minimal trauma. Silk sutures were used to reposition the flaps and ensure complete coverage of the alveolar bone. Periapical radiographs at settings of 15 mA, 75 kV(p), and ½ second were then obtained to verify complete root removal. Postoperatively, the animals received a mixture of penicillin G procaine and penicillin G benzathine (300,000 units/mL) intramuscularly in a dose of 1 mL/5 kg of body weight. The same dose was repeated after 48 hours. Ibuprofen 10 mg/kg (Advil, Whitehall-Robins Healthcare, Madison, NJ) was also administered by mouth twice a day for 2 to 3 days. The dogs were placed on a soft diet until

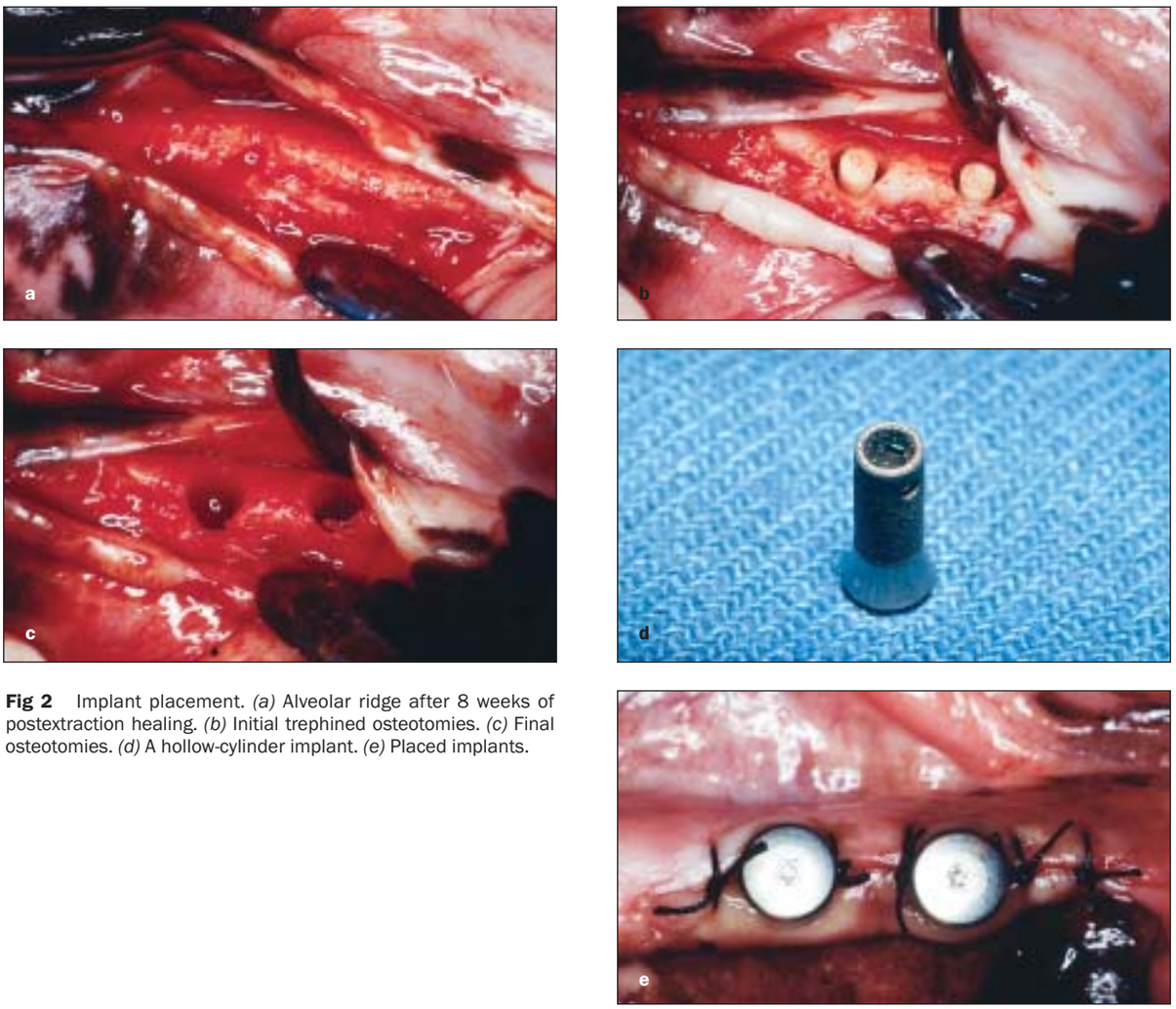

Fig 2 Implant placement. *(a)* Alveolar ridge after 8 weeks of postextraction healing. *(b)* Initial trephined osteotomies. *(c)* Final osteotomies. *(d)* A hollow-cylinder implant. *(e)* Placed implants.

completion of the study. After a healing period of 8 weeks, lateral radiographs were obtained to evaluate the bone quality and quantity, and the animals entered the next phase of the study.

Implant Placement for Histomorphometric Analysis

For the second phase, all 12 dogs (groups 1 and 2) were premedicated and anesthetized using the same protocol described for the first phase. Implant surgery was performed in each of the previously prepared alveolar ridges. Each dog received 4 implants (2 controls on one side of the mandible and 2 experimentals on the contralateral side) scheduled for the 8-week (group 1) or 12-week (group 2) observation periods. Sides (left vs right) and sites (anterior vs posterior) were assigned alternately for each dog. An alveolar crest incision was made, and full-thickness mucoperiosteal flaps were elevated to expose the sites for implant placement (Fig 2a). The osteotomy site was prepared with a trephine drill under constant saline irrigation following the protocol suggested by the manufacturer (Fig 2b). Using surgical forceps, the bone core of the osteotomy site was broken and extracted from the surgical site for subsequent histologic analysis, leaving the osteotomy site as if it had been prepared with a twist drill (Fig 2c).

Following this procedure, titanium plasma-sprayed (TPS) hollow-cylinder ITI implants (3.5-mm diameter and 8-mm length, ITI (042.071S; Straumann, Waldenburg, Switzerland) (Fig 2d) were placed as control implants. In the contralateral sites the hollow implants were filled with a solution of rhBMP-2 (Genetics Institute, Andover, MA) soaked on an absorbable collagen sponge (ACS) (Helistat; Colla-Tec, Plainsboro, NJ) prior to placement. The rhBMP-2 was added to the sponge with a concentration of 0.4 mg/mL; a total of 20 μg of protein was delivered with each implant. Control

Table 1 Timetable of Implant Placement

Group	No. of animals	No. of implants per interval and animal	Total no. of implants per animal	Timing of implant placement (wk)				
				0	4	6	8	12
1	6	2 control 2 experimental	8	X	—	X	S	—
2	6	2 control 2 experimental	8	X	—	—	X	S

X = implant placement; S = sacrifice.

defects were left empty to compare regular bone growth with that in the rhBMP-2–treated defects. It has been shown that the collagen carrier itself does not have an effect on bone formation.[16–18] Thus, the control group in the present study was designed to simulate the clinical situation of guided bone regeneration (GBR), with the titanium walls of the hollow chamber acting like a rigid membrane.

Implants were covered with a large closure screw and the flaps were closed with 3.0 silk sutures (Perma-Hand; Ethicon, Somerville, NJ) allowing transmucosal penetration of the implant (Fig 2e). Twenty-four implants were placed in 6 dogs for 8 weeks (group 1) and 24 implants were placed in 6 dogs for 12 weeks (group 2). Sutures were removed 7 to 10 days later.

At the third phase, which occurred 6 weeks after initial implant surgery in group 1 and 8 weeks after initial implant surgery in group 2, the same surgical procedure was followed for the implantation of an additional 24 implants in each group (Table 1). Groups 1 and 2 were sacrificed 2 and 4 weeks after the third-phase surgery, respectively.

Sacrifice

At the time of sacrifice, all group 1 animals had implants that had been in place for either 2 or 8 weeks, while all group 2 dogs had implants that had been in place for either 4 or 12 weeks. The design described was used to minimize possible unbalanced loss of implants, increase the number of intra-animal observations, and maximize the number of independent observations. Dogs were kept on a soft diet for the duration of the study. Their teeth and implants were rinsed daily with 20 to 30 mL of 2% chlorhexidine solution (Xttrium Laboratories, Chicago, IL).

At sacrifice, the animals were initially anesthetized intramuscularly with 20 mg/kg ketamine HCl and 2 mg/kg xylazine, followed by a mixture of 390 mg/mL phenobarbital sodium and phenytoin sodium 50 mg/ml (Beuthanasia-D; Schering-Plough, Kenilworth, NJ) at a dose of 1 mL/5 kg. The dogs' heads were then perfused with 10% buffered formalin at less than systolic pressure through the carotid arteries. The mandible was removed en bloc using a bone saw (Stryker, Kalamazoo, MI). Subsequently, implant blocks were prepared and stored in numbered vials containing perfusion solution. Implant blocks 10 to 12 mm long mesiodistally were cut along the buccolingual plane; all included the buccal and lingual cortical plates.

Radiographic Evaluation

Initially each specimen was positioned with its cut surface lying flat against a piece of size 2 film of speed D (Eastman Kodak, Rochester, NY). The paralleling technique was used with a focus-film distance of 25 cm. Settings of the x-ray machine (model GX 700; Gendex, Milwaukee, WI) were 15mA, 75kV(p), and ⅛ second. Radiographs produced in this manner were used to calculate the bone-implant contact (BIC) in the buccolingual dimension (Fig 3a). Specimens were then stabilized with a jig to a new position in which the plane of the cut surface was perpendicular to the radiographic film and the implant's long axis was parallel with the horizontal plane. The radiographic cone was adapted to the other side of the jig, directing the x-ray beam perpendicular to the long axis of the implant and the film. The same settings were used for both positions. The radiographs produced were used to calculate BIC in the mesiodistal dimension (Fig 3b). Radiographic films were developed in the same automatic machine (model A/T2000XR; Air Techniques, Hicksville, NY) using fresh chemical solutions.

All radiographic films were mounted in slide frames and scanned in a 35-mm scanner (Nikon LS-1000, Japan). The scanning resolution was 106 pixels/mm with a 9.4 μm^2 pixel and an output pixel format of 8-bit gray. Digitized images were saved as TIFF files and analyzed with Optimas software (Bioscan, Edmonds, WA). A rectangular range of interest (ROI) was selected, including the placement depth of the implant body with surrounding bone.

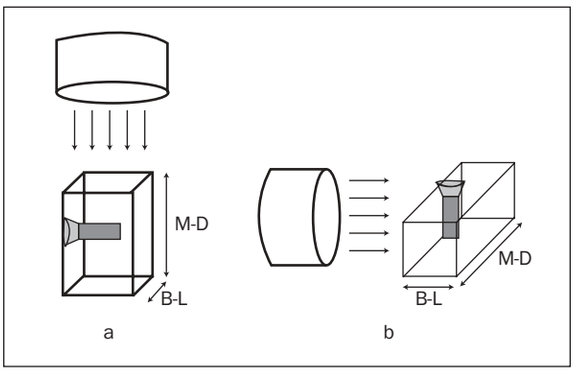

Fig 3 Schematic representation of radiographic setup. *(a)* A specimen lying flat against the cut surface produced radiographs allowing buccolingual (B-L) evaluation. *(b)* The specimen and radiographic cone were rotated 90 degrees for the production of radiographs of the mesiodistal (M-D) plane.

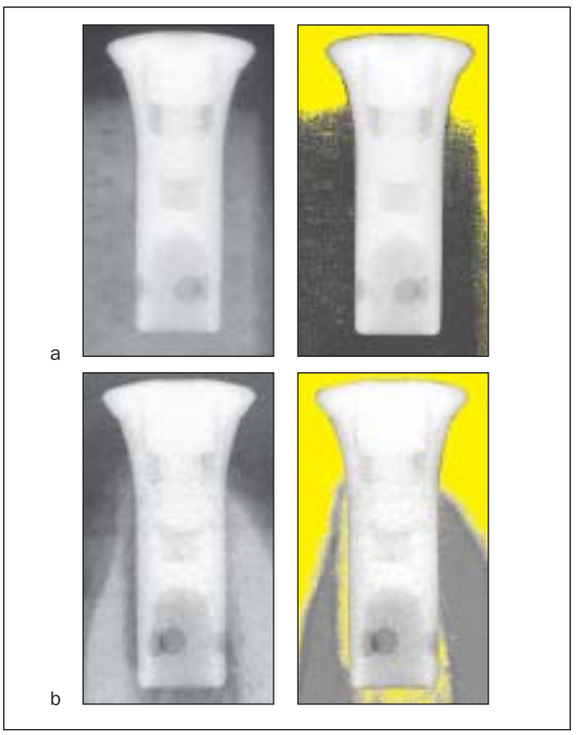

Fig 4 *(Right)* Example of radiographic morphometry. Void spaces are colored yellow based on their threshold luminance. Mesiodistal *(a)* and buccolingual *(b)* bone levels of the same implant are shown.

The image was viewed on a high-resolution screen under 200% magnification. For calibration, manual sampling of the film area outside the specimen boundaries was carried out; 10 points with no tissue presence were registered. Their luminance was automatically calculated on a numeric scale of 0 to 255, and the full range of values was set as the upper and lower threshold limit for detection of void spaces within the ROI. Void spaces were colorized, and BICs were traced and calculated as a percentage of total implant placement length (Fig 4). All analyses were performed by the same examiner, who was trained in the use of the software and the degree to which it was possible for error to relate to the pixel size and density.

Preparation of Undecalcified Specimens

Mandibular blocks containing the implants from groups 1 and 2 were left in the fixation medium (10% buffered formalin) for 7 to 10 days. A series of graded ethanols were used to dehydrate the specimens, which were then placed into embedding molds containing methylmethacrylate resin (Fisher Scientific, Fair Lawn, NJ) and left to cure at room temperature for 2 to 3 weeks. This protocol resulted in well-infiltrated specimens embedded in hard clear acrylic resin blocks. The implant-containing blocks were subsequently divided into parallel sections with the long axis of the implant in a buccolingual direction. Sectioning was performed using a low-speed diamond-blade saw (Isomet; Buehler, Lake Bluff, IL) under constant irrigation, resulting in tissue-implant sections with an initial thickness of 0.5 mm. These sections were reduced to 30 to 50 μm in thickness, using petrographic grinding techniques on a roll grinder containing sandpaper of decreasing grit sizes (Isomet; Buehler). The cut surface was mounted on a microscopic slide with epoxy-resin, polished, stained with Stevenel's blue and Van Gieson picrofuchsin, and protected by a glass coverslip.[19] Two to three sections including the full outline of the apical hollow chamber were obtained from each implant. Photomicrographs were taken using a Zeiss Axiophot photomicroscope (Zeiss, Oberkochen, Germany) with normal transmitted light at 25× magnification. The produced color slides were scanned in a 35-mm film scanner (LS-1000; Nikon, Tokyo, Japan) and the digitized color images were saved as TIFF files. Morphometric analysis of the BIC along the outer surface of the implant and inside the hollow chamber was performed with the Optimas software. Histomorphometric analysis of the % BIC along the outer surface of the implant and inside the hollow chamber was performed with color values threshold detection followed by linear tracing measurements using the same methods and techniques used with radiographic analysis (Fig 5). Qualitative analysis was also performed by studying bone tissue morphology, degree of trabeculation, cell dispersion, and osteogenesis process (distance vs contact).

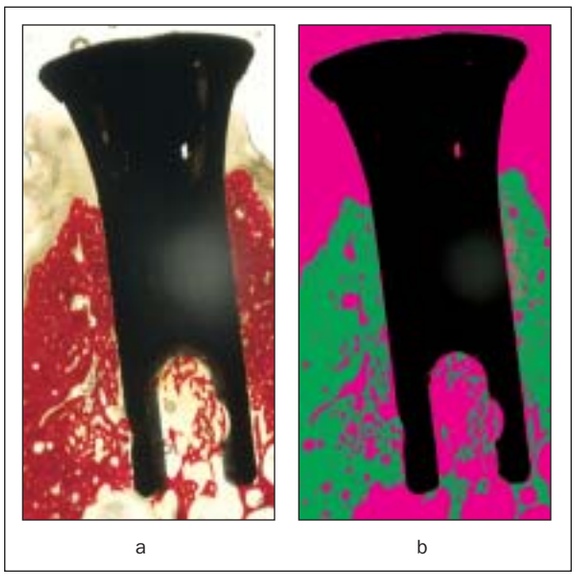

Fig 5 Example of histomorphometric analysis. *(a)* Undecalcified specimen (Stevenel's blue and Van Gieson's picro-fuchsin; original magnification ×15). *(b)* Color threshold detection of the same slide. Bone tissue is green; the remaining tissue is stained pink. Linear measurements of green against black provided BIC values.

Statistical Analysis

The study had a split-mouth, longitudinal design. Subject means for each treatment group at each time point were calculated first. This was done because of the lack of independence between observations within each dog. Summary statistics were then computed from these dog-level means. The dog was considered to be the unit of measure for treatment comparisons of each measure. Two sets of repeated-measures analysis of variance (ANOVA) were fitted for each group. The split-mouth design was taken into account in the fitting of the models. Residuals were tested and plotted to validate models. The variables analyzed included histologic BIC on the outer surface of the implant (% length), total histologic BIC (measured as the proportional average of outer surface and hollow chamber contacts), radiographic BIC (% length) in the buccolingual and mesiodistal planes, and total radiographic BIC measured as the average of the latter 2 values for each specimen. In addition to comparing the rhBMP-2 group to the control group over time, intraclass correlation coefficients (ICCs) using mixed modeling were calculated to assess agreement between histologic and radiographic measures. For each test the significance level was set at $P = .05$.

RESULTS

The overall implant survival rate was 68%; thus, 65 implants were available for histologic and radiographic analysis. Recorded measurements regarding the analyzed variables for all surviving implants are presented in Table 2. Figure 6 shows the mean histologic BIC values along the outer surface of the implant. There was no statistically significant difference between the 2 groups at 2, 8, and 12 weeks ($P > .05$). However, at 4 weeks the mean BIC was 8.58% for the controls and 25.73% for the rhBMP-2 group, a difference which was statistically significant ($P = .017$). Data for the radiographic BIC evaluation in the buccolingual direction are presented in Fig 7. No difference in the osseointegration level was found between the 2 groups at any time period. Results of the ICC analysis are presented in Table 3. ICC analysis between the histologic and radiographic BIC in the buccolingual direction (Fig 8) revealed disagreement between the 2 methods of BIC evaluation (ICC = 0.363).

No significant differences between the control and experimental groups were noted in the radiographic evaluation of BIC along the mesiodistal surfaces (Fig 9). However, radiographic evaluation of BIC along the mesiodistal surfaces disagreed with the radiographic evaluation of BIC along the buccolingual surfaces (Fig 10). ICC analysis of mesiodistal radiographic BIC with the histologic BIC along the implants' outer surface provided evidence for disagreement between the 2 data groups. When the BIC inside the hollow chamber was calculated in addition to the osseointegration level along the outer surface of the implant (Fig 11), the resultant total histologic BIC was not statistically different between the 2 groups at any time interval. Figure 12 shows the mean total radiographic BIC of both groups calculated as the average of the buccolingual and mesiodistal measurements. No significant difference was found between or within the 2 groups at any time point. ICC analysis between total radiographic BIC and total histologic BIC (Fig 13) revealed a poor agreement between the 2 methods (ICC = 0.395).

The histologic observation revealed islands of newly generated bone dispersed throughout the entire defect area for the rhBMP-2 implants, whereas in the control group, bone growth appeared to be initiated at and always connected to the base of the osteotomy site. Bone trabeculae were lined with osteoblast-like cells in both groups, indicating active bone deposition.

Table 2 HIstologic and Radiographic Measurements of BIC

Group/ observation period	Histologic BIC outside (%)	Radiographic BIC buccolingually (%)	Radiographic BIC mesiodistally (%)	Histologic BIC total (%)	Radiographic BIC total (%)
Control					
2 wks	37.36	60	93	24.20	77
	0.00	52	75	0.00	64
	0.00	46	79	8.09	63
	0.00	60	89	4.92	75
	6.83	55	96	4.23	76
	54.12	79	95	37.54	87
	0.00	48	92	0.00	70
	4.85	44	88	4.41	66
	0.00	51	77	0.00	64
	38.11	52	89	38.11	70
	55.82	99	100	35.91	99
4 wks	0.00	29	91	5.16	60
	38.87	85	89	28.06	87
	35.63	81	94	36.42	87
	21.73	36	99	13.73	67
	0.00	77	86	0.00	82
	23.23	38	95	14.65	66
	42.18	78	91	25.54	84
	29.03	25	94	18.31	59
	0.00	10	69	0.00	39
8 wks	60.82	68	86	45.03	77
	58.95	82	93	44.98	88
	25.60	58	92	22.43	75
	28.65	69	88	17.39	79
	10.96	83	85	6.85	84
	41.79	54	95	27.35	75
	53.86	41	79	33.18	60
	40.52	72	89	26.48	81
	65.23	71	74	42.46	72
12 wks	70.74	57	90	62.60	73
	71.59	60	87	50.64	73
	52.66	58	98	46.14	78
	86.57	60	96	57.04	78
	52.55	69	86	37.84	78
rhBMP-2					
2 wks	0.00	36	49	6.03	42
	13.74	50	93	8.33	71
	26.95	73	99	18.22	86
	14.54	49	91	11.86	70
	38.59	26	79	23.84	53
	70.39	45	95	43.63	70
	0.00	59	97	5.51	78
	0.00	40	100	0.00	70
4 wks	44.80	75	88	24.01	82
	0.00	71	96	11.27	84
	56.16	62	91	37.80	76
	69.90	88	95	43.52	91
	0.00	45	102	18.70	73
	66.18	62	97	39.04	79
	0.00	64	58	6.00	61
	51.62	42	81	44.44	61
	68.58	40	88	43.56	64
8 wks	17.22	34	91	22.47	62
	50.45	47	95	40.66	71
	16.79	63	95	28.51	79
	41.82	73	95	30.58	84
	0.00	63	83	4.52	73
	0.00	44	87	6.98	66
	70.88	60	100	47.29	80
	66.66	69	96	40.47	82
12 wks	39.63	52	94	55.41	73
	97.28	40	85	66.87	62
	51.11	83	98	44.63	90
	75.57	77	91	51.53	84
	39.14	75	92	41.75	83
	17.12	60	87	28.63	74

Overall implant survival was 68%, with 65 implants available for analysis.

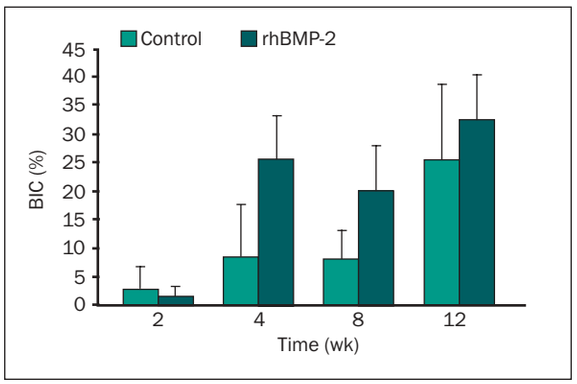

Fig 6 Mean values and standard deviations of the histologic BIC along the outer surface of the implant. Significant difference was noted only at 4 weeks ($P = .017$).

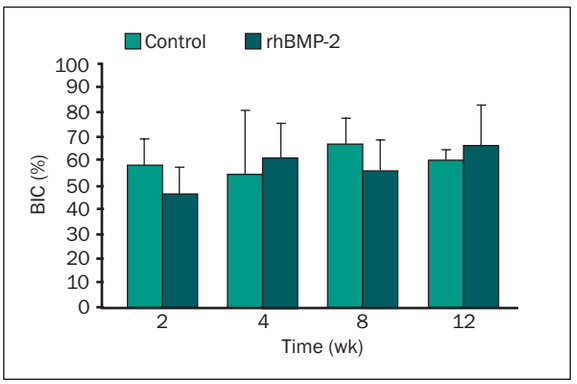

Fig 7 Mean values and standard deviations of the radiographic BIC in the buccolingual direction. No significant difference was found between the 2 groups at any time interval ($P > .05$).

Table 3 ICCs of Histologic and Radiographic BIC in the Mesiodistal and Buccolingual Directions

	Buccolingual histologic BIC (%)	Total histologic BIC (%)	Buccolingual radiographic BIC (%)	Mesiodistal radiographic BIC (%)
Buccolingual radiographic BIC (%)	0.363	0.446	—	0.289
Mesiodistal radiographic BIC (%)	0.181	0.195	0.289	—
Total radiographic BIC (%)	0.290	0.395	0.209	0.233

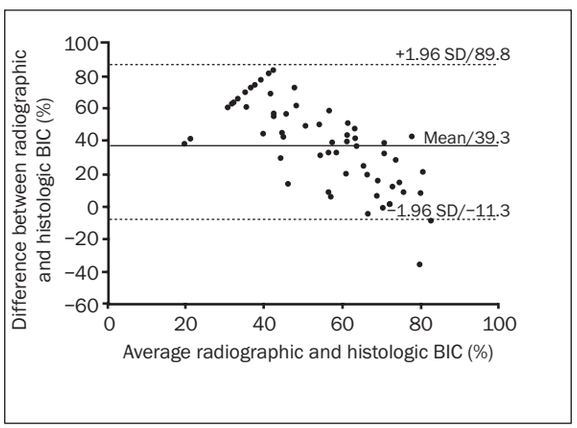

Fig 8 Bland-Altman plot of radiographic and histologic % BIC in the buccolingual direction.

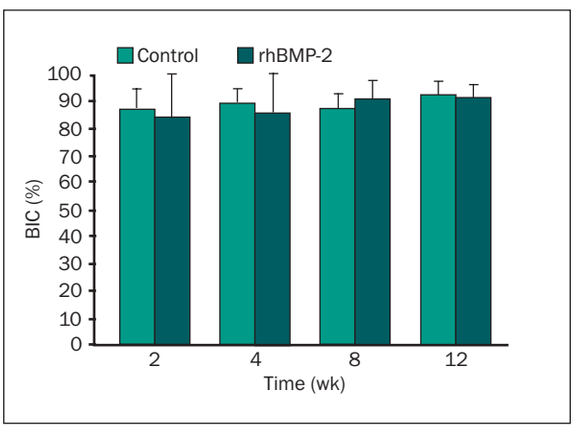

Fig 9 Mean values and standard deviations of the radiographic BIC in the mesiodistal direction. No significant difference was found between the 2 groups at any time interval ($P > .05$).

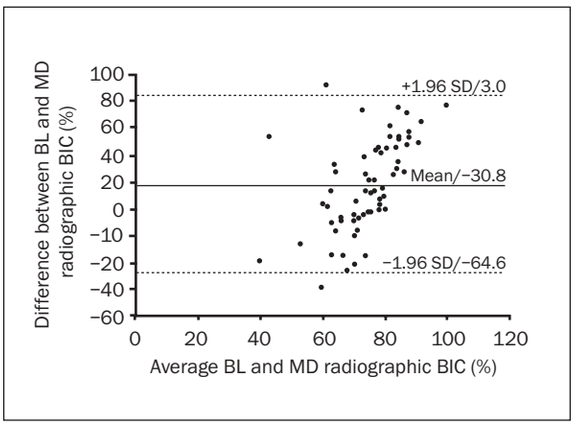

Fig 10 Bland-Altman plot of radiographic % BIC in the buccolingual (BL) and mesiodistal (MD) direction.

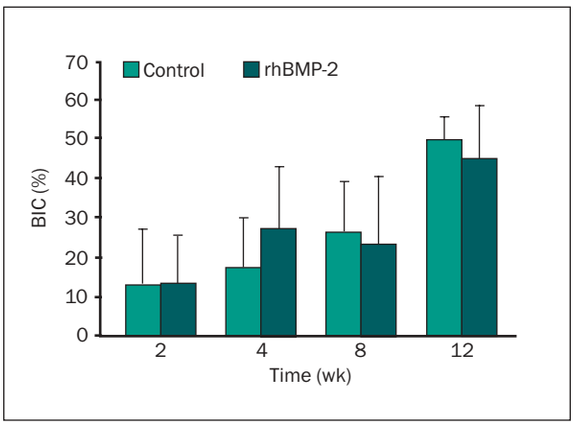

Fig 11 Mean values and standard deviations of the total histologic BIC (outside surface and hollow chamber). No significant difference was found between the 2 groups at any time interval ($P > .05$).

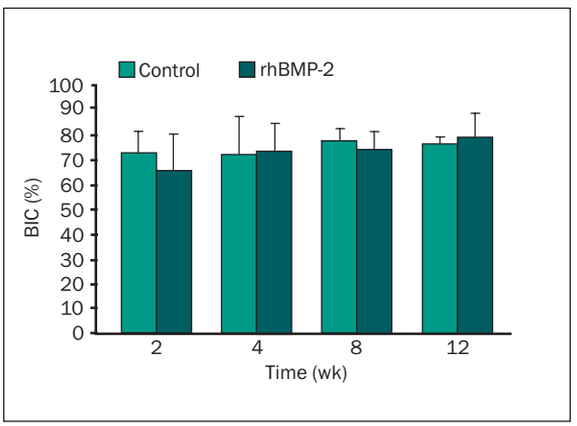

Fig 12 Mean values and standard deviations of the total radiographic BIC. No significant difference was found between the 2 groups at any time interval ($P > .05$).

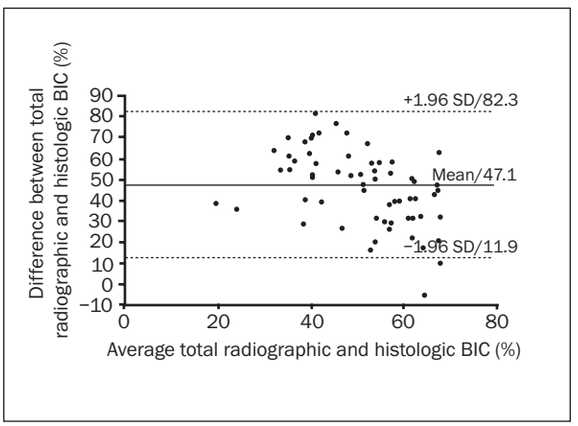

Fig 13 Bland-Altman plot of total radiographic and histologic % BIC.

DISCUSSION

The ability to accurately assess the bone-implant interface is of paramount importance for the clinical evaluation of implant function. In the present study, standard periapical radiographic measurements were compared to histomorphometric data acquired from undecalcified bone-implant specimens, providing information on their diagnostic potential. Statistically significant differences were found between the 2 methods, with radiographic evaluation demonstrating a tendency for overestimation of the actual bone-implant contact.

Implant survival in the present study was 68%. The randomness of failures with equivalent numbers in both rhBMP-2 and control groups indicates that treatment of groups was not a contributing factor. All osteotomy sites were prepared by an experienced oral surgeon with minimal trauma to the soft and hard tissues. Antibiotic coverage and daily application of chlorhexidine prevented infection of the implant sites. Possible reasons for the high failure rate could be the design of the implant and the animal model chosen. More specifically, the short length of the implant placed (8 mm), in combination with its press-fit design and lack of threads for stabilization, may have not provided adequate initial stability. Modification of the suggested surgical protocol by breaking and removing the trephined bone core may have reduced the initial contact surface area and the friction fit resistance of the bone-implant interface. Although dogs were kept on a soft diet, the implants were definitely out of occlusion, and cage openings were narrow enough according to the regulations so

that unintended loading and micromovement could have occurred by interposition and biting of the tongue, foot, and water or food bowl. However, the implants that survived were adequate in number to draw statistically significant conclusions.

Histomorphometric analysis has been accepted as the gold standard for the evaluation of the interfacial zone. However, histomorphometry can be performed in different ways, taking into consideration various parameters; this has resulted in a wide spectrum of reported values. Implant length, implant diameter, implant design, implant material, surface topography, implantation time, implantation site, loading conditions, analyzed length (total length vs 3 best threads), orientation of the histologic section (parallel vs perpendicular to implant's long axis) and specimen thickness are factors that affect and determine histomorphometric results. In the present study, the total BIC was calculated in specimens that were cut in a buccolingual direction along the implant long axis. It has been shown that no significant differences exist for the total BIC between specimens prepared in a transversal direction and those prepared in a longitudinal direction,[20] which suggests that the histomorphometric values reported here represent a valid approximation of the actual BIC.

A gradual increase in the level of osseointegration along the outside surface of the implants was observed, reaching a level of 26% for the control implants and 33% for the rhBMP-2 implants at 12 weeks. The confined nature of the defect, along with the small number of apical perforations, could have limited the diffusion and osseointegration-promoting action of rhBMP-2 along the outside of the implant's surface. Increased trabeculation of bone was observed as a result of rhBMP-2 diffusion. This was achieved through de novo regeneration or remodeling of existing bone. If the implantation site had been less confining, rhBMP-2 could have affected a wider host tissue area, but rapid diffusion of rhBMP-2 would have lowered its concentration and shortened the period of availability. Cells migrating into the hollow chamber in response to the chemotactic effect of rhBMP-2 caused bone induction within the sponge, and the newly matured osteoblasts produced bone matrix that was in contact with the inner implant surface.

When the hollow-chamber BIC values were added to the outside values, total BIC at 12 weeks was 51% for the control group and 48% for the rhBMP-2 group. These values are similar to osseointegration levels reported in other studies, which have ranged from 42% to 70%.[21–24] The slight decrease of BIC between 4 and 8 weeks in the rhBMP-2 group may be related to the local action of the rhBMP-2, which was able to diffuse through the apical perforations to the surrounding tissue. Increased remodeling and trabecular bone regeneration under the influence of rhBMP-2 has been reported in various studies[25,26] and may have affected the measured levels of osseointegration. The concentration of rhBMP-2 in this particular model may have been further modified by the confined nature of the defect. This confinement may have affected collagen sponge degradation, rate of delivery, cell proliferation and maturation, and the rate of mineralization by existing osteoblasts.[27–29]

The osteoinductive and regenerative potential of BMPs could be of tremendous clinical benefit in cases where bone augmentation is indicated. Although formations of branching, organized trabeculae of woven bone were seen filling the defect in both groups, their distribution was different. The observed histomorphometric disparities may reflect differences in the events that accompanied bone regeneration in the 2 groups. These histologic observations, together with the similarity between bone fill and BIC values for the control implants, support the hypothesis that bone growth occurred via osteoconduction rather than osteoinduction for the control implants. In the present study, there was no evidence for endochondral ossification. Bone formation took place via direct induction of mesenchymal cells into osteoblasts with active extracellular matrix deposition. Thus, the present model refutes the currently held belief of bone formation through a cartilaginous intermediate as is always observed in ectopic nonskeletal implantation of rhBMP-2. The process of bone formation through intramembranous or endochondral ossification is primarily dependent on microenvironmental conditions that have an impact on stability and source of cell population rather than carrier characteristics or implantation site per se.[30,31]

The radiographic evaluation of BIC overestimated the actual osseointegration levels by almost 100% in some cases. Radiographic superimposition of the mandibular buccal and lingual cortices precluded trabecular bone from showing, thus generating a false radiographic appearance of increased BIC at the mesiodistal plane. The mean radiographic mesiodistal BIC levels were 85% at 2 weeks and reached the level of 92% at 12 weeks, whereas the mean histologic BIC values did not exceed 58% at 12 weeks. Yet it is these radiographic BIC levels that clinicians usually evaluate in a periapical radiograph in an attempt to monitor and assess implant osseointegration (Fig 14).

Sewerin and associates[14] evaluated the accuracy of diagnosing peri-implant radiolucencies using an "exact-fit" group, a "0.1-mm space" group, and a

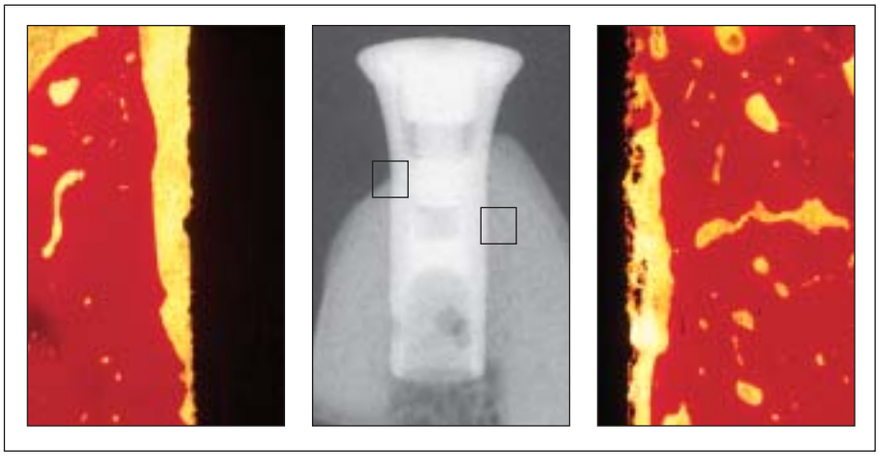

Fig 14 Histologic observation of the same implant seen in Figs 4 and 5 reveals the difference in the diagnostic accuracy of BIC measurements between the 2 techniques (original magnification ×100).

"0.175-mm space" group and concluded that accuracy improved at increased space widths, while specificity was low and sensitivity was moderate. Digital subtraction radiography has increased the sensitivity of the detection of subtle bone density changes which can be interpreted into qualitative assessments,[32] but offers little in terms of quantitative measurement of the interface. If radiographic film with resolution of less than 0.5 μm is used, it has the sensitivity to accurately assess BIC[33] and provide information about the mineralization of bone.[34] In routine clinical practice, however, radiographs are most often analyzed by the naked eye without any standardization that would provide the basis for comparisons. Albrektsson and Jacobsson[35] argued that clinical radiographs cannot be taken as evidence of a lack of fibrous tissue interface because there is a major difference between the cell dimensions and the optimal radiographic resolution. Despite their low diagnostic accuracy in determining % BIC, periapical radiographs remain a valuable tool in the clinical practice for the assessment of parameters that are not so critically dependent on resolution restrictions. Localized radiolucencies beyond the level of 0.1 mm (periapical radiographic resolution), fractured implants, fractured abutment screws, and resorption or remodeling of bone crest are some of the radiographic findings that can help the clinician diagnose clinical symptoms.

CONCLUSIONS

Application of rhBMP-2 within the confined boundaries of the hollow chamber of the implant had a limited effect on the osseointegration level along the implant's outer surface, perhaps because diffusion was physically restricted. Histomorphometrically determined BIC values were significantly higher for the rhBMP-2 group only at 4 weeks after implantation, whereas radiographic analysis did not support any statistical difference between the 2 groups at any time interval. However, radiographic evaluation of BIC in the mesiodistal plane, which is commonly used clinically, was significantly different from radiographic evaluation of the buccolingual plane and the actual histologic measurements. The limited resolution capacity of radiographs was likely responsible for the erroneous overestimation of osseointegration levels, even when computerized analysis was performed. It is suggested that more accurate radiographic imaging techniques be employed if implant osseointegration is to be accurately clinically assessed.

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