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Periodontal Repair in Dogs : Evaluation of rhBMP-2 Carriers

Lauralee Nygaard

ABSTRACT

PERIODONTAL REPAIR IN DOGS: EVALUATION OF rhBMP-2 CARRIERS

by

Lauralee Nygaard

This study evaluated candidate carriers for recombinant human bone morphogenic protein-2 (rhBMP-2) in periodontal reconstructive surgery.

Canine demineralized bone matrix (DBM), bovine inorganic crystallene bone matrix (Bio-Oss), bovine derived microfibrillar collagen matrix (Helistat), poly (D,L-lactide-co-glycolide) microparticles (PLGA), and polylactic acid granules (Drilac) were used with rhBMP-2 (20 μg/100 μl implant volume) in routine critical size canine supraalveolar periodontal defects. Contralateral defects in six beagle dogs were randomly assigned to receive: DBM/rhBMP-2, DBM-control, Bio-Oss/rhBMP-2, Helistat/rhBMP-2, PLGA/rhBMP-2, or Drilac/rhBMP-2, all with autologous blood except for Helistat and PLGA. Animals were sacrificed eight weeks postsurgery and block sections of the defects were processed for light microscopy. Histometric analysis included: defect height, connective tissue repair, cementum height, bone height, bone area, bone density, root resorption, and ankylosis. Mean and standard deviation values for each parameter were calculated for each carrier in each of two dogs, as well as mean values for both animals. Notable differences were evident among the candidate carriers examined, particularly with respect to cementum and bone regeneration. The results suggest that bovine derived bone matrix and canine demineralized bone matrix provide the best support for rhBMP-2 driven periodontal regeneration in the dog.

LOMA LINDA UNIVERSITY

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PERIODONTAL REPAIR IN DOGS: EVALUATION OF rhBMP-2 CARRIERS

by

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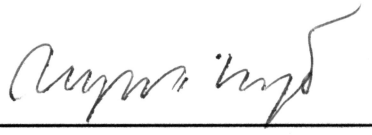
Each person whose signature appears below certifies that this thesis in their opinion is adequate, in scope and quality, as a thesis for the degree Master of Science.



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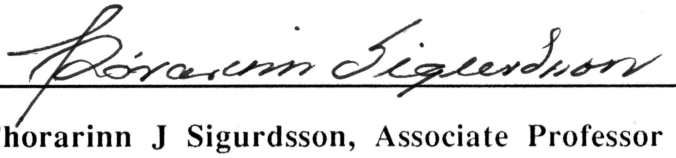

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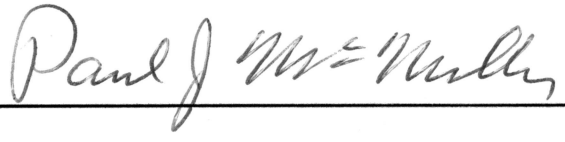

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CHAPTER 1

INTRODUCTION

PERIODONTAL REPAIR IN DOGS: EVALUATION OF rhBMP-2 CARRIERS.

Bone morphogenic proteins (BMPs) comprise a family of related proteins which possess a unique ability to induce cartilage and bone formation (Wang et al. 1990). BMPs have been suggested to induce differentiation of perivascular mesenchymal cells into osteoprogenitor cells (Wozney et al. 1988, Wozney 1995). Likely, it is this property of BMPs which has been shown to support healing of human femur and tibia nonunions (Johnson et al. 1988, Johnson et al. 1990). Recombinant human BMP-2 (rhBMP-2) has been shown to induce ectopic bone formation preceded by cartilage in rodents (Wang et al. 1990). Increasing concentrations of rhBMP-2 may enhance bone formation rate (Wozney et al. 1988, Wozney 1995). When high doses are used, cartilage and bone formation occur simultaneously. Thus, it appears that rhBMP-2 may influence both the endochondral and the intramembranous bone formation pathways. In addition, rhBMP-2 has been shown to induce cross species bone formation in critical size defects (Yasko et al. 1992, Toriumi et al. 1991).

Our studies employing the critical size supraalveolar periodontal defect model in the beagle dog have focus on factors influencing periodontal wound healing (Wikesjö et al. 1990, Wikesjö & Nilvéus 1990, Wikesjö & Nilvéus 1991, Wikesjö et al. 1991, Wikesjö et al. 1991, Wikesjö et al. 1991, Wikesjö et al. 1992, Wikesjö 1991, Wikesjö et al. 1992, Haney et al. 1993, Sigurdsson et al. 1994, Wikesjö et al. 1994, Sigurdsson et al. 1994, Haney et al. 1995, Sigurdsson et al. 1995, Wikesjö et al. 1992). We have shown that regeneration of cementum and alveolar bone in this model generally is limited, even in the presence of extensive new connective tissue attachment formation (Wikesjö & Nilvéus

1991, Wikesjö et al. 1994). We have shown that wound stability is critical for formation of a new connective tissue attachment (Wikesjö & Nilvéus 1990, Wikesjö et al. 1991, Wikesjö et al. 1992, Toriumi et al. 1991). Moreover, we have shown that creation and maintenance of a wound space, and infection control are critical factors supporting the natural regenerative potential of cementum and alveolar bone (Haney et al. 1993, Sigurdsson et al. 1994).

The osteogenic properties of BMPs (Urist et al. 1987, Lindholm et al. 1988, Ferguson et al. 1987, Johnson et al. 1989, Lovell et al. 1989, Wozney et al. 1988) and of growth factors (Joyce et al. 1990, Hollinger & Chaudhari 1992, Mohan et al. 1984, Seyedin et al. 1985, Hauschka et al. 1986, Canalis et al. 1988, Finkelman et al. 1990) have led to their evaluation as adjuncts in periodontal reconstructive therapy. Indeed, enhanced periodontal regeneration has been reported following surgical implantation of BMPs or growth factors (Sigurdsson et al. 1994, Ripamonti et al. 1994, Ishikawa et al. 1994, Bowers et al. 1991, Lynch et al. 1991, Rutherford et al. 1993). We have shown clinically significant cementum and alveolar bone regeneration in the supraalveolar periodontal defect model following surgical implantation of rhBMP-2 in autologous blood and bioerodable microparticles (Sigurdsson et al. 1994) This study evaluated additional candidate carriers for rhBMP-2 in periodontal reconstructive surgery using the supraalveolar periodontal defect model.

MATERIALS AND METHODS

Animals

Six young male beagle dogs 18 to 24 months old were used. Animal selection, management and surgical protocol followed routines approved for this study by Loma Linda University Institutional Animal Care and Use Committee (Wikesjö et al. 1994). Prestudy preparation included calculus removal and daily plaque control to obtain gingival health. A 2% chlorhexidine solution * applied as a rinse was used for the plaque control.

Surgical Procedures

Periodontal defects were surgically created in the mandibular right and left jaw quadrants as previously described (Wikesjö et al. 1994). Briefly, following sulcular incisions and elevation of buccal and lingual mucoperiosteal flaps, alveolar bone was surgically removed around the full circumference of the third and fourth mandibular premolar teeth. Clinical defect height measured 6 mm from the cemento-enamel junction to the alveolar crest (Figure 1). The first and second mandibular premolar teeth were extracted and the first molar amputated at the level of the reduced alveolar crest. Root surfaces were thoroughly planed using curettes and rotary instruments to remove cementum. Defects in alternate quadrants in subsequent dogs were randomly assigned to receive the various carriers with rhBMP-2 (Figure 1). Each carrier was implanted into two jaw quadrants in separate animals. The implants were molded around the premolar teeth. Periostea were then fenestrated at the base of the flaps. Flaps were positioned approximately 2 to 3 mm coronal to the cemento-enamel junction, and were adapted and closed with vertical mattress sutures © (Figure 1). Surgical procedures were performed under intravenous sodium pentobarbital anesthesia. @ A long acting opioid ** was used for immediate postoperative pain control. A broad spectrum antibiotic £ was administered twice daily the first two weeks postsurgery for infection control. Plaque control was accomplished by a daily 2% chlorhexidine rinse. Animals were fed a soft consistency laboratory diet ¶ supplemented with vitamins. ¥ Sutures were removed two weeks postsurgery.

Surgical Implants

Purified (>98%) rhBMP-2 ΩΩ, formulated in storage-stable buffer, and lyophilized, was used. Implants consisting of rhBMP-2 in carrier were formulated at a concentration of 20 µg per 100 µl implant volume. Actual implant volume per defect was 2.0 to 2.5 ml. Candidate carriers with rhBMP-2 in buffer included: canine demineralized bone matrix

(DBM; processed from freshly obtained beagle dog long bones) Ω, bovine inorganic crystallene bone matrix (Bio-Oss) †, bovine derived microfibrillar collagen matrix (Helistat) ‡, poly(D,L-lactide-co-glycolide) microparticles (PLGA) ††, and polylactic acid granules (Drilac) ¢. All carriers additionally included autologous blood except for Helistat and PLGA. Helistat was wetted with rhBMP-2 in buffer only. PLGA microparticles were mixed with glycerol. Since DBM may include endogenous bone morphogenetic activity, a DBM control with autologous blood was added.

Histological Procedures

The dogs were sacrificed eight weeks postsurgery by intravenous injection of concentrated sodium pentobarbital. Block sections including teeth, bone and soft tissues were removed. The blocks were fixed in 10% buffered formalin, decalcified in 5% formic acid, trimmed, dehydrated and embedded in butylmethacrylate and paraffin (McMillan et al. 1983). Serial sections, 7 μm thick, were cut in a buccal-lingual plane throughout the entire mesial-distal extension of the teeth. Every 14th section was stained with Ladewig's connective tissue stain modified by Mallory allowing for observations at 100-μm intervals.

Histometric Analysis

The most central stained section for each premolar tooth root was identified by the size of the root canal. This section and the immediate stained step serial section on either side were subjected to histometric analysis. Thus, three subsequent step serial sections, representing approximately 0.2 mm of the mid-portion of each root, were used. Analysis was performed using a PC based image analysis system β with a customized application for the supraalveolar periodontal defect model.

The following measurements were recorded for the buccal and the lingual surfaces of each root for each section:

Defect Height: Distance between apical extension of root planing and the cemento-enamel junction (CEJ).

Connective Tissue Repair: Distance between apical extension of root planing and apical extension of junctional epithelium.

Cementum Regeneration: Distance between apical extension of root planing and coronal extension of continuous layer of regenerated cementum or cementum-like deposit.

Bone Regeneration (height): Distance between apical extension of root planing and coronal extension of bone regeneration along the root apical to CEJ .

Bone Regeneration (area): Cross-sectional area represented by bone regeneration along the root apical to CEJ.

Bone Regeneration (density): Proportion of the regenerated area occupied by mineralized bone.

Root Resorption: Combined linear heights of distinct resorption lacunae on the root.

Ankylosis: Combined linear heights of ankylotic union of bone regeneration and the root.

Data Analysis

Summary statistics including means and standard deviations for each candidate carrier and the DBM control in each of two dogs were calculated. Due to the small sample size, statistical testing to determine differences between treatments was not considered meaningful.

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* Chlorhexidine Gluconate 20%, ICI Pharmaceutical Group, Wilmington, DE

@ Nembutal® Sodium Solution, Abbott Laboratories, North Chicago, IL

© Gore-Tex™ Suture CV5, WL Gore & Associates Inc, Flagstaff, AZ

** Buprenex Injectable, buprenorphine HCL, Reckitt Colman Pharmaceuticals Inc, Richmond,VA

£ Baytril® Brand of Enrofloxacin, Mobley Corporation, Animal Health Division, Shawnee, KS

¶ Pedigree®, Kal Kan Foods Inc, Vernon, CA

¥ Vet A Mix®, Pet-Form®, Vet-A-Mix Inc, Shenandoah, IA

ΩΩ rhBMP-2, Genetics Institute Inc, Cambridge, MA

Ω DBM, kindly provided by Dr. Anne Prewett, Osiris, OH

† Bio-Oss®, Osteohealth Co, Division of Luitpold Pharmaceuticals Inc, Shirley, NY

‡ Helistat™, Marion Laboratories Inc, Kansas City, MO

†† PLGA, Genetics Institute Inc, Cambridge, MA

¢ Drilac®, THM Biomedical Inc, Duluth, MN

β Image-Pro Plus™, Media Cybernetics ®, Silver Spring, MD

RESULTS

Clinical Observations

Primary wound closure was accomplished in all defects. No adverse reactions or infection occurred during the eight week healing sequence. One premolar tooth in one dog (DBM control) was lost to trauma. Commonly, jaw quadrants in separate animals exhibited carrier related clinical characteristics. General characteristics for defects receiving rhBMP-2 included swelling within the first week of surgery, appearing to culminate within two weeks postsurgery. During week one, the swollen tissue was soft to palpation, however, it became gradually firmer to assume characteristics of a bony alveolar ridge from week two postsurgery. A gradual regress of the tissue mass was usually observed from week two through eight postsurgery.

DBM/rhBMP-2 defects exhibited extensive swelling within a week of surgery. A gradual dimensional regression followed, however, at sacrifice the defects still appeared larger than immediately postsurgery. Limited gingival recession was observed. In some locations, gingival tissue gradually submerged the premolar teeth. Some variation in tissue response was observed between animals.

DBM control defects exhibited minimal swelling postsurgery. At two weeks, the gingival margin was located at the CEJ. At sacrifice the gingiva had receded to below the CEJ. The alveolar ridge had regressed to below presurgery dimensions. Both animals exhibited similar tissue reaction.

Bio-Oss/rhBMP-2 treated defects exhibited substantial initial swelling, some cusps gradually became submerged by the gingiva. The gingival margin remained coronal to the CEJ postsurgery. The granular texture of the implant material could easily be observed and palpated through the gingival and mucosal tissues throughout the healing sequence. Both animals presented similar tissue reaction.

Helistat/rhBMP-2 treated defects displayed moderate initial swelling followed by a gradual regression throughout the healing sequence. At eight weeks, the alveolar process closely resembled its presurgery appearance with the gingival margin located at the CEJ. No obvious differences were observed between animals.

PLGA/rhBMP-2 treated defects featured minimal swelling the first week of healing. During the healing sequence, the alveolar process regressed to or beyond presurgery dimensions. Gingival margins had in some areas receded to slightly apical to the CEJ. Tissue reactions were similar for both animals.

Drilac/rhBMP-2 treated defects exhibited moderate swelling in one animal and considerable swelling in the other. Regression of initial swelling was observed in both animals from week two. The gingival margin receded to slightly below the CEJ in the animal with moderate swelling, however maintained well above the CEJ in the other animal.

Radiographic Observations

Radiographic registrations were made immediately postsurgery and at two, four, six and eight weeks postsurgery.

The DBM/rhBMP-2 implant was evident at baseline as a weak radiopaque shadow. Two, four and eight week observations revealed increasing radiopacity extending beyond the CEJ in all teeth. At eight weeks, the radiopaque structures had assumed features of dense trabecular bone continuous with the reduced alveolar crest. A periodontal ligament space and lamina dura was observed at mesial and distal aspects in most roots from four weeks. Root resorption or ankylosis was not detected with certainty at any time point. Residual carrier could not be observed from two weeks (Figure 2).

Similar to the DBM/BMP-2 implant, the DBM control could be appreciated at baseline as a weak radiopaque shadow. At two weeks, minor evidence of the DBM was detected. Increasing radiopacity was observed in the apical aspect of the defects from week four. At week eight, suggestions of a dense bony structure was observed extending coronally from the reduced alveolar crest to about one third of the defect height. A periodontal ligament space and lamina dura were usually observed. Root resorption and ankylosis were not detected (Figure 3).

Bio-Oss/rhBMP-2 treated defects exhibited characteristic tightly packed granular radiopaque particles at baseline. A distinct change in radiopacity could not be observed through week eight, however, the extension of the implant appeared to increase from baseline through eight weeks, always exceeding the height of the defects. A periodontal ligament space could not be detected with certainty, nor could root resorption or ankylosis be detected (Figure 4).

Helistat/rhBMP-2 implanted sites displayed a weak radiopaque shadow suggestive of the carrier at baseline. A gradual increase in radiopacity was observed through week eight. At eight weeks, the defects featured suggestions of dense cortical bone continuous with the resident alveolar bone. The new bone extended to or slightly below the CEJ. A periodontal ligament space was observed in some locations, however, frequently alveolar bone appeared continuous with the root. Root resorption was diagnosed at the CEJ in

some teeth at eight weeks. Residual carrier could not be observed at any time point after baseline (Figure 5).

PLGA/rhBMP-2 implants could not be detected at baseline. At two weeks some radiopacity was seen in the apical aspect of PLGA/rhBMP-2 implanted defects. The density and extension of the radiopaque structure gradually increased throughout the healing sequence assuming characteristics of dense cortical bone sometimes extending to the CEJ at eight weeks postsurgery. A periodontal ligament space was occasionally observed. Root resorption was observed at the CEJ in two teeth (Figure 6).

Drilac/rhBMP-2 implants were hardly detectable at baseline. At two weeks, limited radiopacity was observed. At four weeks, a distinct radiopaque structure was observed extending to or slightly below the CEJ. This structure exhibited increasing radiopacity throughout the healing sequence and had assumed appearance of a dense trabecular bone structure at eight weeks postsurgery. A periodontal ligament space could be observed in some areas. There was no evidence of root resorption or ankylosis (Figure 7).

Histological Observations

DBM/rhBMP-2 defects exhibited extensive bone regeneration extending coronal to the CEJ, often covering a large portion of the clinical crown (Figure 8). Numerous well-defined bone trabeculae exhibiting mature physiologic form were apparent. Trabeculae were frequently lined with cuboidal osteoblast-like cells. Narrow marrow spaces were evenly dispersed among bone trabeculae. No evidence of residual DBM could be observed. Cementum regeneration appeared as a continuous cellular layer from the base of the defect at times merging into and becoming indistinguishable from ankylosed bone in the coronal aspect of the defect. A fibrous attachment was observed to the new cementum, however, could not be clearly defined more coronally, i.e. to apparently denuded dentin. Functionally oriented fibers were occasionally observed in the periodontal ligament space.

Limited root resorption and ankylosis were observed. When present, root resorption and ankylosis were observed immediately apical to the CEJ.

DBM control defects healed in part with junctional epithelium. Bone regeneration was usually limited to the apical third of the defect (Figure 9). Regenerated bone appeared dense and distinguished minimally from the resident bone of the alveolar crest. At higher magnifications, osteoblast-like cells were seen lining the bone trabeculae. No evidence of residual DBM was noticed. Cementum regeneration was limited to the apical aspect of the defect. Newly formed cementum appeared acellular. The thin cementum layer extended coronally from the defect base. A fibrous attachment was observed to the newly formed cementum, at times extending to apparently denuded dentin. Limited root resorption and ankylosis was observed.

Bio-Oss/rhBMP-2 defects exhibited bone regeneration extending coronal to the CEJ (Figure 10). Bone trabeculae, lined with osteoblast-like cells, were observed in wide marrow spaces. Implant particles lined with bone were continuously observed within the marrow spaces. Cementum regeneration presented as a continuous layer from the base of the defects. The cementum frequently appeared relatively thick with embedded cell structures. A periodontal ligament space including a fibrous attachment to the newly formed cementum and also more coronally where cementum regeneration could not be clearly defined was observed. Limited root resorption was evident. Ankylosis, when present, was generally limited to just apical to the CEJ.

Helistat/rhBMP-2 specimens presented with bone regeneration extending to or slightly short of the CEJ. The newly formed bone exhibited features of dense cortical bone and assumed physiologic form at some sites. At other sites, thin bone lamellae approximated the root (Figure 11). No evidence of residual Helistat could be observed. Cementum regeneration appeared acellular, extending coronally from the base of the defect. A periodontal ligament space was observed in the apical part of the defect including a

fibrous attachment to the newly formed cementum and extended coronally until regenerated bone ankylosed the root. Root resorption and ankylosis, frequently present, were commonly located immediately apical to the CEJ. Some specimens exhibited root resorption extending into the coronal two-thirds of the root.

PLGA/rhBMP-2 defects exhibited partial epithelization of the gingival flap-tooth interface in some teeth. Teeth presenting with a junctional epithelium were located in the same animal. Bone regeneration extended variably along the root in a coronal direction at various tooth surfaces (Figure 12). Regenerated bone exhibited features of dense cortical bone with narrow marrow spaces. Osteoblast-like cells were noticed on bone trabeculae. Residual PLGA could not be observed. Cementum regeneration was limited to the apical portion of the defect. The cementum appeared thin and acellular. A fibrous attachment was observed to the newly formed cementum and more coronally to the apparently denuded dentin. The periodontal ligament space variably extended coronally as ankylosis, at times, was present in the coronal aspect of the root. Similarly, root resorption was noted in the coronal aspect of the root.

Drilac/rhBMP-2 specimens exhibited partial epithelization in the defects in one animal. Bone regeneration generally extended to the height of the defect (Figure 13). At times, newly formed bone exhibited features of dense cortical bone and assumed physiologic form. In other sites, small islands of bone were immersed in wide marrow spaces and connective tissue. Numerous osteoblasts were seen on the bone trabeculae. Remnants of the polylactic acid implant particles were evident within the defects. A thin, seemingly acellular cementum was usually observed in the apical aspect of the defect. A periodontal ligament space was observed in all teeth including a fibrous attachment to the new cementum and more coronally to the apparently denuded dentin. Root resorption and ankylosis were common features in the coronal third of the root.

Histometric Observations

Table 1 and 2, and Figure 14 describes the results of the histometric analysis. Means and standard deviations for each treatment in separate animals are shown (Table 1) along with the overall means and standard deviations for each treatment (Table 2, Figure 14).

Histologic defect height was similar for the various treatments, ranging form 5.1 to 5.6 mm. Generally, connective tissue repair comprised the entire defect height except for defects receiving the DBM control, which, in part, healed with a junctional epithelium. Similarly, the PLGA/ rhBMP-2 and Drilac/rhBMP-2 treated defects exhibited some epithelialization. Bio-Oss/rhBMP-2 sites exhibited numerically increased cementum regeneration compared to the other candidate carriers and the DBM control. Bone regeneration ranged from 2.1 mm for the DBM control to the full extent of the defect height for DBM/rhBMP-2 and Bio-Oss/rhBMP-2 implants (5.6 and 5.1 mm, respectively). Compared to the other treatments, DBM/rhBMP-2 and Bio-Oss/rhBMP-2 implanted defects displayed a wider alveolar process (expressed histometrically as a larger area of regenerated bone. Bone quality, herein assessed as bone density, ranged from 23% for Bio-Oss/rhBMP-2 implants to 46% for DBM/rhBMP-2 implants. When comparing bone density values, it must be noted that Bio-Oss/rhBMP-2 and Drilac/rhBMP-2 implanted defects were occupied, in part, by residual carrier. Bio-Oss particles were embedded in bone, however, they were not included in bone density calculations. Similarly, residual Drilac carrier was not included in the bone density assessment. Root resorption and ankylosis values were within limits previously observed in this model.

DISCUSSION

This study evaluated candidate carriers for rhBMP-2 in periodontal reconstructive surgery. All carriers combined with rhBMP-2 exhibited a periodontal regenerative potential. There was generally limited clinical, radiographic, histological, and histometric variance between teeth and consequently animals within the same treatment group.

Candidate carriers most notable for enhancing regeneration of cementum and bone were DBM and Bio-Oss. As observed in our previous study, the periodontium regenerated to a considerable extent in critical size defects treated with rhBMP-2 (Sigurdsson et al. 1994). In contrast to our previous study, the premolar teeth were left in a transgingival position at wound closure. Even so, limited epithelization of the tooth-gingival flap interface was observed in defects receiving rhBMP-2, with the outcome obviously being carrier dependent.

Clinical and histological differences were observed between carriers. Test quadrants displayed initial swelling due to an inflammatory response commonly observed following periodontal surgery including rhBMP-2 (Sigurdsson et al. 1994). Increased tissue volume slowly regressed to, or below, presurgery dimensions, except for in defects receiving DBM/rhBMP-2 or Bio-Oss/rhBMP-2. These defects exhibited increased volume compared to presurgery dimensions throughout the observation interval. Histological differences included variability in quantity and quality of regenerated bone and cementum. Bio-Oss and Drilac carriers were still present at eight weeks postsurgery. Bio-Oss particles were usually embedded in bone. This is in concert with previous observations of bovine derived bone matrix (Fukuta et al. 1992, Boyne 1989). and may, in part, account for low bone density values in defects receiving Bio-Oss. Similarly, polylactic acid matrices such as Drilac may erode over considerable time and thus prevent rapid and/or extensive bone formation (Wikesjö & Nilvéus 1990). Bone density in PLGA/rhBMP-2 defects in the present study appeared higher than in our previous study using the same bioerodable microparticles (Sigurdsson et al. 1994). However, in our previous study the carrier additionally included autologous blood, in contrast to glycerol in the present study. Further evaluation is necessary to explain this apparent difference between studies.

The defects exhibited varying amounts of root resorption. Helistat/rhBMP-2 and PLGA/rhBMP-2 treated defects apparently exhibited increased root resorption compared to

the other carriers. Resorption in these carriers was not always limited to immediately apical to the CEJ. There also appeared to be a difference in amount of root resorption compared to our previous study (Sigurdsson et al. 1994). Frequency and amount of root resorption in PLGA/rhBMP-2 defects appeared more extensive in the present study. This may possibly be explained by differences in mechanical integrity between the PLGA/blood versus the PLGA/glycerol carrier. The blood containing carrier was more robust to wetting from wound moisture, maintaining physical contact with the tooth surfaces which was not always the case for the glycerol containing version. Thus, the blood containing carrier may more effectively present rhBMP-2 to the wound which may influence the cementum regeneration/root resorption complex. Although root resorption is common to periodontal wound healing, it seems that initial resorption may be a necessary event in the process of tissue remodeling following periodontal reconstructive therapy (Wikesjö et al. 1991, Wikesjö et al. 1994, Wikesjö et al. 1992, Pindborg 1970, Andreasen 1981, Shafer 1983). In many specimens root resorption was noted coronal to the new cementum formation. This supports the suggestion that cementum matrix apposition may prevent root resorption (Schroeder 1992).

Ankylosis is believed to be associated with physiologic resorption of primary teeth and it may result in infraocclusion, reimpaction, and incomplete development of the alveolar process (Andreasen 1981, Shafer 1983). In the permanent dentition, ankylosis rarely causes clinical symptoms and requires no treatment unless there is an underlying problem (Shafer 1983). We have suggested ankylosis following periodontal reconstructive surgery including rhBMP-2 being model dependent and unrelated to the protein in the canine supraalveolar defect model (Sigurdsson et al. 1994). This is supported by observations of the location ankylosis just apical to the CEJ and distribution between experimental and control conditions (Sigurdsson et al. 1994, Sigurdsson et al. 1995). In the present study, few DBM control defects exhibited extensive bone regeneration, and

consequently only few teeth exhibited ankylosis. In contrast, defects treated with DBM/rhBMP-2, Bio-Oss/rhBMP-2, and Helistat/rhBMP-2, all exhibiting extensive bone regeneration, displayed frequent ankylosis. Ankylosis was observed in the coronal one-third and occasionally in the coronal two-thirds of the defects.

Two pathways of periodontal regeneration have been hypothesized: 1) growth and migration of differentiated cells into the wound from immediate existing tissue resources such as resident alveolar bone and periodontal ligament, (Ripamonti & Reddi 1994) and 2) regeneration through growth and differentiation of pluripotent progenitor cells (Sigurdsson et al. 1994, Wozney 1995). rhBMP-2 has been demonstrated to induce endochondral ossification through differentiation of mesenchymal cells into cartilage and bone cells (Wang et al. 1990). This may in part explain periodontal reconstruction following implantation of rhBMP-2. Interaction of BMPs with extracellular matrix macromolecules has formed the basis for a concept of a regulatory role of BMPs in osteogenesis (Reddi 1992). It seems that optimal bone formation is dependent on the combined action of BMPs and a complementary substratum (Ripamonti & Reddi 1994). It is this combination of extracellular matrix substratum in association with soluble BMPs which triggers a bone differentiation cascade (Reddi 1992). These morphogenic actions of BMPs may also trigger the cascade of events necessary for periodontal reconstitution. Thus, it may not only be the placement of rhBMP-2 in a periodontal defect but also choice of carrier which may influence the regenerative result.

A collagenous bone matrix provides optimal substratum for recruitment, anchorage of progenitor cells, and subsequent proliferation and differentiation into osteoblasts (Rath & Reddi 1979, Muthukumaran et al. 1988). Moreover, a collagenous matrix may prevent premature diffusion and protect BMPs from nonspecific proteolysis (Sampath 1981). The fact that an insoluble collagen component is resorbed and replaced by bone appears advantageous for its application in periodontal reconstructive therapy (Wikesjö et al. 1992).

Demineralized freeze-dried allogeneic bone is suggested to have therapeutic potential in regeneration of bone and connective tissue in humans (Mellonig 1992). DBM may also prevent premature diffusion of rhBMP-2 by space maintenance and increased wound stability (Wikesjö & Nilvéus 1990). Further, DBM is thought to be resorbed and replaced by resident bone and histological evidence of new attachment and bone regeneration has been demonstrated at root surfaces previously exposed to periodontal disease (Bowers et al. 1989). Thus, DBM appears to have several properties which make it advantageous as a carrier for osteoinductive proteins in periodontal reconstructive therapy.

Weak mechanical performance, immunogenic response, and potential transmission of viral antigens are factors to be considered with the use of proteinaceous carrier systems (Ripamonti & Reddi 1994). Thus, formulation and use of inorganic carriers may have significant therapeutic benefit over organic implants. Bio-Oss, a porous inorganic implant material, has been shown to support bone and connective tissue growth (Fukuta et al. 1992). One benefit of a porous carrier may be a spatially controlled osteogenesis restricting bone differentiation to the local site. All these properties appear valuable for a carrier system for rhBMP-2.

Of the candidate carriers evaluated, Helistat exhibited outstanding clinical handling. The collagen matrix, however had weak mechanical performance for space maintenance under the gingival flaps. In the supraalveolar defects this property may be of greater importance than in intraalveolar defects where bone walls would help support the flaps. Moreover, BMPs are closely associated with the collagenous part of bone tissue and may even be difficult to separate (Urist et al. 1968). Thus, BMPs likely have chemical affinity for the microfibrillar collagen and the Helistat vehicle may act in a controlled release fashion for rhBMP-2. This may be beneficial for this type of carrier system. The supraalveolar defect model may, however, not be ideal the carriers with weak biomechanical properties such as Helistat and PLGA with glycerol.

Our studies support a potential for alveolar bone and cementum regeneration in the presence of wound stability and space provision (Wikesjö et al. 1991, Wikesjö et al. 1994, Wikesjö et al. 1992). The results of this study suggest a significant potential for periodontal regeneration following surgical implantation of rhBMP-2 with various candidate carriers. It is suggested that an ideal carrier system for periodontal reconstructive therapy should be inorganic, non-immunogenic, resorbable, and adaptable (Ripamonti & Reddi 1994). Importantly, it should provide space, provide support of the mucoperiosteal flap, and allow rapid mesenchymal cell differentiation and vascular invasion when combined with BMPs. This study indicates, that out of the five candidate carriers DBM and Bio-Oss were most suitable to enhance the periodontal regenerative potential of rhBMP-2. Further studies are needed, however, to refine these rhBMP-2 carriers for periodontal reconstruction.

Figure 1. Preparation of supraalveolar periodontal defect model (A); application of rhBMP-2 in carrier (B); following suturing of gingival flaps (C).

Figure 2. Representative radiographs from 2 and 8 weeks postsurgery for DBM/rhBMP-2.

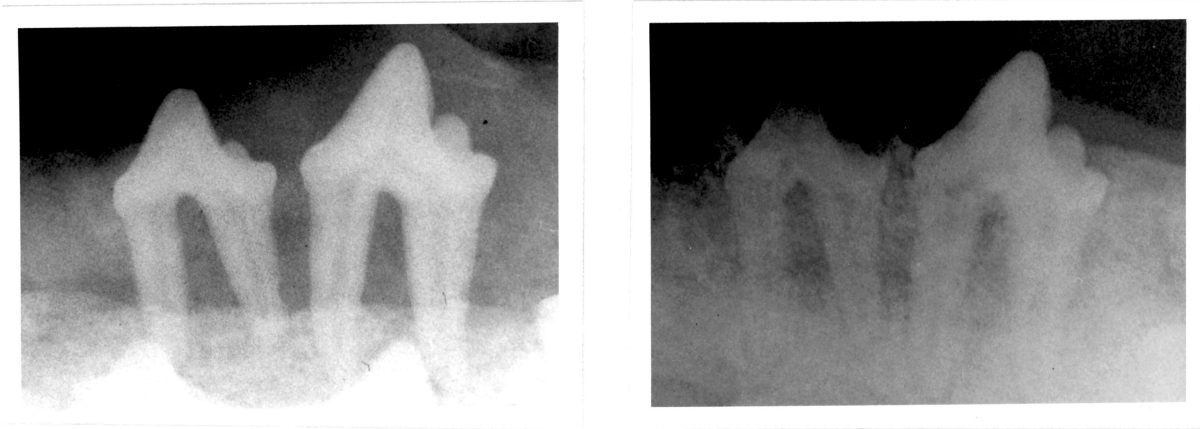

Figure 3. Representative radiographs from 2 and 8 weeks postsurgery for DBM Control.

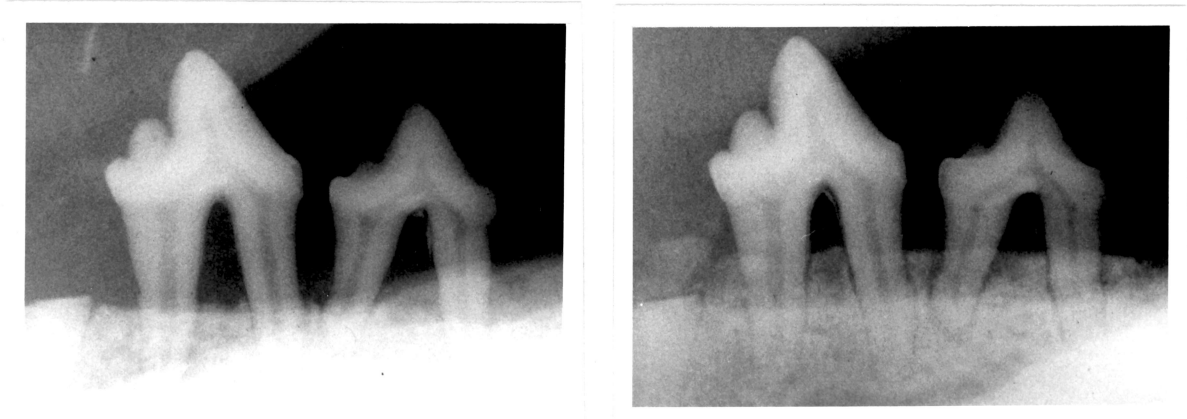

Figure 4. Representative radiographs from 2 and 8 weeks postsurgery for Bio-Oss/rhBMP-2.

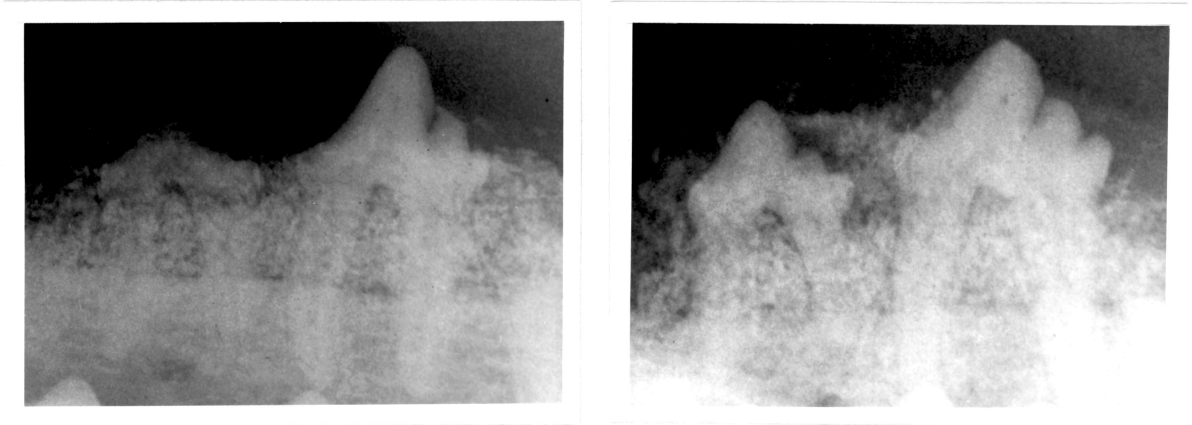

Figure 5. Representative radiographs from 2 and 8 weeks postsurgery for Helistat/rhBMP-2.

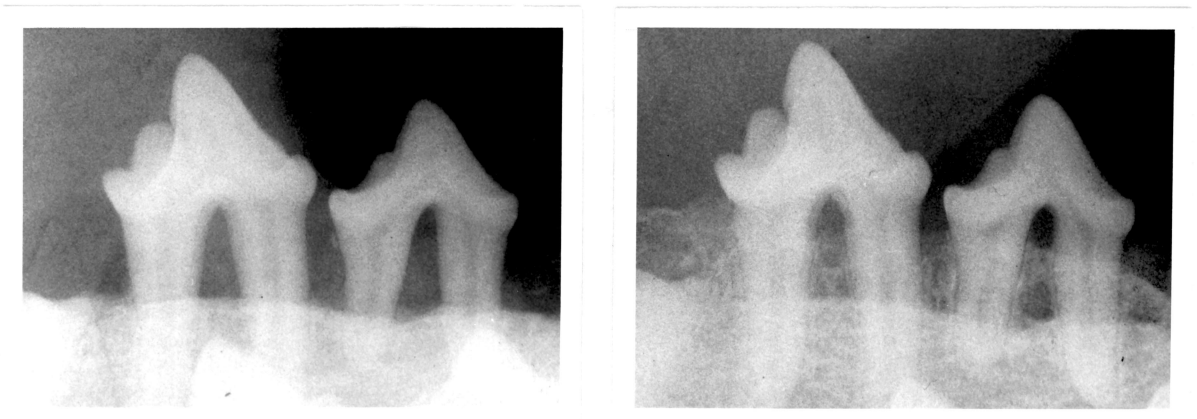

Figure 6. Representative radiographs from 2 and 8 weeks postsurgery for PLGA/rhBMP-2.

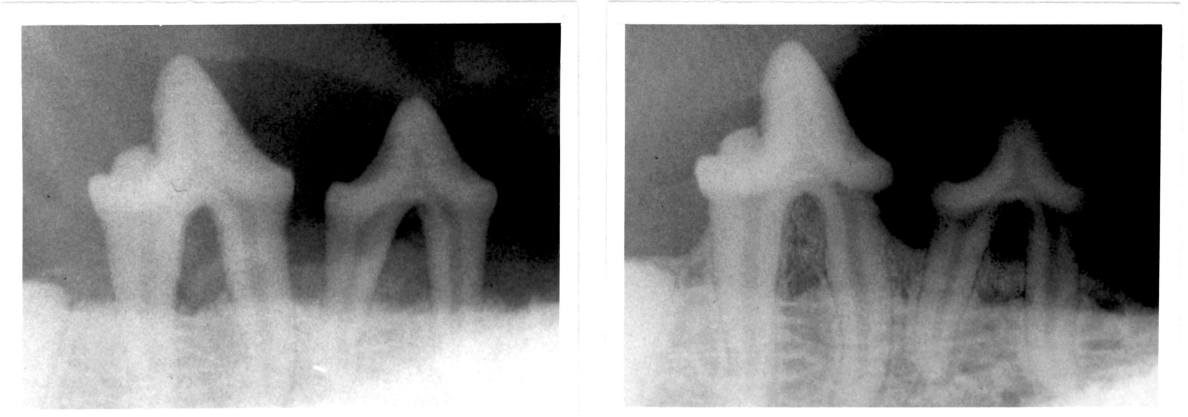

Figure 7. Representative radiographs from 2 and 8 weeks postsurgery for Drilac/rhBMP-2.

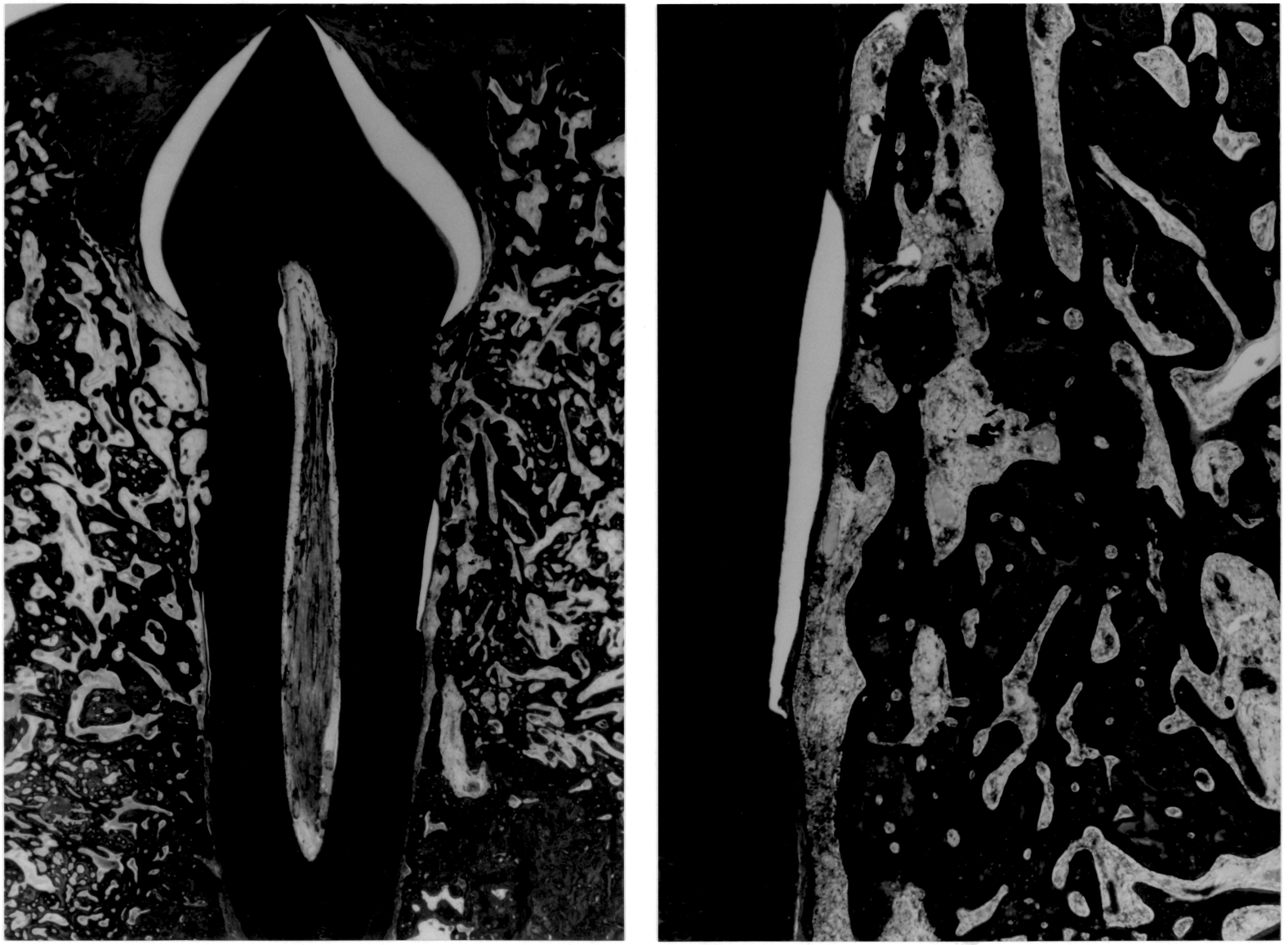

Figure 8. Photomicrograph of defect treated with DBM/rhBMP-2.

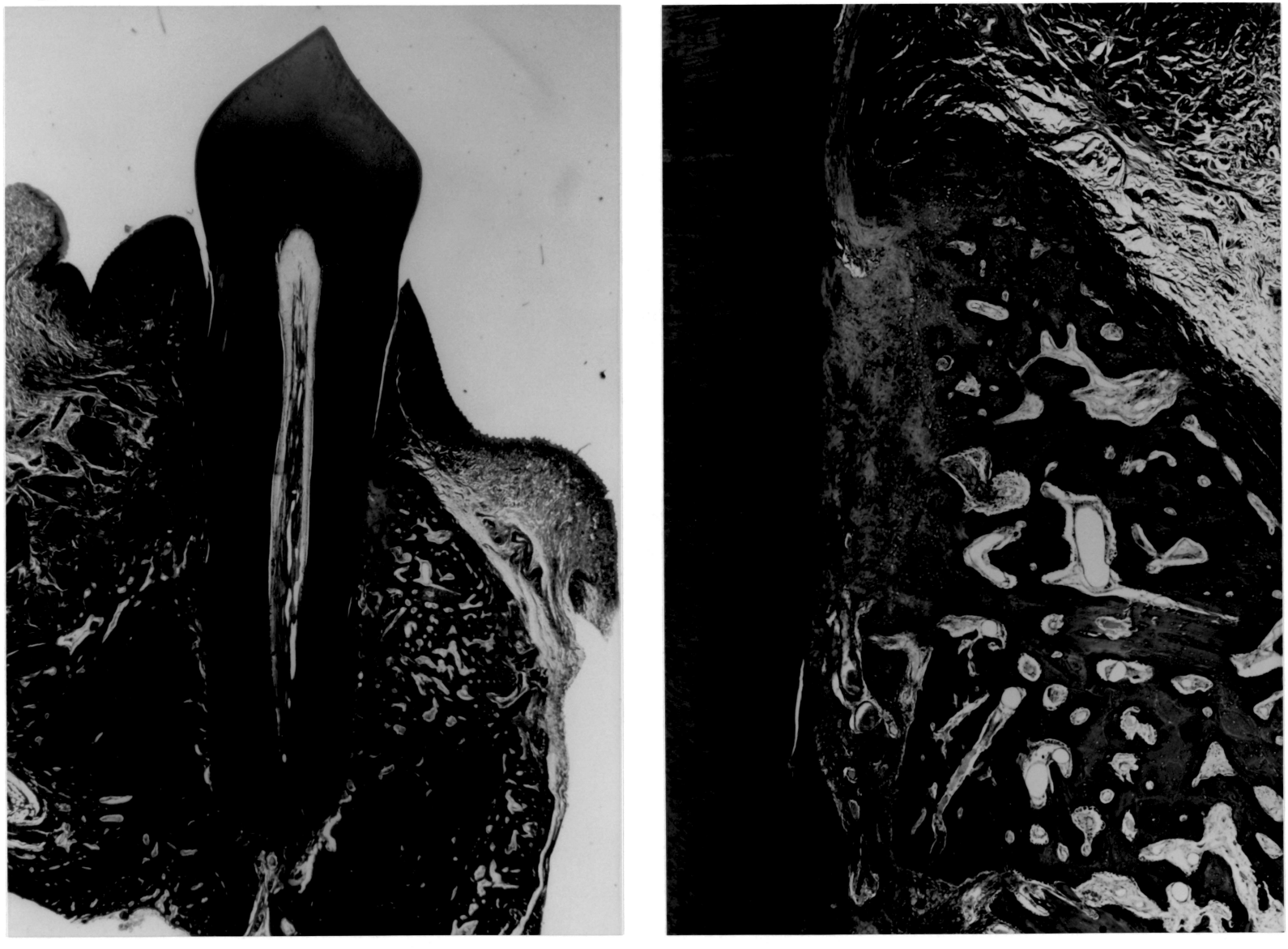

Figure 9. Photomicrograph of defect treated with DBM control.

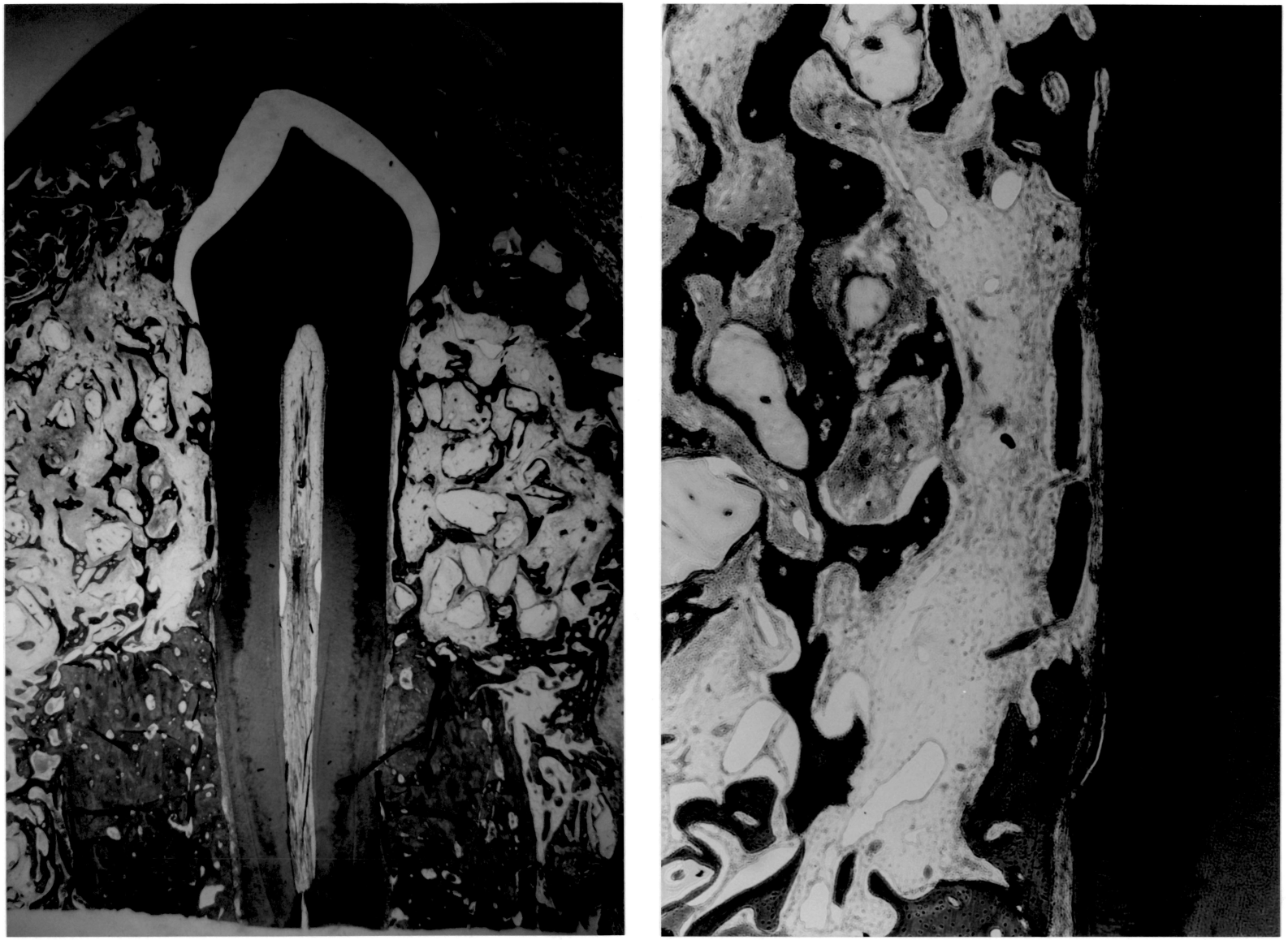

Figure 10. Photomicrograph of defect treated with Bio-Oss/rhBMP-2.

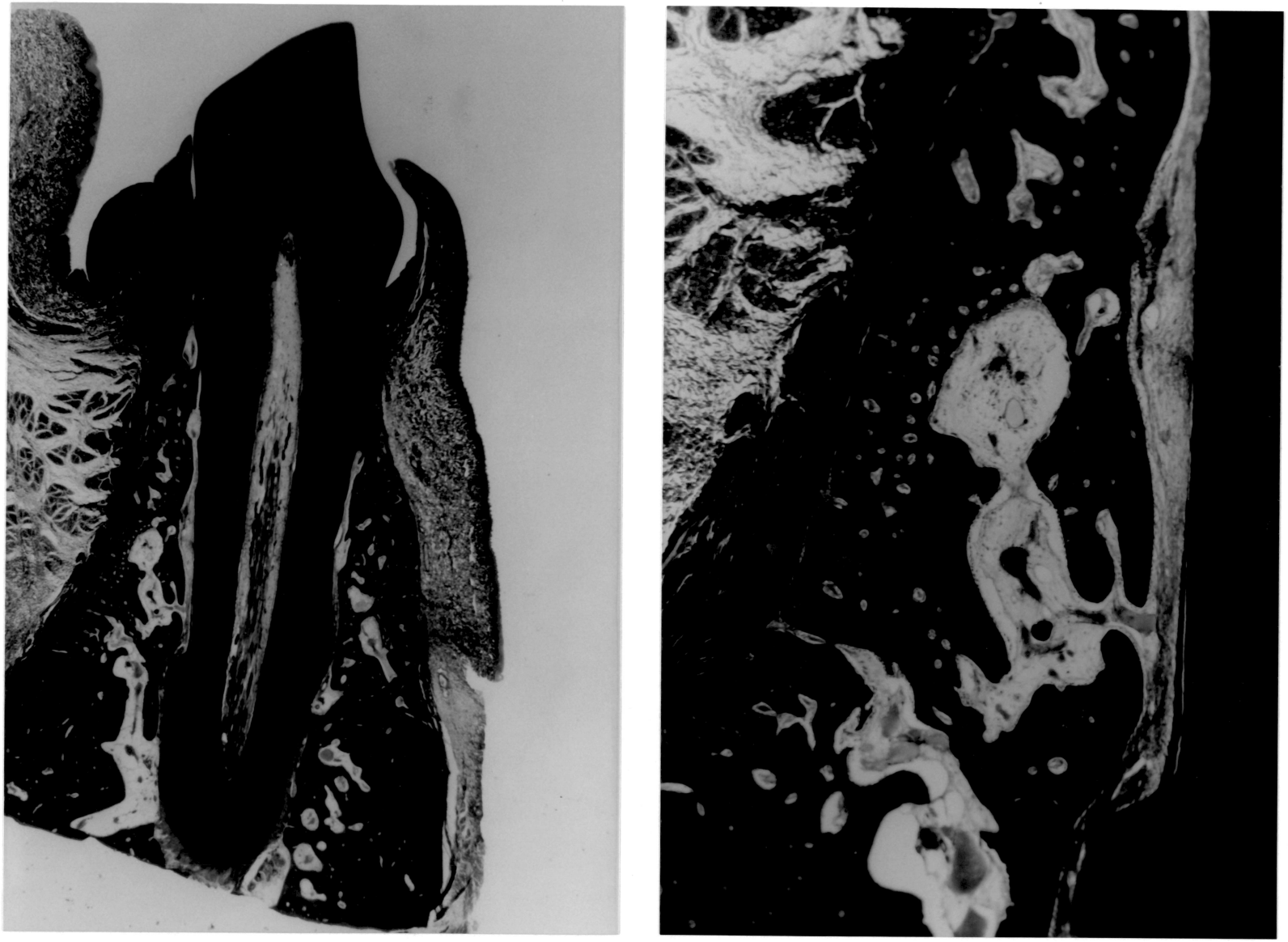

Figure 11. Photomicrograph of defect treated with Helistat/rhBMP-2.

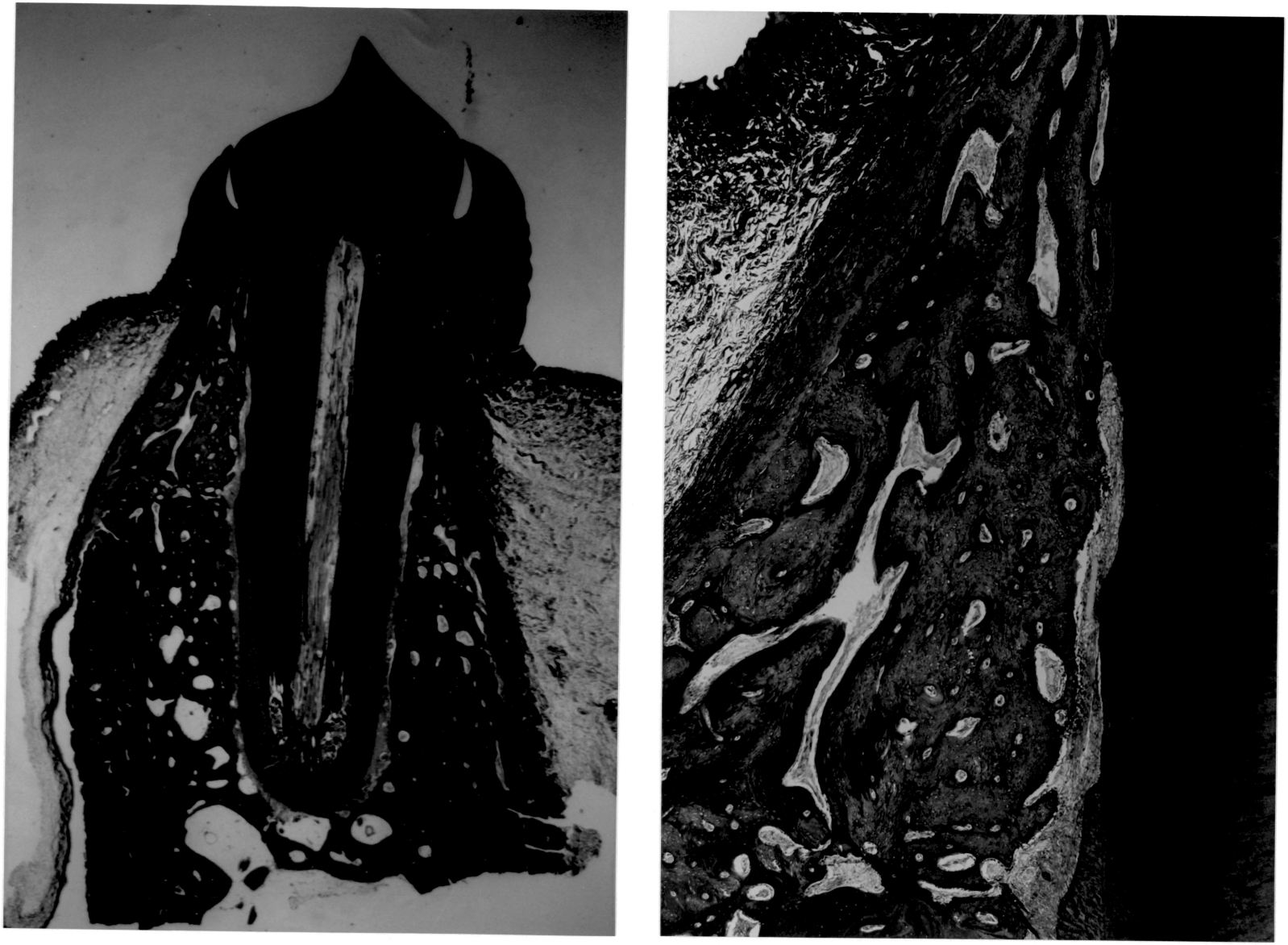

Figure 12. Photomicrograph of defect treated with PLGA/rhBMP-2.

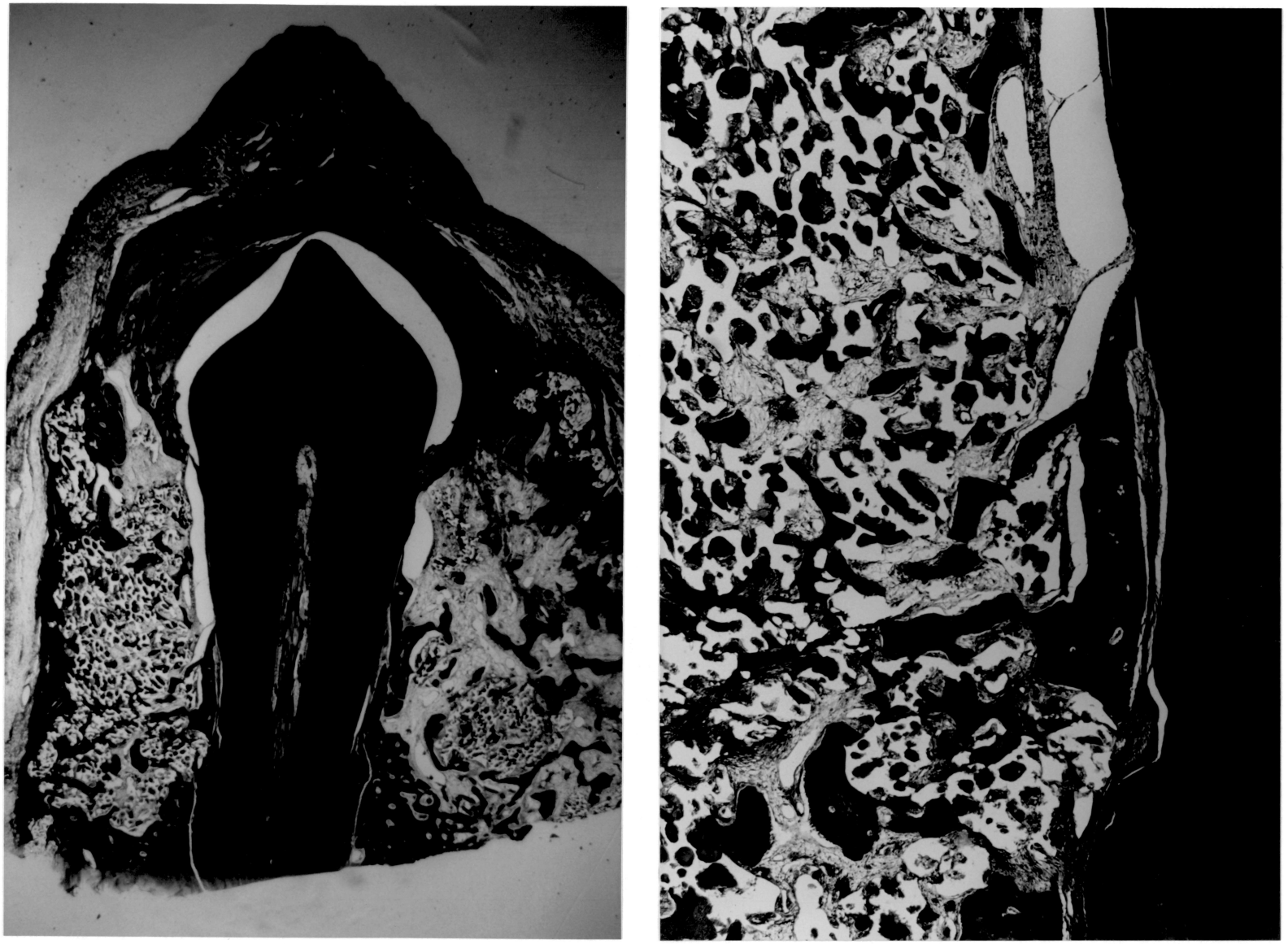

Figure 13. Photomicrograph of defect treated with Drilac/rhBMP-2.

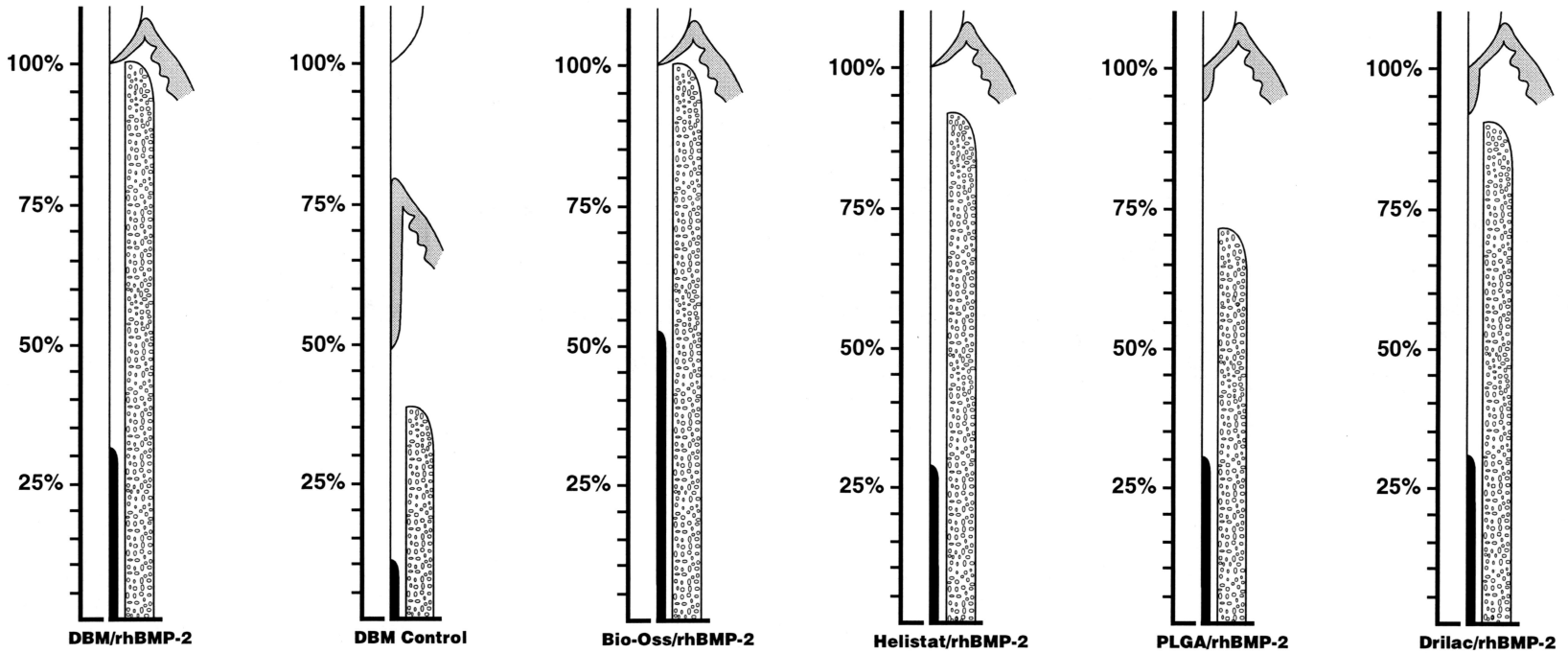

Figure 14. Histometric results expressed in percent (%) of the defect height.

Table 1. Mean defect height (DH), connective tissue repair (CTR), cementum height (CH), bone height (BH), bone area (BA), bone density (BD), root resorption (RR), and ankylosis (ANK) (± s.d. in mm; BA in mm^2; BD in %) for treatments with DBM/rhBMP-2, DBM control, Bio-Oss/rhBMP-2, Helistat/rhBMP-2, PLGA/rhBMP-2, and Drilac/rhBMP-2 in each of two dogs.

DBM/rhBMP-2

	DH	CTR	CH	BH	BA	BD (%)	RR	ANK
dog 1	5.5 ± 0.7	5.5 ± 0.7	2.1 ± 1.1	5.5 ± 7.0	27 ± 5.3	40 ± 8	1.1 ± 0.7	2.6 ± 0.7
dog 2	5.7 ± 0.5	5.7 ± 0.5	1.4 ± 0.8	5.7 ± 0.5	18 ± 2.7	50 ± 10	1.4 ± 0.7	0.7 ± 0.6

DBM control

	DH	CTR	CH	BH	BA	BD (%)	RR	ANK
dog 1	5.5 ± 0.7	3.0 ± 0.6	0.6 ± 0.7	2.0 ± 0.7	4.4 ± 2.1	40 ± 10	0.7 ± 0.5	0.4 ± 0.2
dog 2	5.5 ± 0.7	2.3 ± 0.4	0.5 ± 0.3	2.1 ± 0.9	4.4 ± 2.9	40 ± 9	0.5 ± 0.5	0.0 ± 0.0

Bio-Oss/rhBMP-2

	DH	CTR	CH	BH	BA	BD (%)	RR	ANK
dog 3	5.2 ± 0.8	5.2 ± 0.8	2.7 ± 0.8	5.2 ± 0.9	15 ± 4.1	30 ± 8	0.9 ± 0.7	2.0 ± 1.5
dog 4	5.0 ± 0.6	5.0 ± 0.6	2.7 ± 0.7	5.0 ± 0.6	19 ± 3.4	20 ± 10	1.1 ± 0.8	1.6 ± 0.7

Helistat /rhBMP-2

	DH	CTR	CH	BH	BA	BD (%)	RR	ANK
dog 5	5.2 ± 0.8	5.1 ± 0.8	1.5 ± 0.7	5.0 ± 0.7	3.1 ± 2.0	40 ± 10	1.7 ± 1.2	2.7 ± 1.1
dog 6	5.6 ± 0.9	5.6 ± 0.9	1.4 ± 0.8	5.0 ± 1.0	5.0 ± 2.2	50 ± 10	1.6 ± 0.7	2.0 ± 1.3

PLGA/rhBMP-2

	DH	CTR	CH	BH	BA	BD (%)	RR	ANK
dog 4	5.3 ± 0.5	5.3 ± 0.5	1.2 ± 0.6	4.3 ± 9.8	3.0 ± 1.3	50± 10	2.3 ± 1.1	2.0 ± 1.1
dog 6	5.1 ± 0.8	4.5 ± 1.0	2.0 ± 1.0	3.0 ± 1.1	3.6 ± 2.1	40 ± 10	1.6 ± 1.4	0.4 ± 0.5

Drilac/rhBMP-2

	DH	CTR	CH	BH	BA	BD (%)	RR	ANK
dog 3	5.1 ± 0.9	5.1 ± 0.9	1.7 ± 1.1	5.1 ± 0.9	12.0 ±	20 ± 7	1.3 ± 0.8	1.5 ± 1.1
dog 5	5.0 ± 0.5	4.3 ± 1.2	1.1 ± 0.7	3.6 ± 1.8	5.8 ± 5.1	40 ± 10	1.1 ± 1.0	1.4 ± 0.3

Table 2. Mean defect height (DH), connective tissue repair (CTR), cementum height (CH), bone height (BH), bone area (BA), bone density (BD), root resorption (RR), and ankylosis (ANK) (± s.d. in mm; BA in mm^2; BD in %) for treatments with DBM/rhBMP-2, DBM control, Bio-Oss/rhBMP-2, Helistat/rhBMP-2, PLGA/rhBMP-2, and Drilac/rhBMP-2 based on means from two dogs.

	DH	CTR	CH	BH	BA	BD (%)	RR	ANK
DBM/rhBMP-2	5.6±0.1	5.6±0.4	1.8±0.6	5.6±0.1	22.5±6.0	46±9	1.3±0.3	1.7±1.3
DBM/control	5.5±0.0	2.7±0.4	0.6±0.1	2.1±0.0	4.4±0.0	41±3	0.6±0.2	0.02±0.0
Bio-Oss/rhBMP	5.1±0.2	5.1±0.2	2.7±0.0	5.1±0.2	17.0±3.4	23±5	1.0±0.5	1.8±1.0
Helistat/rhBMP-2	5.4±0.1	5.4±0.3	1.5±0.0	5.0±0.0	4.1±1.2	45±5	1.7±0.1	2.4±0.5
PLGA/rhBMP-2	5.2±0.1	4.9±0.5	1.6±0.7	3.7±0.9	3.3±0.4	45±4	2.0±0.5	1.2±1.1
Drilac/rhBMP-2	5.1±0.1	4.7±0.6	1.6±0.5	4.6±1.1	9.0±4.7	30±13	1.2±0.2	1.5±0.1

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