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Periodontal repair in dogs: evaluation of a bioresorbable calcium phosphate cement (Ceredex™) as a carrier for rhBMP-2

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Abstract

Background: Recombinant human bone morphogenetic protein-2 (rhBMP-2) has been shown to induce clinically relevant bone formation for orthopedic, craniofacial, and oral indications. It appears critical, in particular for onlay indications, that the associated carrier technology exhibits structural integrity to offset compressive forces in support of rhBMP-2-induced bone formation. The objective of this study was to evaluate a calcium phosphate (CP) cement, Ceredex™, as a candidate carrier for rhBMP-2 in a defect model with limited osteogenic potential.

Materials: Bilateral, critical size, 6-mm, supra-alveolar, periodontal defects were created in six, adult, male, Hound Labrador mongrels. Three animals received rhBMP-2/Ceredex™ (rhBMP-2 at 0.20 and 0.40 mg/ml) in contralateral defect sites (implant volume/defect $\sim$ 1 ml). One defect site in each of the three remaining animals received Ceredex™ without rhBMP-2 (control). The animals were euthanized at 12 weeks postsurgery for histologic and histometric analysis.

Results: Mean induced bone height exceeded 80% of the defect height for supra-alveolar periodontal defects receiving rhBMP-2/Ceredex™ without major differences between rhBMP-2 concentrations compared with approximately 40% for the control. The newly formed bone, a mixture of lamellar and woven bone in fibrovascular tissue, circumscribed relatively large portions of the residual Ceredex™ biomaterial. Inflammatory lesions were associated with limited bone formation in some sites. From a periodontal perspective, sites receiving rhBMP-2/Ceredex™ exhibited increased cementum formation compared with control, but without a functionally oriented periodontal ligament, and increased ankylosis and root resorption. Control sites exhibited early wound failure and exposure, loss of the Ceredex™ biomaterial, and limited bone formation.

Conclusions: The Ceredex™ CP cement appears a potentially promising carrier technology for rhBMP-2 onlay indications. However, a slow resorption rate may prevent its wider use. This study does not support use of the rhBMP-2/Ceredex™ combination for periodontal indications.

Key words: bone induction; calcium phosphate; periodontal regeneration; tissue engineering

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Recombinant human bone morphogenetic protein-2 (rhBMP-2) has been shown to induce clinically relevant bone formation for a variety of orthopedic, craniofacial and oral indications (Valentin-Opran et al. 2002). It appears critical that the associated carrier technology exhibits innate structural integrity to offset compressive forces in

support of rhBMP-2-induced bone formation or that the rhBMP-2 implant is shielded by a space-providing device (Wikesjö et al. 2002, 2003a, b). In particular, for onlay indications, mechanical forces may compress the rhBMP-2 construct thus limiting bone formation. Technology limitations have also been associated with space providing candidate carriers such as biphasic calcium phosphate (CP), DL-polylactic acid (PLA), demineralized bone matrix (DBM), and hydroxyapatite (Wikesjö et al. 2001). These limitations include slow resorption rates of the carrier biomaterial impairing or preventing bone formation, carrier-specific biodegradation processes interfering with bone formation and bone maintenance, inadequate rhBMP-2 release profiles for bone induction, and unacceptable clinical swelling.

CP cements have been reported to be osteoconductive and biodegradable, possessing a crystalline structure similar to that of bone (for a review see Ishikawa et al. 1997, Schmitz et al. 1999). The ability of CP cements to influence bone formation and bone ingrowth has been shown in supra-orbital ridge defects, skull base defects, craniofacial defects, canine femoral slot defects, for mandibular augmentation, and in long-bone fractures (for a review see Schmitz et al. 1999). CP cements approved by the FDA for craniofacial indications include Bone Source® (Howmedica Leibinger Inc., Dallas, TX, USA), α-BSM® (ETEX Corporation, Cambridge, MA, USA), and Norian SRS/CRS (Norian Corporation, Cupertino, CA, USA). Each of the CP cements react to form hydroxyapatite. Study-specific complications have arisen based on the particular chemical composition of the CP biomaterials. For example, Bone Source® has a long setting time (up to 4 h) and a slow resorption time (up to 2 years).

Ceredex™ (ETEX Corporation) and α-BSM® are synthetic, space-providing CP putties that endothermically set into cement form within 15 min of application at body temperature (Knaack et al. 1998, Lee et al. 1999, Schmitz et al. 1999). α-BSM® has been evaluated as an injectable carrier for rhBMP-2 in long bone fractures including non-human primate fibula osteotomy defects (Seeherman et al. 2001, 2002), non-human primate core defects, the rabbit ulnar osteotomy model (Li et al. 2003), and onlay indications in the critical size supra-alveloar peri-implant defect model (Wikesjö et al. 2002). Radiographic and histologic evidence suggest that the material sets into cement, provides and maintains space, typically resorbs within 12 weeks (with the proper liquid/solid ratio), and has an acceptable rhBMP-2 release profile. The objective of this study was to evaluate Ceredex™ CP cement as a candidate carrier for rhBMP-2 in a defect model with limited osteogenic potential.

Materials and Methods

Animals

Six, male, 18-month-old Hound Labrador mongrels, approximate weight 25 kg, were used. Animal selection and management, and surgical protocol followed routines approved by the Institutional Animal Care and Use Committees at Wyeth Research (Cambridge, MA, USA) and Temple University (Philadelphia, PA, USA). The animals had access to a standard laboratory diet and water until the beginning of the experimental segment of the study. Oral prophylaxis was performed within 1 week before surgical procedures.

rhBMP-2 reconstitution and dilutions

Using aseptic routines, lyophilized rhBMP-2 (Wyeth Research) was reconstituted with sterile water for injection (Abbott Laboratories, Abbott Park, IL, USA) to produce a 4.45 mg/ml stock solution. The 4.45 mg/ml solution was diluted with MFR 00842 buffer (5 mM L-glutamic acid, 2.5% glycine, 0.5% sucrose, 0.01% polysorbate 80, pH 4.5; Wyeth Research) to create rhBMP-2 dilutions at 0.20 and 0.40 mg/ml.

rhBMP-2/Ceredex™ preparation

To produce the rhBMP-2/Ceredex™ construct, 1.1 ml of the rhBMP-2 solution was added per 1.0 g Ceredex™. The 0.20 or 0.40 mg/ml rhBMP-2 solution was injected into a pouch containing the Ceredex™ powder. The pouch was palpated until the content was thoroughly mixed. The rhBMP-2/Ceredex™ construct was withdrawn into a 3-cc syringe to measure the implant volume and placed into the defect within 15 min (Ceredex™ hardens to a cement-like consistency within 15 min at 37°C). The total implanted volume of each rhBMP-2/Ceredex™ construct ranged from 1.2 to 1.4 ml. For the control sites, 0.8 ml MFR00842 buffer was added per 1.0 g Ceredex™ yielding a total implant volume of 1.5–2.0 ml.

Surgical procedure

Food was withheld the night preceding surgery. Animals were preanesthetized with buprenorphine HCl (0.01–0.03 mg/kg)/acepromazine (0.1 mg/kg)/atropine (0.02–0.04 mg/kg) SQ, sedated with methohexital (4–8 mg/kg to effect), and maintained on gas anesthesia (1–2% isoflurane/O_2 to effect). To maintain hydration, a sterile IV catheter was placed and animals received a constant rate infusion of lactated Ringer's solution (10–20 ml/kg/h IV) while anesthetized. Prophylactic antibiotics (cefazolin; 22 mg/kg SQ) were administered within 1 h of surgery and redosed postsurgery.

Bilateral, critical size, 6-mm, supra-alveolar, periodontal defects were created around the mandibular third and fourth mandibular premolar teeth (Wikesjö et al. 1994, Wikesjö & Selvig 1999). Briefly following sulcular incisions reflecting buccal and lingual mucoperiosteal flaps, alveolar bone was removed around the circumference of the premolar and first molar teeth using chisels and water-cooled rotating burs. The first and second premolars were extracted and the first molars were amputated at the level of the reduced alveolar crest. The root surfaces of the third and fourth premolar teeth were instrumented with curettes, chisels, and water-cooled rotating diamonds to remove the cementum. The crowns of the teeth were reduced to approximately 2 mm coronal to the cemento-enamel junction (CEJ) and the cut surfaces smoothed. Exposed pulpal tissues were sealed (Cavit®, ESPE, Seefeld/Oberbayern, Germany) (Fig. 1). To alleviate potential trauma to the experimental mandibular sites postsurgery, the first, second and third maxillary premolar teeth were surgically extracted and the maxillary fourth premolars reduced in height and exposed pulpal tissues sealed (Cavit®).

Supra-alveolar periodontal defect sites in three animals received rhBMP-2/Ceredex™ (rhBMP-2 at 0.20 and 0.40 mg/ml) in contralateral jaw quadrants (Fig. 1). Periostea were then fenestrated at the base of the flaps to allow tension-free flap apposition, the flaps were advanced and the flap margins adapted 3–4 mm coronal to

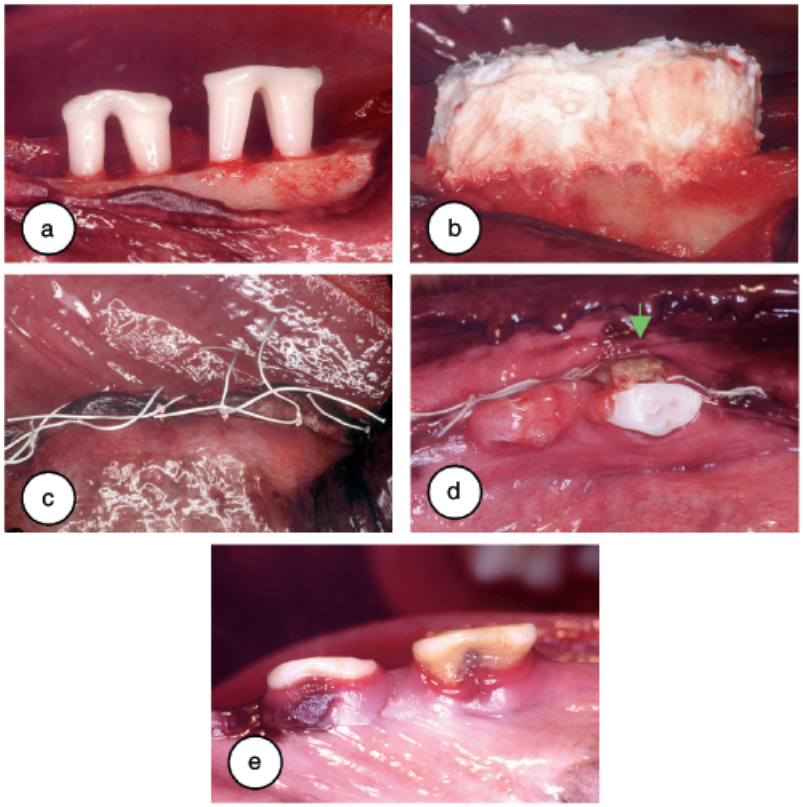

Fig. 1. Supra-alveolar periodontal defect implanted with rhBMP-2/Ceredex™ (a, b, c). Healing at Ceredex™ control sites at suture removal (d) and week 16 postsurgery (e). Note the exposed Ceredex™ biomaterial at suture removal (arrow).

the teeth and sutured (GORE-TEX™ Suture CV5, W.L. Gore & Associates Inc., Flagstaff, AZ, USA). The defect sites were hot-packed with sterile, saline-soaked gauze for approximately 20 min to accelerate hardening of the Ceredex™ biomaterial. Supra-alveolar defect sites in the three remaining animals received Ceredex™ without rhBMP-2 (control) as above in the left or right jaw quadrant only.

Postsurgery care

The animals were fed a canned soft dog food diet postsurgery. Buprenorphine HCl (0.015 mg/kg IM bid for 48 h) was administered for pain control. A broad-spectrum antibiotic (enrofloxacin, 2.5 mg/kg, IM, twice daily for 14 days) was used for infection control. Plaque control was maintained by daily topical application of chlorhexidine (Chlorhexidine Gluconate 20%, Xttrium Laboratories Inc., Chicago, IL, USA; 40 ml of a 2% solution) until gingival suture removal, thereafter once daily, Monday–Friday, until the completion of study. Sutures were removed under sedation at approximately 8 days postsurgery.

Clinical recordings

Observations of experimental sites with regard to gingival/mucosal health, maintenance of suture line closure, edema, and evidence of tissue necrosis or infection were made daily until suture removal, and at least twice weekly thereafter. Radiographs were obtained immediately postsurgery, and at 4, 8, 12, and 16 weeks postsurgery.

Histological processing

At 12 and 16 (controls) weeks postsurgery, animals were preanesthetized with buprenorphine HCl (0.01–0.03 mg/kg)/acepromazine (0.1 mg/kg)/atropine (0.02–0.04 mg/kg), anesthetized with pentobarbital (30 mg/kg IV bolus), and euthanized with Euthanasia-5 solution (1 ml/10 kg IV; Sparhawk-Veterinary Laboratories, Lenexa, KS, USA). Following euthanasia, block sections including teeth, bone and soft tissues were collected and radiographed to estimate bone regeneration. The specimens were rinsed in sterile saline, sectioned, and fixed in 10% neutral buffered formalin for 8–10 weeks.

The distal roots of the third and fourth premolar teeth were processed for undecalcified histology (Schenk et al. 1984). The mesial roots were decalcified with EDTA (Luna 1992) and subsequently processed for undecalcified histology (Schenk et al. 1984). Specifically, all specimens were washed after 10% neutral buffered formalin and/or EDTA, dehydrated with gradients of alcohol, and cleared in xylene using an automatic tissue processor (Tissue-Tek; Sakura, Torrance, CA, USA). Specimens were infiltrated and embedded in methyl-methacrylate, and allowed to polymerize for 3–5 days, at room temperature. Using a Reichert Jung Polycut (Leica, Deerfield, IL, USA), 5-µm sections were taken 100 µm apart through the root canal area and stained with a modified Goldner's trichrome stain.

Analysis

One experienced masked examiner evaluated the radiographs from immediately postsurgery and 4, 8, and 12 weeks postsurgery for bone formation and resorption, seroma formation, root resorption, ankylosis, and residual carrier biomaterial using a magnifier/masking device (Viewscope 2 ×, Flow X-Ray Corp., Hempstead, NY, USA). The histopathologic evaluation included observations of new bone formation and resorption, woven and lamellar bone, cortex formation, seroma formation, fibrovascular tissue and marrow, vascularity, cementum formation, fibrous attachment, root resorption, ankylosis, residual carrier biomaterial, and tissue reactions relative to the carrier biomaterial.

One experienced masked examiner performed the histometric analysis using image analysis software with a custom program for the supra-alveolar periodontal defect model (Image-Pro Plus™, Media Cybernetic, Silver Springs, MD, USA). The most central stained section of each root for the third and fourth premolar teeth was identified by the size of the root canal. This section and the immediate stained step serial section on either side were subjected to the histometric analysis. Thus, three subsequent step serial sections, representing 0.2 mm of the mid-portion of the mesial and the distal root for each premolar tooth, were used for analysis. The following measurements were recorded for the buccal and lingual tooth surfaces of each section:

- Defect height (mm): distance between apical extension of root planing and CEJ.
- Cementum regeneration (mm): distance between apical extension of root planing and coronal extension of a continuous layer of new cementum or cementum-like deposit on the planed root.
- Bone regeneration/induced bone height (mm): distance between apical extension of root planing and coronal extension of new alveolar bone along the planed root.
- Bone regeneration/induced bone area (mm^2): area represented by new alveolar bone along the planed root.
- Bone regeneration/induced bone density (%): ratio of regenerated bone/marrow spaces.
- Root resorption (mm): combined linear heights of distinct resorption lacunae on the planed root.
- Ankylosis (mm): combined linear heights of ankylotic union between new alveolar bone and the planed root.
- Residual Ceredex™ (mm^2): combined area of residual Ceredex™ in defect area.

Summary statistics (means ± SD) based on animal means for the experimental conditions were calculated using the selected step serial sections.

Results

Clinical and radiographic observations

All defect sites receiving rhBMP-2/Ceredex™ exhibited uneventful primary healing. The sites appeared hard to palpation from week 2 throughout the healing interval. Fistulas were observed at all sites irrespective of rhBMP-2 dose. Evidence of seroma formation was observed from week 4 postsurgery in one animal. The seroma did not appear to resolve by week 12. All sites exhibited some degree of exposure of the teeth irrespective of dose and typically increased throughout the healing interval. The time of exposure ranged from day 12 to week 4 postsurgery.

The controls exhibited less postsurgery swelling compared with sites receiving rhBMP-2/Ceredex™. At suture removal, all control sites exhibited some exposure (Fig. 1). Exposed Ceredex™ was removed from one site. At week 4 postsurgery, two of three animals exhibited almost complete exposure of the supra-alveolar defects while the defects in the third animal remained partially exposed throughout the healing interval. The fourth premolars were extracted in two animals at week 8 due to radiographic evidence of peri-apical infections.

Representative radiographs are shown in Fig. 2. Some resorption of the Ceredex™ biomaterial was apparent at suture removal in sites receiving rhBMP-2/Ceredex™. A radiolucent line that became less distinctive over the healing interval indicative of localized resorption was apparent between the reduced alveolar crest and the Ceredex™ biomaterial. The resorption of Ceredex™ appeared to continue although some material apparently remained in the furcation area and immediately adjacent to the roots through week 12. Bone formation appeared to increase through week 8 postsurgery irrespective of dose and appeared greater in the periphery, surrounding the Ceredex™ biomaterial. Some sites exhibited evidence of root resorption. Two animals implanted with rhBMP-2/Ceredex™ exhibited evidence of peri-apical infections at week 12 postsurgery. To prevent any effect on adjacent sites and out of concern for animal welfare, all animals implanted with rhBMP-2/Ceredex™ were euthanized at week 12 postsurgery.

The control sites exhibited limited new bone formation. At week 4, residual Ceredex™ was difficult to detect in two animals. At week 8, residual Ceredex™ was only apparent in one animal. This animal exhibited the greatest amount of bone regeneration and Ceredex™ resorption throughout the healing interval, however, some Ceredex™ appeared to remain at sacrifice (Fig. 2).

Histological evaluation

Defect exposure generally including the top surface of the teeth extending to the CEJ was observed at seven of the 12 teeth receiving rhBMP-2/Ceredex™ without apparent influence of rhBMP-2 concentration. Defect exposures did not appear to have an immediate effect on new bone formation. Extensive bone formation was observed in jaw quadrants receiving rhBMP-2/Ceredex™ except for in sites apparently affected by local inflammatory reactions. The newly formed bone, a mixture of lamellar and woven bone in fibrovascular tissue, circumscribed the Ceredex™ biomaterial like coral reefs (Figs 3 and 4). Woven bone was actively remodeling into lamellar bone with new Haversian systems. Active osteoblasts, osteoclasts, vascular cells, and marrow cells were observed. Osteoclasts were observed resorbing bone along cementum reversal lines. Osteoblasts were concurrently observed forming new bone. Control sites all exhibited exposure of a large portion of the teeth and consequently exhibited limited bone formation (Fig. 4).

There was a substantial amount of residual Ceredex™ biomaterial in most

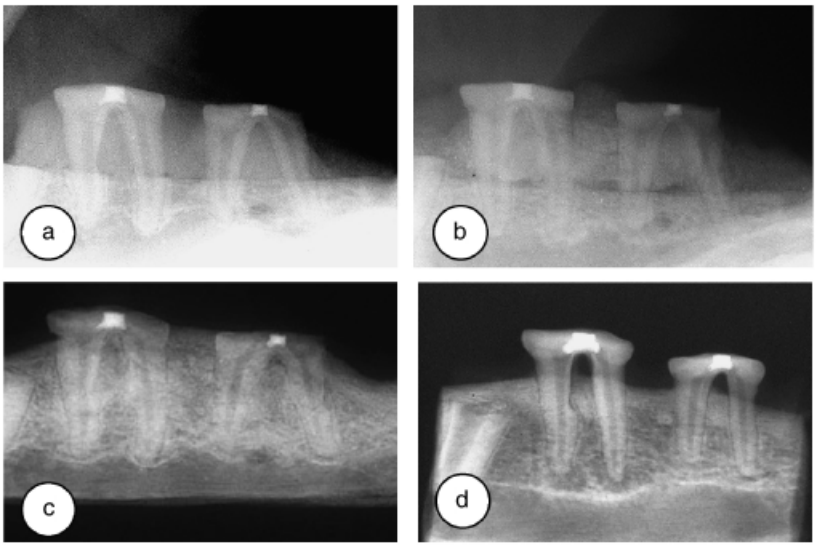

Fig. 2. Radiographs of supra-alveolar periodontal defect implanted with rhBMP-2/Ceredex™ (rhBMP-2 at 0.2 mg/mL) at implantation (a), week 4 (b), and week 12 (c). Ceredex™ control site at week 16 (d).

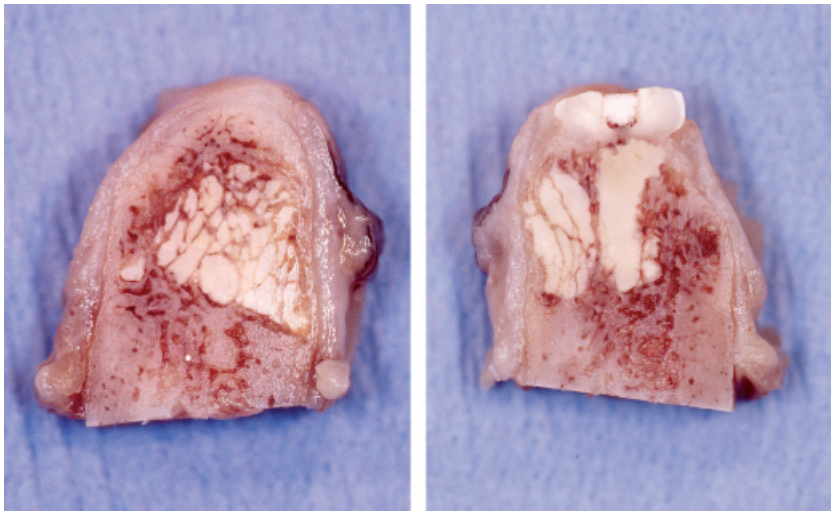

Fig. 3. Gross specimens of interproximal (left) and interradicular sites implanted with rhBMP-2/Ceredex™. Note new bone formation within the rhBMP-2/Ceredex™ constructs.

defect sites, particularly in the absence of defect exposure and inflammatory reactions, irrespective of rhBMP-2 dose (Figs 3 and 4). Bone formation was observed around the rhBMP-2/Ceredex™ construct and forming cutting cones into the Ceredex™ biomaterial, e.g. osteoclasts tunneled into the biomaterial leading to biomaterial resorption (Fig. 5). Newly formed bone, circumscribing residual Ceredex™, was often found adjacent and ankylosed to the root. Limited or no residual Ceredex™ was observed in two defect sites as these sites harbored local inflammatory reactions. Residual Ceredex™ was not observed in any of the control animals (Fig. 4).

Newly formed cementum was present to variable degrees in all sites receiving rhBMP-2/Ceredex™ (Fig. 5). The extent of cementum formation did not appear related to the rhBMP-2 dose. Although a periodontal ligament space was evident in all specimens, functionally oriented fibers inserting into the newly formed cementum were a rare observation. In contrast, newly formed cementum with an inserting functionally oriented periodontal ligament was usually observed in the Ceredex™ controls, in the presence of bone regeneration.

Ankylosis located to the coronal third of the root surface was common in sites receiving rhBMP-2/Ceredex™ and observed in one site receiving the Ceredex™ control, in other words, ankylosis was not present in the absence of extensive bone formation. Five of 12 teeth receiving rhBMP-2/Ceredex™ exhibited significant cervical resorption commonly associated with an inflammatory reaction. In the absence of local inflammatory reactions, there was minimal cervical resorption. Seromas or traces thereof were observed in one defect site.

Histometric evaluation

The results of the histometric analysis are shown in Table 1. Mean defect height ($\pm$ SD) ranged from 5.6 ± 0.3 to 5.7 ± 0.5 mm for the experimental and control groups. Induced bone height in sites receiving rhBMP-2/Ceredex™ was twofold greater than that observed in the Ceredex™ control approximating 82% and 84% (rhBMP-2 at 0.2 and 0.4 mg/ml, respectively) versus 41% of the defect height. The corresponding mean values for induced bone area were 11.4 ± 4.4 and 16.2 ± 10.1 mm^2 versus 1.9 ± 1.3 mm^2. Bone density values appeared somewhat smaller for sites receiving rhBMP-2/Ceredex™ (31.1 ± 4.3% and 26.5 ± 3.9%; rhBMP-2 at 0.2 and 0.4 mg/ml, respectively) compared with that of the Ceredex™ control (40.9 ± 7.2%). Differences in bone density values appeared, in part, to be due to differences in residual Ceredex™ between the experimental and control conditions. Ceredex™ comprised 21% and 25% of the induced bone area in sites receiving rhBMP-2/Ceredex™ (rhBMP-2 at 0.2 and 0.4 mg/ml, respectively). Residual Ceredex™ was not observed in the control sites.

Cementum regeneration encompassed 60% and 35% of the defect height in sites receiving rhBMP-2/Ceredex™ (rhBMP-2 at 0.2 and 0.4 mg/ml, respectively) compared with 27% for the Ceredex™ control. The extent of root resorption and ankylosis appeared greater in sites receiving rhBMP-2/Ceredex™ compared with that observed for the control, ankylosis and root resorption being commonly observed in the cervical third of the experimental teeth.

Discussion

The objective of this study was to evaluate Ceredex™ CP cement as a carrier for rhBMP-2 in a defect model with limited osteogenic potential. Supra-alveolar, critical size, periodontal defects in six mongrel dogs were surgically implanted with Ceredex™, with or without rhBMP-2. Defect sites receiving rhBMP-2/Ceredex™ exhibited enhanced bone formation at week 4 that increased through week 12, irrespective of dose. The CP cement was not completely resorbed by 12 weeks. Sites receiving rhBMP-2/Ceredex™ exhibited increased cementum regeneration compared with control, but without a functionally oriented periodontal ligament as well as increased root resorption and ankylosis. Ceredex™ control sites commonly exhibited early wound failure and exposure, loss of the biomaterial, and limited bone formation.

Induced bone height exceeded 80% of the defect height for supra-alveolar periodontal defects implanted with rhBMP-2/Ceredex™ without major differences between rhBMP-2 concentrations compared with approximately 40% for the Ceredex™ control. The induced bone area was also considerably greater in defects receiving rhBMP-2/Ceredex™ compared with control. Although caution must be exerted when comparing data from different studies, these results compare favorably to that previously reported from our laboratory utilizing the same experimental model implanted with rhBMP-2 and a variety of carrier technologies including bioerodable poly(D,L-lactide-*co*-glycolide) microspheres (PLGA) formulated with autologous blood (Sigurdsson et al. 1995) or formulated with carboxymethyl cellulose in aqueous glycerol (Sigurdsson et al. 1996), freeze-dried demineralized allogeneic bone matrix (DFDBA), bovine bone mineral matrix (Bio-Oss®, Osteohealth Company, Shirley, NY, USA), and PLA granules, all formulated with autologous

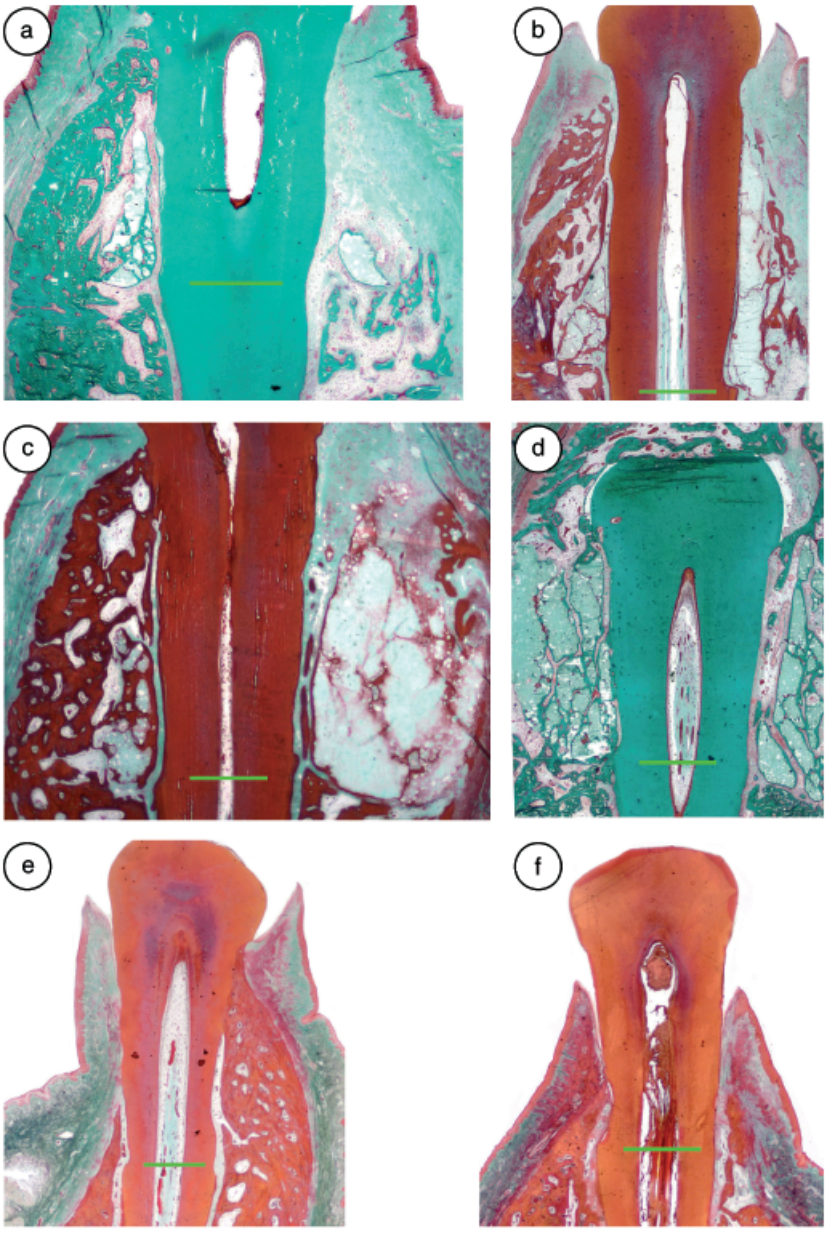

Fig. 4. Representative photomicrographs of supra-alveolar periodontal defect sites implanted with rhBMP-2/Ceredex™ (a & c: rhBMP-2 at 0.20 mg/mL; b & d: rhBMP-2 at 0.40 mg/mL), and Ceredex™ controls (e & f). The green line represents the apical extent of the supra-alveolar defects.

Table 1. Results of the histometric analysis of supra-alveolar periodontal defects implanted with the Ceredex™ calcium phosphate cement with or without rhBMP-2 (group means ± SD)

	rhBMP-2/Ceredex™, 0.2 mg/ml	rhBMP-2/Ceredex™, 0.4 mg/ml	Ceredex™ (control)
defect height (mm)	5.6 ± 0.3	5.7 ± 0.5	5.6 ± 0.2
cementum regeneration (mm)	3.4 ± 0.4	2.0 ± 0.4	1.5 ± 0.3
new bone height (mm)	4.6 ± 0.5	4.9 ± 0.5	1.9 ± 1.3
new bone area (mm^2)	11.4 ± 4.4	16.2 ± 10.1	1.9 ± 2.0
new bone density (%)	31.1 ± 4.3	26.6 ± 3.9	40.9 ± 7.2
residual Ceredex™ (mm^2)	2.4 ± 1.5	4.0 ± 2.8	0.0 ± 0.0
root resorption (mm)	0.7 ± 0.5	1.3 ± 1.1	0.4 ± 0.0
ankylosis (mm)	0.5 ± 0.1	0.7 ± 0.2	0.1 ± 0.2

rhBMP-2, recombinant human bone morphogenetic protein-2.

blood (Sigurdsson et al. 1996), or ACS (Sigurdsson et al. 1996, Wikesjö et al. 1999, 2003a, 2004, Selvig et al. 2002). In contrast, bone regeneration (height and volume) following guided tissue regeneration (GTR) (Haney et al. 1993, Sigurdsson et al. 1994, Wikesjö et al. 2003a,c,d,e), implantation of particulate DBM/DFDBA or in block preparations (Sigurdsson et al. 1996, Kim et al. 1998), rhTGF-β_1 with or without GTR (Wikesjö et al. 1998, Tatakis et al. 2000), a prostaglandin E_1 analog with or without GTR (Trombelli et al. 1999), or a coral-derived calcium carbonate bone biomaterial with or without GTR (Koo et al. 2003, Wikesjö et al. 2003e) appears considerably more modest than that generally observed following implantation of rhBMP-2 constructs. Apparently, the osteogenic/osteoinductive/osteoconductive potential of these therapy concepts is considerably weaker than that of rhBMP-2.

Bone density appeared lower in defect sites receiving rhBMP-2/Ceredex™ compared with control averaging 31% and 27% (rhBMP-2 at 0.2 and 0.4 mg/ml), and 43%, respectively. A relatively large amount of residual Ceredex™ comprised 20–26% of the bone area in sites receiving rhBMP-2/Ceredex™, but not in the Ceredex™ control (0%). Newly formed bone was observed surrounding the biomaterial. Ceredex™ resorption appeared to occur by (osteoclast) cutting-cone formation into the biomaterial. Previous studies have evaluated still other biomaterials as candidate carriers for rhBMP-2 including Bio-Oss® bovine bone mineral, poly-α-hydroxy acid technologies such as PLA particles and PLGA microspheres, the bovine ACS, allogeneic DBM/DFDBA, and hyaluronan in the supra-alveolar, periodontal defect model (Sigurdsson et al. 1995, 1996, Wikesjö et al. 1999, 2003a, d, f, 2004, Selvig et al. 2002). It was shown that rhBMP-2 combined with DBM and autologous blood produced a large bone area (23 mm^2) of high density (46%). The other biomaterials combined with rhBMP-2 produced either a small bone area of high density or a large bone area of lesser bone density. The latter was typically observed for the Bio-Oss® or PLA preparations. Bio-Oss® appeared to impede bone formation by obstructing the space for bone to form into. In contrast to Ceredex™, there was no apparent tendency for conversion of the Bio-Oss® biomaterial into new bone. The

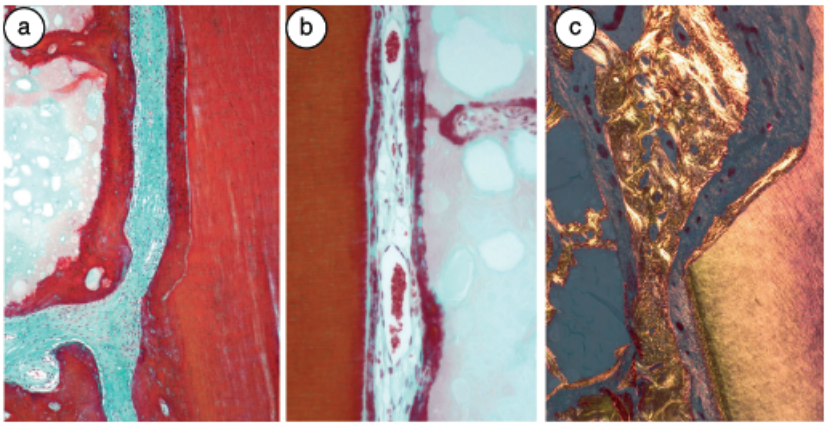

Fig. 5. Representative high power photomicrographs of supra-alveolar periodontal defect sites implanted with rhBMP-2/Ceredex™ showing new cementum formation at the apical extent of the defect (a & c) and at a more coronal location. Note cutting cone formation within the Ceredex™ biomaterial (b). The periodontal ligament space is characterized by fibrovascular tissue (a & b) without a functionally oriented periodontal ligament (c; polarized light).

Bio-Oss® particles remained embedded in fatty marrow without any associated bone metabolic activity (bone formation or biomaterials resorption). The PLA biomaterial, on the other hand, was predictably associated with an inflammatory reaction including accumulation of foamy macrophages as it fragmented during the degradation process. Both newly formed and resident bone resorbed as a consequence of this process.

A concern of this study is the relatively large amount of residual Ceredex™ CP cement, which was significantly greater than that observed in a previous study evaluating rhBMP-2/α-BSM® in the supra-alveolar peri-implant defect model (Wikesjö et al. 2002). This may relate to differences in observation interval between studies (12 versus 16 weeks) or differences in healing rate between periodontal and peri-implant defect sites. However, this may also relate genuine differences in the CP cement formulation. The Ceredex™ Type 2.1 clinical material used herein consists of diphasic calcium phosphate (DPCP). The α-BSM® biomaterial is a variation of the clinical material consisting of less reactive amorphous calcium phosphate (ACP). The ACP exhibits much weaker bonds than the DPCP ultimately resulting in a macro-particulate *in vivo* more readily resorbed by osteoclasts. Given that the same amount of rhBMP-2 was added to each formulation in this and the study evaluating rhBMP-2/α-BSM®, it is conceivable that the relatively large differences in residual biomaterial between studies may mainly be an effect of the chemical variation between the CP cements. Longer observation intervals are desirable to determine the eventual resorption of the Ceredex™ biomaterial and maturation of associated induced bone formation to ultimately understand its clinical value.

The primary reason for the differences between rhBMP-2/Ceredex™ and control sites is in all likelihood wound failure, exposure, infection and loss of the Ceredex™ biomaterial in the control sites, rarely encountered in sites receiving rhBMP-2/Ceredex™. Thus, the genuine biologic potential of the Ceredex™ biomaterial, if any, to support bone formation in this onlay model system could not be discerned. These observations may also suggest that rhBMP-2 not only supports clinically relevant bone formation, as shown in several previous preclinical studies, but also has significant effects on soft tissue healing as observed herein and previously in the supra-alveolar periodontal defect model (Wikesjö et al. 1999, 2002, 2003d). These observations may also suggest that surgical implantation of biomaterials such as Ceredex™ herein without rhBMP-2 or barrier devices (Kim et al. 1998, Wikesjö et al. 2003c, d) to support regeneration of alveolar bone may be associated with significant morbidity to the overlying mucoperiosteal flap due to compromised vascular support or due to other unique effects of the biomaterial or device.

From a periodontal perspective, mean cementum formation encompassed 35–60% of the defect height in sites receiving rhBMP-2/Ceredex™ compared with 27% for the control. Overall cementum regeneration parallels that of previous studies in this model system evaluating rhBMP-2 and a variety of carrier technologies (Sigurdsson et al. 1995, 1996, Wikesjö et al. 1999, 2003a, d, f, 2004, Selvig et al. 2002). Common to all studies, the newly formed cellular cementum merged with ankylotic bone in the coronal third of the large supra-alveolar defect. Moreover, the newly formed cementum did not include a functionally oriented periodontal ligament anchoring the tooth to the newly formed bone. Importantly, when this model system was used to evaluate the biologic potential of GTR, healing was characterized by regeneration of a cellular cementum *including* a functionally oriented periodontal ligament anchoring the previously denuded root to newly formed bone and limited, if any, ankylosis (Sigurdsson et al. 1994, Wikesjö et al. 2003c, d, f). Surgical controls have been characterized by limited cementum formation without a functionally oriented periodontal ligament (Wikesjö & Nilvéus 1991, Sigurdsson et al. 1994, Wikesjö et al. 1994). Nevertheless, we, and others, have reported formation of a functional periodontal attachment following application of rhBMP-2 and other BMP technologies for periodontal regeneration (Ishikawa et al. 1994, Ripamonti et al. 1994, 1996, 2001, Kinoshita et al. 1997, Giannobile et al. 1998, Kuboki et al. 1998, Blumenthal et al. 2002, Choi et al. 2002, Wikesjö et al. 2004). Such observations may be explained by the fact that some of the model systems have been insufficiently discriminating, however, it cannot be excluded that genuine differences in biologic potential exist among the technologies evaluated.

Root resorption and ankylosis in the cervical third of the defects, observed also in this study, is a common observation in this defect model following surgical implantation of rhBMP-2 (Sigurdsson et al. 1994, 1995, Wikesjö et al. 1999, 2003a, d, f, 2004, Selvig et al. 2002). Root resorption and ankylosis are rarely encountered in the apical aspect of the supra-alveolar periodontal defect and thus may not become a healing aberration in more limited periodontal defects or defects exhibiting limited healing (Blumenthal et al. 2002, Choi et al. 2002). Ankylosis has also not

been a healing aberration following surgical implantation of BMPs in the absence of extensive bone regeneration (Ripamonti et al. 1994, Kinoshita et al. 1997, Blumenthal et al. 2002, Choi et al. 2002).

Conclusions

The Ceredex™ CP cement appears a potentially promising carrier technology for rhBMP-2 onlay indications. However, a slow resorption rate may prevent its wider use. This study does not support use of the rhBMP-2/Ceredex™ combination for periodontal indications.

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References

Blumenthal, N. M., Koh-Kunts, G., Alves, M. E. A. F., Miranda, D., Sorensen, R. G., Wozney, J. M. & Wikesjö, U. M. E. (2002) Effect of surgical implantation of recombinant human bone morphogenetic protein-2 in a bioabsorbable collagen sponge or a calcium phosphate putty carrier in intrabony periodontal defects in the baboon. *Journal of Periodontology* **73**, 1494–1506.

Choi, S.-H., Kim, C.-K., Cho, K.-S., Huh, J.-S., Sorensen, R. G., Wozney, J. M. & Wikesjö, U. M. E. (2002) Effect of recombinant human bone morphogenetic protein-2/absorbable collagen sponge (rhBMP-2/ACS) on healing in 3-wall intrabony defects in dogs. *Journal of Periodontology* **73**, 63–72.

Giannobile, W. V., Ryan, S., Shih, M.-S., Su, D. L., Kaplan, P. L. & Chan, T. C. K. (1998) Recombinant human osteogenic protein-1 (OP-1) stimulates periodontal wound healing in Class III furcation defects. *Journal of Periodontology* **69**, 129–137.

Haney, J. M., Nilvéus, R. E., McMillan, P. J. & Wikesjö, U. M. E. (1993) Periodontal repair in dogs: expanded polytetrafluoroethylene barrier membranes support wound stabilization and enhance bone regeneration. *Journal of Periodontology* **64**, 883–890.

Ishikawa, I., Kinoshita, A., Oda, S. & Roongruangphol, T. (1994) Regenerative therapy in periodontal diseases. Histological observations after implantation of rhBMP-2 in the surgically created periodontal defects in adult dogs. *Dentistry in Japan* **31**, 141–146.

Ishikawa, K., Miyamoto, Y., Takechi, M., Toh, T., Kon, M., Nagayama, M. & Asaoka, K. (1997) Non-decay type fast-setting calcium phosphate cement: hydroxyapatite putty containing an increased amount of sodium alginate. *Journal of Biomedical Materials Research* **36**, 393–399.

Kim, C.-K., Cho, K.-S., Choi, S.-H., Prewett, A. & Wikesjö, U. M. E. (1998) Periodontal repair in dogs: effect of allogeneic freeze-dried demineralized bone matrix implants on alveolar bone and cementum regeneration. *Journal of Periodontology* **69**, 26–33.

Kinoshita, A., Oda, S., Takahashi, K., Yokota, S. & Ishikawa, I. (1997) Periodontal regeneration by application of rhBMP-2 to horizontal circumferential defects created by experimental periodontitis in beagle dogs. *Journal of Periodontology* **68**, 103–109.

Knaack, D., Goad, M. E., Aiolova, M., Rey, C., Tofighi, A., Chakravarthy, P. & Lee, D. D. (1998) Resorbable calcium phosphate bone substitute. *Journal of Biomedical Materials Research* **43**, 399–409.

Koo, K.-T., Polimeni, G., Qahash, M., Kim, C.-K. & Wikesjö, U. M. E. (in press) Periodontal repair in dogs: guided tissue regeneration enhances bone regeneration associated with a coral calcium carbonate implant. *Journal of Clinical Periodontology*.

Kuboki, Y., Sasaki, M., Saito, A., Takita, H. & Kato, H. (1998) Regeneration of periodontal ligament and cementum by BMP-applied tissue engineering. *European Journal of Oral Sciences* **106** (Suppl. 1), 197–203.

Lee, D. D., Tofighi, A., Aiolova, M., Chakravarthy, P., Catalano, A., Majahad, A. & Knaack, D. (1999) Alpha-BSM: a biomimetic bone substitute and drug delivery vehicle. *Clinical Orthopaedics and Related Research* **367**, S396–S405.

Li, R. H., Bouxsein, M. L., Blake, C. A., D'Augusta, D., Li, X. J., Wozney, J. M. & Seeherman, H. J. (2003) rhBMP-2 injected in a calcium-phosphate paste (alpha-BSM) accelerates healing in the rabbit ulnar osteotomy model. *Journal of Orthopaedic Research* **21**, 997–1004.

Luna, L. G. (1992) *Histologic Methods and Color Atlas of Special Stains and Tissue Artifacts*. Gaithersburg, MD: American Histolab, Inc., Publications Division, p. 113.

Ripamonti, U., Crooks, J., Petit, J. C. & Rueger, D. C. (2001) Periodontal tissue regeneration by combined applications of recombinant human osteogenic protein-1 and bone morphogenetic protein-2. A pilot study in Chacma baboons (*Papio ursinus*). *European Journal of Oral Sciences* **109**, 241–248.

Ripamonti, U., Heliotis, M., Rueger, D. C. & Sampath, T. K. (1996) Induction of cementogenesis by recombinant human osteogenic protein-1 (hop-1/bmp-7) in the baboon (*Papio ursinus*). Short communication. *Archives of Oral Biology* **41**, 121–126.

Ripamonti, U., Heliotis, M., van den Heever, B. & Reddi, A. H. (1994) Bone morphogenetic proteins induce periodontal regeneration in the baboon (*Papio ursinus*). Brief communication. *Journal of Periodontal Research* **29**, 439–445.

Schenk, R. K., Olah, A. J. & Herrmann, W. (1984) Preparation of calcified tissues for light microscopy. In: *Methods of Calcified Tissue Preparation*, eds. Dickson, G. R., pp. 1–42. Amsterdam: Elsevier Science Publishers BV.

Schmitz, J. P., Hollinger, J. O. & Milam, S. B. (1999) Reconstruction of bone using calcium phosphate bone cements: a critical review. *Journal of Oral and Maxillofacial Surgery* **57**, 1122–1126.

Seeherman, H., Aiolova, M., Bouxsein, M. & Wozney, J. (2001) *A single injection of rhBMP-2/calcium phosphate paste accelerates healing in a nonhuman primate osteotomy model*. Transactions of the Annual Meeting of the Orthopaedic Research Society, 2001, San Francisco, CA.

Seeherman, H., Li, R., Blake, C., Bouxsein, M., Cooper, J., Gavin, D., Li, J. & Wozney, J. (2002) *A single injection of rhBMP-2/calcium phosphate paste given one week after surgery accelerates osteotomy healing by 50% in a non-human primate fibula osteotomy model*. Transactions of the 48th Annual Meeting of the Orthopaedic Research Society, 2002, Dallas, TX.

Selvig, K. A., Sorensen, R. G., Wozney, J. M. & Wikesjö, U. M. E. (2002) Bone repair following recombinant human bone morphogenetic protein-2 stimulated periodontal regeneration. *Journal of Periodontology* **73**, 1020–1029.

Sigurdsson, T. J., Hardwick, R., Bogle, G. C. & Wikesjö, U. M. E. (1994) Periodontal repair in dogs: space provision by reinforced ePTFE membranes enhances bone and cementum regeneration in large supraalveolar defects. *Journal of Periodontology* **65**, 350–356.

Sigurdsson, T. J., Lee, M. B., Kubota, K., Turek, T. J., Wozney, J. M. & Wikesjö, U. M. E. (1995) Periodontal repair in dogs: recombinant human bone morphogenetic protein-2 significantly enhances periodontal regeneration. *Journal of Periodontology* **66**, 131–138.

Sigurdsson, T. J., Nygaard, L., Tatakis, D. N., Fu, E., Turek, T. J., Jin, L., Wozney, J. M. & Wikesjö, U. M. E. (1996) Periodontal repair in dogs: evaluation of rhBMP-2 carriers. *The International Journal of Periodontics and Restorative Dentistry* **16**, 525–537.

Tatakis, D. N., Wikesjö, U. M. E., Razi, S. S., Sigurdsson, T. J., Lee, M. B., Nguyen, T., Ongpipattanakul, B. & Hardwick, R. (2000) Periodontal repair in dogs: effect of transforming growth factor-ß$_1$ on alveolar bone and cementum regeneration. *Journal of Clinical Periodontology* **27**, 698–704.

Trombelli, L., Lee, M. B., Promsudthi, A., Guglielmoni, P. G. & Wikesjö, U. M. E. (1999) Periodontal repair in dogs: histologic observations of guided tissue regeneration with a prostaglandin E_1 analog/methacrylate composite. *Journal of Clinical Periodontology* **26**, 381–387.

Valentin-Opran, A., Wozney, J., Csimma, C., Lilly, L. & Riedel, G. E. (2002) Clinical

evaluation of recombinant human bone morphogenetic protein-2. *Clinical Orthopaedics and Related Research* **395**, 110–120.

Wikesjö, U. M. E., Guglielmoni, P. G., Promsudthi, A., Cho, K.-S., Trombelli, L., Selvig, K. A., Jin, L. & Wozney, J. M. (1999) Periodontal repair in dogs: effect of rhBMP-2 concentration on regeneration of alveolar bone and periodontal attachment. *Journal of Clinical Periodontology* **26**, 392–400.

Wikesjö, U. M. E., Kean, C. J. C. & Zimmerman, G. J. (1994) Periodontal repair in dogs: supraalveolar defect models for evaluation of safety and efficacy of periodontal reconstructive therapy. *Journal of Periodontology* **65**, 1151–1157.

Wikesjö, U. M. E., Lim, W. H., Razi, S. S., Sigurdsson, T. J., Lee, M. B., Tatakis, D. N. & Hardwick, W. R. (2003e) Periodontal repair in dogs: a bioresorbable calcium carbonate coral implant enhances space provision for alveolar bone regeneration in conjunction with guided tissue regeneration. *Journal of Periodontology* **74**, 955–962.

Wikesjö, U. M. E., Lim, W. H., Thomson, R. C., Cook, A. D., Wozney, J. M. & Hardwick, W. R. (2003d) Periodontal repair in dogs: evaluation of a bioresorbable space-providing macro-porous membrane with recombinant human bone morphogenetic protein-2. *Journal of Periodontology* **74**, 635–647.

Wikesjö, U. M. E., Lim, W. H., Thomson, R. C. & Hardwick, W. R. (2003c) Periodontal repair in dogs: gingival tissue exclusion, a critical requirement for guided tissue regeneration? *Journal of Clinical Periodontology* **30**, 655–664.

Wikesjö, U. M. E. & Nilvéus, R. (1991) Periodontal repair in dogs: healing patterns in large circumferential periodontal defects. *Journal of Clinical Periodontology* **18**, 49–59.

Wikesjö, U. M. E., Qahash, M., Thomson, R. C., Cook, A. D., Rohrer, M. D., Wozney, J. M. & Hardwick, W. R. (2003b) Space-providing expanded polytetrafluoroethylene devices define alveolar augmentation at dental implants induced by recombinant human bone morphogenetic protein-2. *Clinical Implant Dentistry and Related Research* **5**, 112–123.

Wikesjö, U. M. E., Razi, S. S., Sigurdsson, T. J., Tatakis, D. N., Lee, M. B., Ongpipattanakul, B., Nguyen, T. & Hardwick, R. (1998) Periodontal repair in dogs: effect of recombinant human transforming growth factor-beta$_1$ on guided tissue regeneration. *Journal of Clinical Periodontology* **25**, 475–481.

Wikesjö, U. M. E. & Selvig, K. A. (1999) Periodontal wound healing and regeneration. *Periodontology 2000* **19**, 21–39.

Wikesjö, U. M. E., Sorensen, R. G., Kinoshita, A., Li, X. J. & Wozney, J. M. (2004) Periodontal repair in dogs: effect of rhBMP-12 on regeneration of alveolar bone and periodontal attachment. A pilot study. *Journal of Clinical Periodontology* **31**, 662–670.

Wikesjö, U. M. E., Sorensen, R. G., Kinoshita, A. & Wozney, J. M. (2002) rhBMP-2/α-BSM® induces significant vertical alveolar ridge augmentation and dental implant osseointegration. *Clinical Implant Dentistry and Related Research* **4**, 173–181.

Wikesjö, U. M. E., Sorensen, R. G. & Wozney, J. M. (2001) Augmentation of alveolar bone and dental implant osseointegration: clinical implications of studies with rhBMP-2. A comprehensive review. *The Journal of Bone and Joint Surgery. American Volume* **83-A** (Suppl. 1, Part 2), S136–S145.

Wikesjö, U. M. E., Xiropaidis, A. V., Thomson, R. C., Cook, A. D., Selvig, K. A. & Hardwick, W. R. (2003a) Periodontal repair in dogs: space-providing ePTFE devices increase rhBMP-2/ACS induced bone formation. *Journal of Clinical Periodontology* **30**, 715–725.

Wikesjö, U. M. E., Xiropaidis, A. V., Thomson, R. C., Cook, A. D., Selvig, K. A. & Hardwick, W. R. (2003f) Periodontal repair in dogs: rhBMP-2 significantly enhances bone formation under provisions for guided tissue regeneration. *Journal of Clinical Periodontology* **30**, 705–714.

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