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Effect of recombinant human bone morphogenetic protein-12 (rhBMP-12) on regeneration of periodontal attachment following tooth replantation in dogs

A pilot study

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Abstract

Objectives: Subcutaneous and intramuscular implants of bone morphogenetic protein-12 (BMP-12) have been shown to induce formation of tendon and ligament tissue. BMP-12 induced a new attachment with a distinct fibrocartilaginous zone at the tendon-bone interface in the rat tendon-bone attachment model. Surgical controls showed poor healing and failure to reform the appropriate tendon–bone attachment morphologically. Application of recombinant human BMP-12 (rhBMP-12) to periodontal defects suggests that rhBMP-12 has the potential to support regeneration of the periodontal ligament (PDL). The objective of this pilot study was to evaluate this effect of rhBMP-12 in a tooth replantation model.

Methods: Six, young adult, male Hound Labrador mongrel dogs were used. Maxillary and/or mandibular incisor and premolar teeth were extracted and the PDL was either left ''intact'' or removed by root planing. rhBMP-12 (1.0 mg/ml) or a buffer control was topically applied to teeth with ''intact'' PDL in contralateral jaw quadrants in each of 3 animals. The teeth were immersed in 1.0 ml of the rhBMP-12 or the buffer solution for 10 min and then replanted. The remaining three animals received rhBMP-12 (1.0 mg/ml) and the buffer control in a similar fashion applied to teeth instrumented to remove the PDL and cementum, and surface demineralized with citric acid. The animals were euthanized at 8 weeks postsurgery and block sections were collected and processed for histopathologic analysis.

Results: No dramatic differences were found between teeth receiving topical rhBMP-12 and the buffer control. Application of rhBMP-12 did not have an apparent effect on new cementum and PDL formation in the tooth replantation model. Moreover, application of rhBMP-12 did not increase nor did it decrease the apparent presence and extent of ankylosis along the root surface compared to the control.

Conclusions: The observations from this study do not support the use of topical rhBMP-12 to support the reestablishment of the PDL including regeneration of cementum and functionally oriented fibers, and to prevent ankylosis and root resorption following replantation of teeth.

Key words: ankylosis; cementum; periodontal ligament; periodontal regeneration; root resorption

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Dento-alveolar ankylosis is a common complication following replantation of teeth (Løe & Waerhaug 1961, Andersson 1988, Hammarström et al. 1989, Andersson & Bodin 1990, Skoglund 1991, Trope & Friedman 1992, Andreasen & Andreasen 1992, Hellsing et al. 1993, Andreasen et al. 1995a, b, Gunday et al. 1995, Oikarinen et al. 1996, Kratchman 1997). Ankylosis leads to progressive root resorption whereby roots are gradually replaced by bone and fibrovascular tissue eventually necessitating surgical removal and prosthetic replacement of the injured teeth (Skoglund 1991). Most resorptive processes are diagnosed within the first 2–3 years but have been diagnosed as late as 10 years following tooth replantation (Andreasen et al. 1995b).

The development of replacement resorption appears dependent on the degree of injury to the periodontal ligament (PDL) at the time of avulsion and the extent to which the PDL has been compromised (Trope & Friedman 1992, Hellsing et al. 1993). Anti-resorption factors are believed to be present in the PDL given that healthy permanent teeth are not compromised by osteoclasts and teeth with a necrotic or absent PDL are compromised (Hellsing et al. 1993). The PDL appears to prevent ankylosis by maintaining separation between the alveolar socket and the root surface protecting the root surface from osteoclastic activity (Andreasen & Andreasen 1992) in addition to providing structural support. If there is loss of tissue integrity, root resorption results especially if non-PDL-derived cells gain access to the injured site.

Andreasen et al. (1995b) have reported that the strongest impacts on healing of the PDL are: the stage of root development, length of dry extra-alveolar storage, immediate replantation time, and length of wet extra-oral storage. The extent of ankylosis is increased with an increased extra-oral storage period. Non-physiologic storage (home-made saline, sterilizing solutions) always leads to root resorption (Hammarström et al. 1989). The common denominator affecting these factors is the survival of PDL cells along the root surface. Immediate replantation (within 15 min) is therefore recommended to maintain the integrity of the PDL (Andersson & Bodin 1990).

Bone morphogenetic protein-12 (BMP-12), a member of the transforming growth factor-β/BMP gene family, is the human homologue of mouse growth/differentiation factor-7 (GDF-7) (Chang et al. 1994, Wolfman et al. 1997). BMP-12 and BMP-13 (GDF-6) have been found to induce connective tissue formation rich in collagen type I fibers resembling neonatal tendon and ligament when implanted in ectopic sites in vivo (Wolfman et al. 1997). Analysis of tissue induced by BMP-12 and BMP-13 demonstrated that proteins specific to bone (osteocalcin, alkaline phosphatase) were absent but those specific to tendon and ligament were present (Chang et al. 1994, Wolfman et al. 1997, Hattersley et al. 1998).

rhBMP-12 and rhBMP-2 were evaluated in the rat model of tendon-to-bone attachment (Hattersley et al. 1998). rhBMP-12-treated sites induced a new attachment with a distinct fibrocartilaginous zone at the bone-tendon interface. The untreated tendons showed poor healing response and failure to reform morphologically to the normal attachment site. rhBMP-2 led to tendon ossification and a narrow fibrocartilaginous interface with a lower failure load. High doses of rhBMP-12 ($>100\,\mu$g), and rhBMP-13, could induce ectopic bone formation in vivo and direct injection into adult rat tendons/ligaments resulted in new connective tissue and endochondral ossification.

The ability of rhBMP-2 to stimulate significant regeneration of alveolar bone and dental cementum in preclinical models has been established (Ishikawa et al. 1994, Sigurdsson et al. 1995, 1996, Kinoshita et al. 1997, King et al. 1997, Wikesjö et al. 1999, 2003a, b, Selvig et al. 2002). A common limitation of rhBMP-2-induced bone in the periodontal model is the development of dento-alveolar ankylosis (Ishikawa et al. 1994, Sigurdsson et al. 1995, 1996, King et al. 1997, Wikesjö et al. 1999, 2003a, b, Selvig et al. 2002). Moreover the newly formed cementum does not appear to be adjoined by the formation of inserting, functionally oriented PDL fibers (Wikesjö et al. 1999, 2003a, b, Selvig et al. 2002). A recent pilot study has evaluated a possible influence of rhBMP-12 and rhBMP-2 on regeneration of the PDL (Wikesjö et al. 2003b). rhBMP-12 but not rhBMP-2 was associated with formation of a new PDL. As BMP-12 has been evaluated for tendon and ligament repair, its ability to support repair of the PDL, prevent ankylosis and subsequent resorptive activity, and allow replanted teeth to be maintained lifelong needs to be evaluated. The objective of this pilot study was to evaluate the effect of rhBMP-12 on regeneration of the PDL following replantation of teeth.

Material and Methods

Animals

Six young adult, male, Hound Labrador mongrels, age 18–24 months, weight approximately 20 kg, obtained from an USDA-approved dealer, were used. Animal selection, management, and experimental protocol followed routines approved by the Animal Care and Use Committee, Wyeth Research, Cambridge, MA, USA. The animals had access to a standard laboratory diet and water until the beginning of the study. Oral prophylaxis was performed within 2 weeks prior to surgery.

rhBMP-12

Using aseptic routines rhBMP-12 (Wyeth Research) supplied at a liquid concentration of 1.5 mg/ml in MFR 00842 buffer (0.5%sucrose, 2.5% glycine, 5 mM L-glutamatic acid, 5 mM NaCl, 0.01% polysorbate 80, pH 4.5; Wyeth Research) was diluted with MFR 00842 to a final concentration of 1.0 mg/ml.

Surgery procedure

Food was withheld the night preceding surgery. Animals were prepared for surgery, pre-anesthetized with atropine (0.02–0.04 mg/kg)/buprenorphine (0.01–0.03 mg/kg)/acepromazine (0.1 mg/kg) SQ, induced with methohexital (4–8 mg/kg to effect i.v.) and maintained on gas anesthesia (1–3% isoflurane/O_2 to effect). To maintain hydration, a sterile i.v. catheter was placed and animals received a constant rate infusion of lactated Ringer's solution (10–20 mg/kg/h i.v.) while anesthetized. Prophylactic antibiotics (cefazolin; 22 mg/kg i.v.) were administered within 1 h of surgery and redosed postsurgery.

A maximum of three maxillary or mandibular incisor and/or premolar teeth per side and animal were extracted and replanted. Care was taken to avoid damage to the PDL by avoiding the walls of the socket and minimally handling the extracted teeth (Oikarinen et al. 1996, Kratchman 1997). The PDL of the extracted teeth was left ''intact'' in three animals. The extracted teeth from the remaining three animals were planed with a curette to remove the PDL and cementum. A saturated citric acid solution was applied to the root-planed teeth for 3 min. (Wikesjö et al. 1991, Zervas et al. 1991, Chaves et al. 1993). This treatment will surface demineralize the root to increase its wettability supporting adsorption and adhesion of rhBMP-12. The teeth were rinsed in sterile saline and then stored in air for 20–30 min. Before replantation, the teeth were immersed in 1.0 ml of the rhBMP-12 or the MFR 00842 buffer (control) for 10 min. Treatments, i.e., rhBMP-12 versus buffer control, were alternated between left and right jaw quadrants in subsequent animals. The teeth were replanted into their original sockets. A sling suture was used to secure the replanted teeth.

Postsurgery care

Animals were fed a canned soft dog food diet throughout the healing sequence. A long-acting opioid (buprenorphine HCl; 0.015 mg/kg i.m. bid for 48 h) was administered for pain control. A broad-spectrum antibiotic (enrofloxacin, 2.5 mg/kg, i.m., twice daily) was used for infection control for 14 days. Plaque control was maintained by once 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 through Friday) until completion of study. Gingival sutures were removed under sedation at approximately 8 days postsurgery.

Clinical recordings

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

Histological processing

At 8 weeks postsurgery, animals were anesthetized and euthanized by an IV bolus of pentobarbital (100 mg/kg). Following euthanasia, block sections including teeth, bone and soft tissues were collected and radiographed. The specimens were rinsed in sterile saline and fixed in 10% neutral buffered formalin for 7 days. Three non-extracted/replanted teeth, untreated controls, were also removed for histologic evaluation.

The tissue blocks were trimmed, washed, and subsequently decalcified with EDTA (Luna 1992). The specimens were washed, 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, 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.

Histopathologic analysis

The most central stained section from each root was identified by the size of the root canal. This section and the immediate stained step serial section on either side were used for the histopathologic analysis by two examiners. The descriptive analysis focused on presence/absence of native cementum and PDL, new formation of cementum and a functionally oriented PDL, and ankylosis and root resorption.

Results

Clinical and radiographic observations

A total of five teeth (two maxillary incisors, two maxillary premolars, one mandibular incisor) with ''intact'' PDL received the buffer control and six teeth (three maxillary incisors, two maxillary premolars, one mandibular incisor) received topical rhBMP-12 in the three animals designated for this protocol. These teeth were replanted and maintained in situ throughout the healing interval without apparent clinical complications. The remaining three animals provided 16 teeth, root planed to remove the PDL and surface demineralized with citric acid, that either received the buffer control (three maxillary premolars, five mandibular incisors) or topical rhBMP-12 (one maxillary incisor, two maxillary premolars, five mandibular incisors). Six teeth, three receiving rhBMP-12, three the buffer control, all from the same animal, exfoliated during the healing interval and were consequently unavailable for evaluation. The radiographs exhibited evidence of various degrees of root resorption of the replanted teeth at 8 weeks postsurgery without remarkable differences between experimental conditions. Some teeth exhibited periapical radiolucencies.

Histopathological observations

Untreated controls

Three teeth from two animals served as untreated controls. The native root cementum covered the root surface without any discontinuity. Functionally oriented PDL fibers were observed along the root surface without evidence of root resorption or erosion. There were also no evidence of ankylosis between the cementum and the alveolar bone (Fig. 1).

Replanted teeth with intact PDL

There were no noteworthy or consistent differences among replanted teeth with ''intact'' PDL receiving rhBMP-12 or the buffer control (Figs. 2 and 3). The specimens were all characterized by presence of occasional scattered PDL fibers. However a few teeth within each group exhibited a considerable higher density of PDL fibers attached to the native cementum in certain areas. Most of the observed PDL fibers appeared oriented in a perpendicular fashion relative to the root surface. However, collagen fibers with a parallel orientation were also observed. Functionally oriented PDL fibers appeared more consistently at native rather than newly formed cementum.

Limited areas of root resorption of surface erosion character removing the native cementum were observed in all specimens irrespective of treatment protocol. A few teeth receiving the buffer control (2/5) showed signs of undermining root resorption (Fig. 3). The majority of the teeth exhibited new cementum formation on the eroded/resorbed root surface (Fig. 3). Limited ankylosis was present in 2/5 and 2/3 teeth receiving the buffer control and topical rhBMP-12, respectively (Fig. 2).

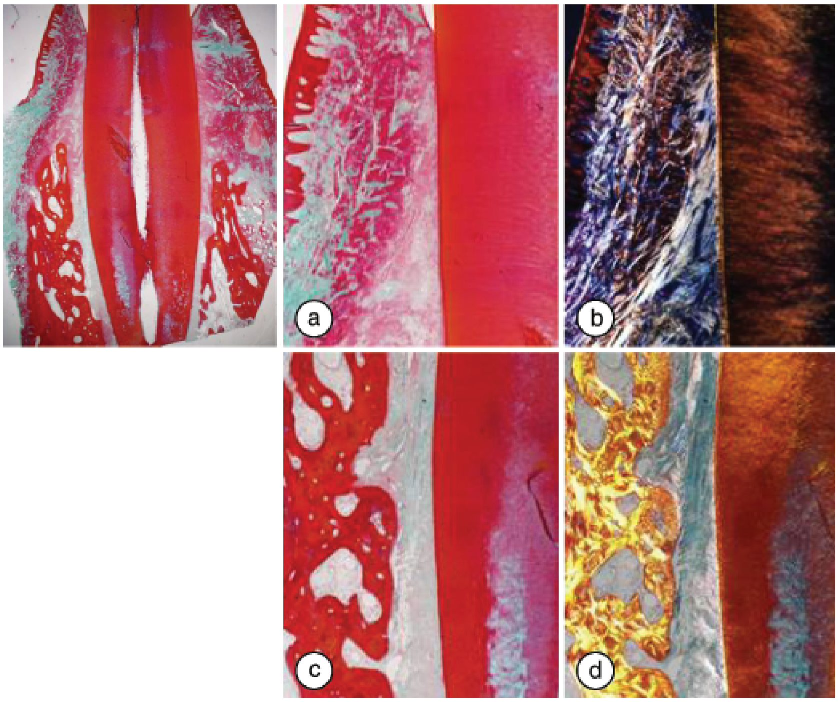

Fig. 1. Photomicrographs (original magnification × 2.5 and × 4; Goldner's trichrome) of native control. Note functionally oriented PDL fibers inserting into the native cementum without evidence of root resorption or ankylosis in the cervical (a/b; b polarized light) as well as the alveolar crest (c/d; d polarized light) region.

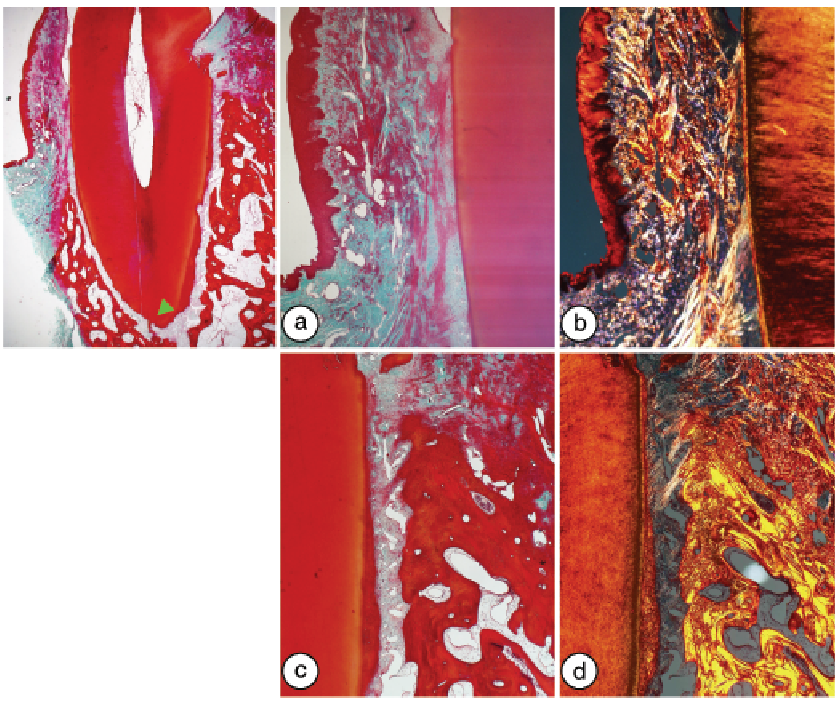

Fig. 2. Photomicrographs (original magnification × 2.5 and × 4; Goldner's trichrome) of replanted incisor treated with topical rhBMP-12 showing functionally oriented fibers inserted into native cementum in the cervical (a/b; b polarized light) and into newly formed and native cementum in the alveolar crest region (c/d; d polarized light). Limited areas of root resorption. Limited ankylosis at arrowhead.

Replanted root-planed teeth

In the replanted teeth, root planed with intent to remove all PDL fibers and the native cementum and root conditioned with citric acid, no dramatic differences were found between those treated with rhBMP-12 or buffer control (Figs. 4 and 5). Newly formed, functionally oriented PDL fibers were observed along the root surface however the density and location of fibers exhibited a high degree of variability for both treatments.

A limited number of cases showed small areas of root resorption of surface erosion character while most of the teeth presented with severe undermining root resorption. Large areas of the root surface showed signs of new cementum formation irrespective of treatment protocol. Limited areas of residual native cementum were seen as well. Ankylosis was observed in a few teeth.

Replanted teeth with intact PDL versus root-planed teeth

Variable PDL fiber density and orientation was observed without remarkable differences between teeth with an ''intact'' PDL and teeth instrumented to remove the PDL and cementum. New cementum formation was a consistent finding for both groups where the native cementum had been removed mechanically or due to erosion/resorption.

Teeth replanted with ''intact'' PDL showed limited areas of root surface erosion or resorption of the native cementum. In contrast, teeth depleted of the PDL and native cementum and subsequently citric acid surface demineralized presented with much larger areas of root surface erosion and, in most cases, undermining root resorption sometimes compromising the entire structure of the tooth.

Discussion

The objective of this pilot study was to evaluate the effect of rhBMP-12 on regeneration of the PDL following tooth replantation. Six, young adult, male Hound Labrador mongrels were used. Maxillary and/or mandibular incisor and premolar teeth were extracted and the PDL was either left ''intact'' or removed by root planing. The teeth were immersed in a rhBMP-12 or a buffer (control) solution for 10 min and were then replanted. Three animals received topically applied rhBMP-12 or a buffer

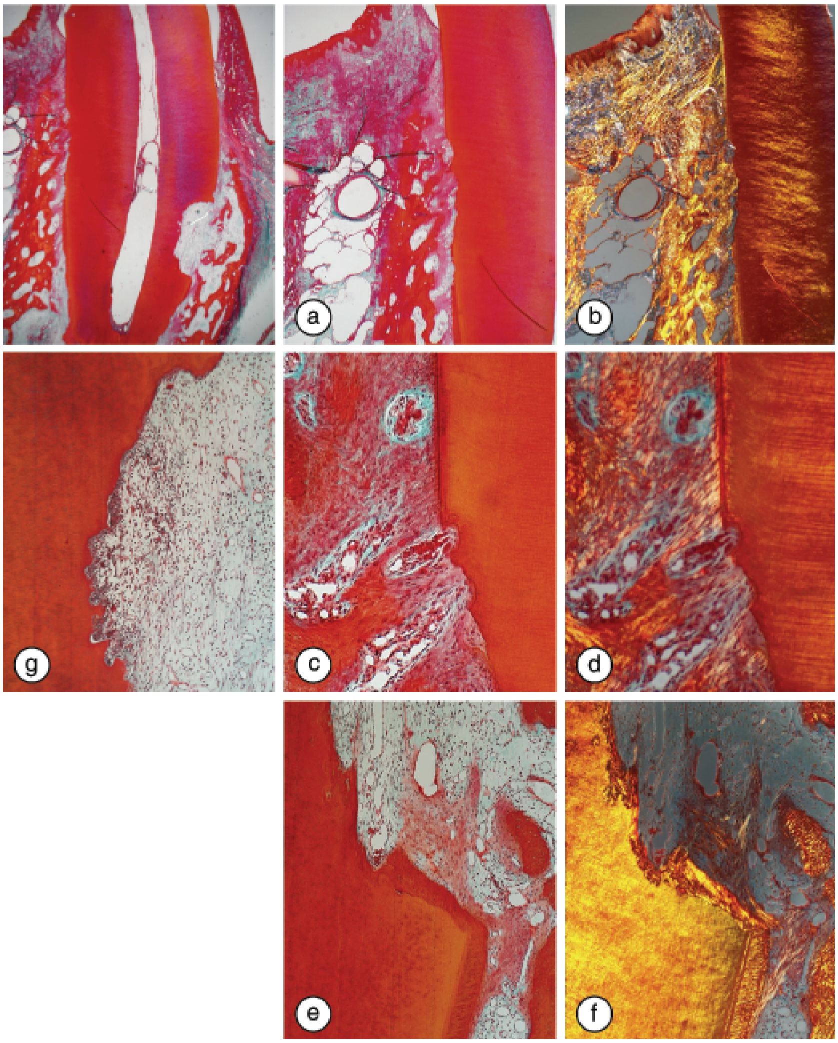

Fig. 3. Photomicrographs (original magnification × 2.5 and × 4; Goldner's trichrome) of replanted incisor (control) showing functionally oriented fibers inserted into new cementum, limited areas of root resorption, and no evidence of ankylosis. Native and newly formed cementum may be observed on the root surface. Bone metabolic activity (bone formation/ remodeling) may be observed in limited areas along the root surface (a/b and c/d; b and d polarized light). Evidence of new cementum formation with inserting PDL fibers may be observed in the area of root resorption (e/f; f polarized light). The area of root resorption also exhibits an inflammatory infiltrate. Cementum formation cannot be observed in this area (g).

control applied to teeth with ''intact'' PDL. Remaining 3 animals received rhBMP-12 or the buffer control applied to teeth instrumented to remove the PDL and the native root cementum and then surface demineralized with citric acid. Animals were euthanized at 8 weeks postsurgery and block biopsies of the teeth processed for histopathologic analysis.

The two experimental groupings, intact PDL and depleted PDL including citric acid root conditioning, showed various amounts of new functionally oriented PDL fibers with unremarkable differences between groupings or treatments, i.e., rhBMP-12 versus buffer control. It appears that intra-alveolar tissue resources along the root surface may play a role in the re-establishment of the PDL. This observation corroborates that of previous studies of replanted teeth in canine and non-human primate models (Løe & Waerhaug 1961, Polson & Proye 1982, 1983).

Under certain circumstances teeth depleted of the PDL by root planing do not posses the ability to re-establish a fibrous attachment. As a consequence, the healing is characterized by a downgrowth of the epithelium along the instrumented root surface (Proye & Polson 1982a, b). In this study, teeth with depleted PDL were also root conditioned with citric acid before replantation. This procedure will remove a surface smear layer disclosing the dentinal tubules and expose intra- and inter-tubular collagen fibers (Baker et al. 2000). Teeth receiving this treatment have been shown to reestablish a connective tissue fiber attachment to the root surface (Polson & Proye 1982, 1983). Early healing observations of the forming attachment have revealed arcade-like fibrin structures attached to the root surface. Late healing observations have shown connective tissue fibers attached to the root suggesting a critical role for fibrin clot adsorption/ adhesion to the root surface for the regeneration of the PDL. Other reports with similar or different methodologies have shown analogous outcomes (Hanes et al. 1985, Hanes & Polson 1989).

The location of the newly formed fibers in this study was characteristic. The fibers were never observed in areas of apparently active root surface erosion/resorption where an inflammatory infiltrate was evident. Rather, the fibrous attachment, when present, was observed in areas of apparently completed root surface erosion/resorption without an inflammatory process. It appears reasonable to speculate that inflammatory processes associated with root surface erosion/resorption may inhibit the differentiation/proliferation of cells deputed to establish a fibrous attachment to the root.

In this study, evidence of new cementum formation was common both in areas of root erosion/resorption in replanted teeth with an ''intact'' PDL as well as in teeth in which the PDL had been removed by instrumentation. Similar to that observed for the new attachment, cementum formation seemed inhibited in presence of inflammatory processes. The quantity, morphology and location of newly formed cementum did not appear different between the treatment conditions and in most cases apparently composed by a single layer of cementoblasts. It appeared that newly formed cementum showed less functionally oriented fibers compared to the native cementum. However, due to the observational nature of this study and the limited sample size, a histometric analysis was not performed to support this observation.

Teeth depleted of their PDL exhibited more advanced patterns of root

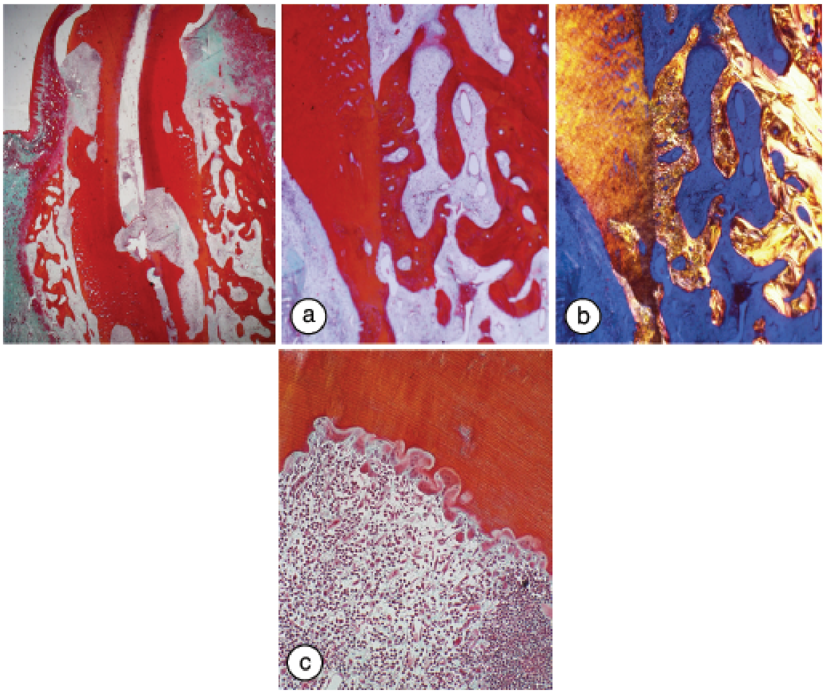

Fig. 4. Photomicrographs (original magnification × 2.5 and × 4; Goldner's trichrome) of replanted incisor depleted from its periodontal ligament and treated with root surface demineralization and topical rhBMP-12 showing limited presence of functionally oriented fibers inserting into new cementum, limited areas of ankylosis (a/b; b polarized light), and severe root resorption (c). New cementum formation may be observed on areas of the root surface exhibiting surface erosion/resorption with limited presence of PDL fibers. Inflammatory infiltrates may be observed in the area of advanced root resorption including evidence of dentinoclastic activity (c).

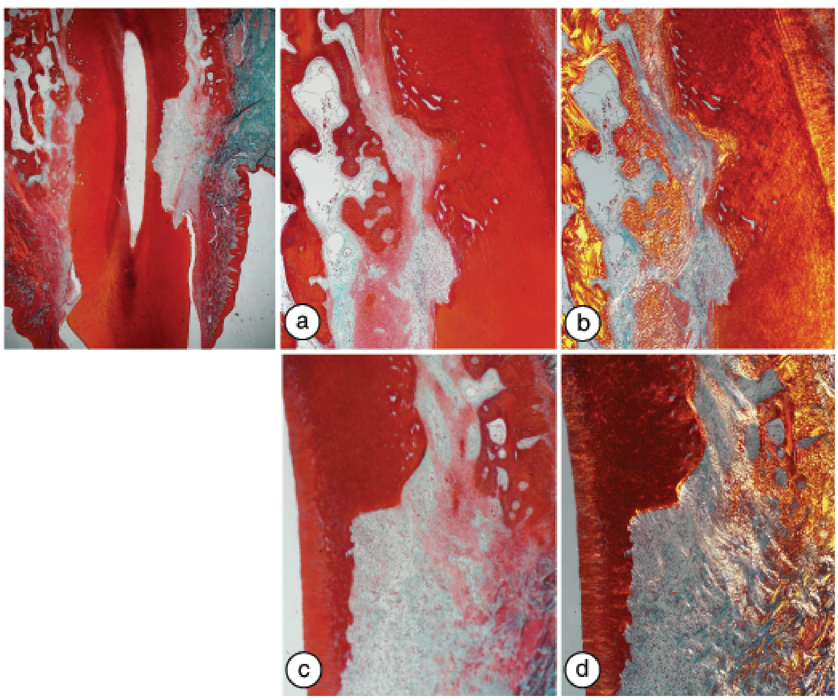

Fig. 5. Photomicrographs (original magnification × 2.5 and × 4; Goldner's trichrome) of replanted incisor (control) depleted from its periodontal ligament and treated with root surface demineralization showing functionally oriented fibers inserting into new cementum, severe root resorption, some residual cementum, but no evidence of ankylosis. New cementum is covering large areas of the root surface (a/b and c/d; b and d polarized light) and may also be observed in areas of severe resorption with inserting PDL fibers (c/d).

resorption than replanted teeth with an ''intact'' PDL. It appears that the PDL in spite of the relatively long extraoral exposure protected these teeth from resorptive processes within the 8-week healing interval. Possibly the native PDL maintained a population of cells that may have altered the healing patterns and/or the PDL per sé provided a buffer zone that may have influenced healing. It remains to be shown whether the citric acid root conditioning may advance the resorptive processes. Observations from other studies may suggest that citric acid root conditioning may not essentially promote root resorption since similar healing patterns as herein has been observed following replantation of root planed teeth without citric acid root conditioning (Løe & Waerhaug 1961).

Limited areas of ankylosis were found in this study compared to previous reports (Løe & Waerhaug 1961). Apparent differences between the present and previous studies relative to presence and extent of ankylosis may relate to the 8-week healing interval; however, it has been suggested that ankylosis may establish within 4 weeks following tooth replantation (Løe & Waerhaug 1961). In the present study, areas of complete maturation of the ankylotic union between the root surface and the alveolar bone as well as areas exhibiting earlier stages of ankylosis were observed. The earliest stage was represented by condensations of granulation tissue adjacent to the root surface while more mature sites showed osteoid formation. Still other sites were represented by ankylotic bone exhibiting features of woven bone. Interestingly, areas of root erosion/resorption did not appear more susceptible to ankylosis than areas exhibiting an intact root surface.

No dramatic differences were found between teeth receiving rhBMP-12 and the buffer control. Application of rhBMP-12 did not have an apparent effect on new cementum and PDL formation in the tooth replantation model. Moreover, rhBMP-12 did not increase nor did it decrease the apparent presence and extent of ankylosis along the root surface compared to control. Several previous studies have shown that rhBMP-2 in various carrier systems may significantly influence regeneration of alveolar bone in periodontal defects whereas regeneration of cementum appears compromised by ankylosis

(Sigurdsson et al. 1996, Wikesjö et al. 1999, 2003a, b, Selvig et al. 2002). Moreover, observed cementum regeneration was not accompanied by formation of a PDL. In a parallel study evaluating surgical implantation of rhBMP-12 and rhBMP-2 into large supraalveolar periodontal defects, it was observed that rhBMP-12 but not rhBMP-2 may have a significant influence on re-establishment of the PDL (Wikesjö et al. 2003b). The observations in the present study could not discern any such effect by rhBMP-12. Possibly the residual native PDL in the periodontal defect model may have influenced healing to support regeneration of the PDL.

Conclusion

The observations made in this study do not support the use of topical rhBMP-12 to support the reestablishment of the PDL including regeneration of cementum and functionally oriented fibers, and to prevent ankylosis and root resorption following replantation of teeth.

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