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Effect of platelet-derived growth factor-BB on root resorption after reimplantation of partially denuded tooth in dog

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Abstract – The prognosis for a reimplanted tooth depends largely on the condition of the root. Platelet-derived growth factor (PDGF)-BB has been shown to regenerate periodontal tissue in animal and human clinical studies. However, information regarding the effect of PDGF-BB on tooth reimplantation is limited. The objective of this study was to investigate the effect of PDGF-BB on root resorption after reimplantation of a partially denuded tooth in dog. A total of 15 healthy female beagle dogs were used. Mandibular third and fourth premolars were endodontically treated and then extracted as atraumatically as possible. The coronal portion of each root was carefully scaled and planed. The roots on the right side of the mandible were treated with PDGF-BB and reimplanted, while the roots on the left side served as controls. After 2, 4, or 8 weeks, specimens were collected and processed for histopathological examination. By the 4th week after reimplantation, new periodontal ligament (PDL)-like tissue had formed around the PDGF-BB-treated root surfaces and new bone. By the 8th week, healing of the PDGF-BB-treated roots was characterized by newly formed PDL with inserting attachment formation. In contrast, control roots showed multiple areas of replacement resorption. Immunohistochemical staining of proliferating cell nuclear antigen (PCNA) performed at 2 weeks after reimplantation showed that the number of PCNA-positive cells in the connective tissue area was statistically significantly greater in the PDGF-BB-treated group than in the control group ($P < 0.001$). The application of PDGF-BB resulted in a significantly lower occurrence and extent of root resorption and ankylosis. These results suggest that the use of PDGF-BB reduces occurrence of ankylosis and root resorption in tooth reimplantation.

Tooth reimplantation is an alternative treatment modality for avulsed teeth or teeth with poor or hopeless prognosis where conventional or surgical endodontic treatment(s) are not possible. Severe attachment injury on root surfaces of reimplanted teeth results in extensive necrosis of periodontal ligament (PDL), which could lead to ankylosis and replacement resorption with eventual tooth loss (1).

One reimplantation study using monkey incisors revealed that periodontal tissue can be regenerated as long as viability of PDL cells was maintained (1). Atrizadeh et al. (2) showed that injury to and necrosis of PDL from the cementum to the alveolar bone induced ankylosis. Melcher (3) suggested that PDL cells and their progeny have the capacity to inhibit osteogenesis. When cells proliferating in damaged PDL are derived from non-PDL cells, root resorption and ankylosis may occur. To delay or prevent the root resorption and increase the long-term success rate of avulsed teeth, treatment modalities including fluoride (4), sodium alendronate (5), dexamethasone (6), enamel matrix derivatives (7–9), triamcinolone (10), and basic fibroblast growth factor (FGF-2) (11) were tested. Further research is required on improving periodontal tissue healing in tooth reimplantation.

Periodontal ligament fibroblasts play a critical role in regeneration of the periodontium. Early recruitment and restoration of fibroblasts in periodontal lesions and acceleration of PDL formation are of vital importance in successful regeneration of periodontal tissue (12–14). Polypeptide growth factors are a class of molecular biological mediator that regulates the proliferation, differentiation, and matrix synthesis of nearly all cell types (15). Platelet-derived growth factor (PDGF)-BB has been found to play an important role in a number of fibroblast activities such as cell proliferation, chemotaxis, and collagenous protein synthesis, both in PDL and other types of connective tissue (16, 17). PDGF-BB may contribute to periodontal regeneration, and its clinical applications in various forms of periodontal treatment have been shown to be effective (18–22). With regard to the use of PDGF-BB in reimplantation, Ninomiya et al. (23), using a canine model, reported that PDGF-BB may be effective in regeneration of the PDL and cementum.

When added to preservation media containing insulin growth factor, PDGF-BB demonstrated mitogenic and clonogenic effects on PDL fibroblasts (24). However, information regarding the effect of PDGF-BB on the periodontal healing and root resorption of reimplanted tooth is limited.

The objective of this study was to investigate the *in vivo* effects of PDGF-BB on periodontal healing following reimplantation of partially denuded root, with a particular focus on the root resorption.

Material and methods

Experimental animals

Fifteen healthy female beagle dogs (8–12 kg) were used in this study. All experiments were performed in accordance with the Guidelines for the Treatment of Experimental Animals at Tokyo Dental College (No. 222206).

Surgical procedure

The animals were placed under general anesthesia with sodium pentobarbital (Somnopentyl®; Kyoritsu Seiyaku, Tokyo, Japan) at a dose of 35 mg kg^{-1}. One week prior to surgical intervention, plaque control consisting of brushing and application of 0.2% chlorhexidine gluconate solution was given. Supragingival scaling was also performed.

All surgical procedures were performed with local infiltration anesthesia (2% xylocaine, 1:80 000 adrenaline). Tooth reimplantation was performed as follows: on the day of reimplantation, after isolation with a rubber dam, third and fourth mandibular premolars (3P3 and 4P4) were endodontically treated to prevent inflammatory root resorption from root canal infection. After endodontic access cavities were produced, the root canals were biomechanically prepared using K- and H-type files. During instrumentation, the root canals were irrigated with 10% NaOCl and 3% H_2O_2 solutions and later dried with sterile paper points. Then, the root canals were filled with gutta-percha and hydroxyapatite-based root canal sealer (Finapec APC; Kyosera Co., Kyoto, Japan), using the lateral condensation method. A crestal incision was then made from the second to the fourth premolar. Buccal and lingual full thickness flaps were elevated. An apical-coronal cut was made in the crown and furcation areas of 3P3 and 4P4 with a fissure bur. The distal and mesial tooth segments were gently luxated with an elevator and extracted with forceps using rotary movements. No attempt was made to debride the socket walls. Each extracted tooth was held by its crown with the forceps and a notch prepared with a round bur in each root halfway between the cemento-enamel junction (CEJ) and the root apex (Fig. 1). The partially denuded tooth was prepared according to the method by Seshima et al. (11). The coronal portion of the area between the CEJ and the notch was carefully scaled and planed. During this procedure, the apical portion of the area between the root apex and the notch was kept moist by flushing the root with a sterile saline solution. In the experimental group, 180 μl of rhPDGF-BB solution (0.3 mg ml^{-1}; BioMimetic Therapeutics, Franklin, TN, USA) was applied to the coronal and apical portions of the roots and the sockets on the right side (3P and 4P: experimental group) of the mandible. The left side (P3 and P4: control group) was treated in an identical manner, but without application of PDGF-BB. The premolars (3P3 and 4P4) were reimplanted. The crowns were severed with a horizontal cut and removed, after which the submerged roots were completely covered with flaps and sutured (Fig. 2).

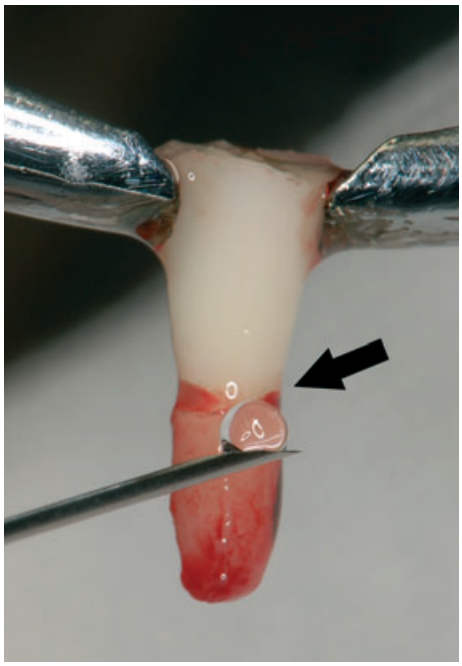

Fig. 1. Coronal portion of root was scaled and planed as far as the notch (arrow) prepared with a round bur in each root halfway between the cemento-enamel junction. For the experimental group, platelet-derived growth factor-BB was applied to cover the root.

All dogs received antibiotics for 7 days after surgery. They also received daily plaque control consisting of gentle wiping of the adjacent teeth with gauze soaked with 0.2% chlorhexidine gluconate solution to maintain healthy gingival conditions.

Histological processing

The animals were euthanized with an intravenous overdose of sodium pentobarbital at 2, 4, or 8 weeks following the procedure described earlier. The jaw of each animal was removed and specimens containing the experimental areas placed in buffered formalin. Specimens were decalcified with 10% ethylenediamine tetraacetic acid (EDTA) (Wako, Tokyo, Japan) over 3 months. The specimens were then dehydrated in ethanol, embedded in paraffin, and serially sectioned to 5 μm in thickness in the bucco-lingual direction. The sections were then stained using hematoxylin–eosin (H & E). Azan-Mallory's connective tissue stain was applied to some sections.

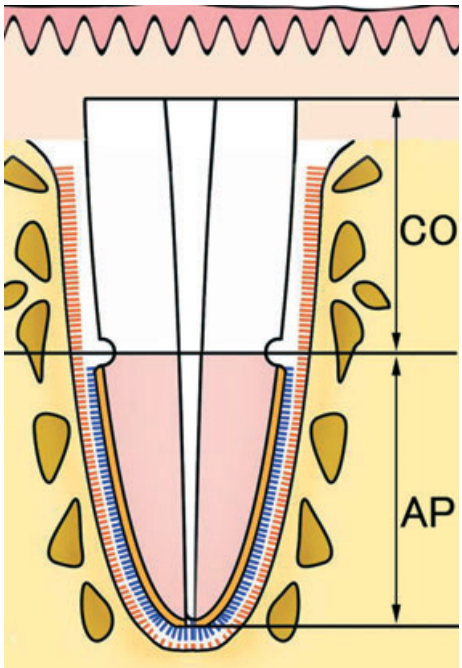

Fig. 2. Diagram illustrating reimplantation. CO, coronal portion: this area was scaled and planed. AP, apical portion; in this area, periodontal ligament was retained.

Immunohistochemical staining

Immunohistochemical staining of proliferating cell nuclear antigen (PCNA) was performed using an immunoperoxidase staining kit [Histofine SAB-PO (MULTI); Nichirei, Tokyo, Japan]. The sections were incubated with mouse anti-PCNA primary antibody (PC-10; DAKO Corporation, Carpinteria, CA, USA) at a dilution of 1:100. Next, each section was incubated with biotinylated secondary antibody and streptavidin peroxidase reagents. The presence of peroxidase complexes was visualized by 3-3′ diaminobenzidine tetrahydrochloride (0.1 mg ml^{-1}) solution with 0.65% H_2O_2. Sections were counterstained with Mayer's hematoxylin. A brown coloration indicated a PCNA-positive reaction.

For quantitation of PCNA-positive cells, magnification was set at ×100. In each section, three 0.12-mm^2 (0.2 × 0.6 mm) areas were randomly selected within a field of connective tissue in the middle portion of root and submitted to quantitative analysis, as described previously (25).

Histomorphometric assessment

Three sections (the central part of the tooth and 200-μm sections on either side of that area) from each reimplanted tooth were used for morphometric evaluation, as described previously (11). The analysis of the root resorption was performed based on a classification suggested by Andreasen (26) with modification. Briefly, the resorption patterns were classified as the following: (i) dentin resorption, condition where bowl-shaped areas of resorption, involving both cementum and dentin was the characteristic feature, and (ii) replacement resorption, condition where direct contact between the alveolar bone and the mineralized root substance (ankylosis) was the characteristic feature. The extent of each resorption pattern on the root periphery was expressed as a percentage of the total length of the peripheral contour of the root surface, that is, the distance between the distal and mesial portions of the CEJ, according to the method described by Katayama et al. (27).

All measurements were taken with an image analysis system comprising a light microscope (Olympus BX51 Microscope; Olympus, Tokyo, Japan) with a 4 × objective equipped with a digital camera (HC-2500; Fujifilm, Tokyo, Japan). A computer (Precision Work Station 220 System; Dell, Round Rock, TX, USA) employing an image processing software (Image Pro Plus v 3.0; Media Cybernetic, Silver Spring, MD, USA) was used.

Statistical analysis

For statistical analysis, one section was randomly selected among specimens from each of five dogs used for each experimental period (2, 4, and 8 weeks). Student's *t*-test was used to compare the differences between experimental and control groups. *P*-values < 0.05 were considered statistically significant.

Results

Clinically, soft tissues healed uneventfully in all dogs tested. The crest of the ridge was consistently covered with non-inflamed keratinized mucosa.

Histology

Coronal portion of root

Week 2. Experimental (PDGF-BB-treated) group: At 2 weeks after reimplantation, spaces apical to the notch (Fig. 3a) and coronal to the notch (Fig. 3b) were filled with connective tissue composed of spindle-shaped cells and capillaries. In the area coronal to the notch, collagen bundles had been inserted into the adjacent alveolar bone (Fig. 3c, d).

Control group: Replacement resorption was observed at a number of sites in the area coronal to the notch (Fig. 4a). Cell-rich fibrous connective tissue had invaded spaces in the PDL from adjacent bone marrow. Collagen bundles in the remaining PDL on the alveolar side were sparsely and randomly distributed throughout the connective tissue (Fig. 4b).

Week 4, 8. Experimental group: At 4 weeks after reimplantation, cementum formation had advanced as far as the coronal portion of the root surface (Fig. 5a). As a result, new PDL had formed around the root surfaces and new bone. Furthermore, numerous cells in the PDL were located in connective tissue along the newly formed cementum surface, which was frequently lined with cementoblast-like cells. High-power magnification of the area coronal to the notch demonstrated collagen fibers inserting directly into newly formed cementum (Fig. 5b).

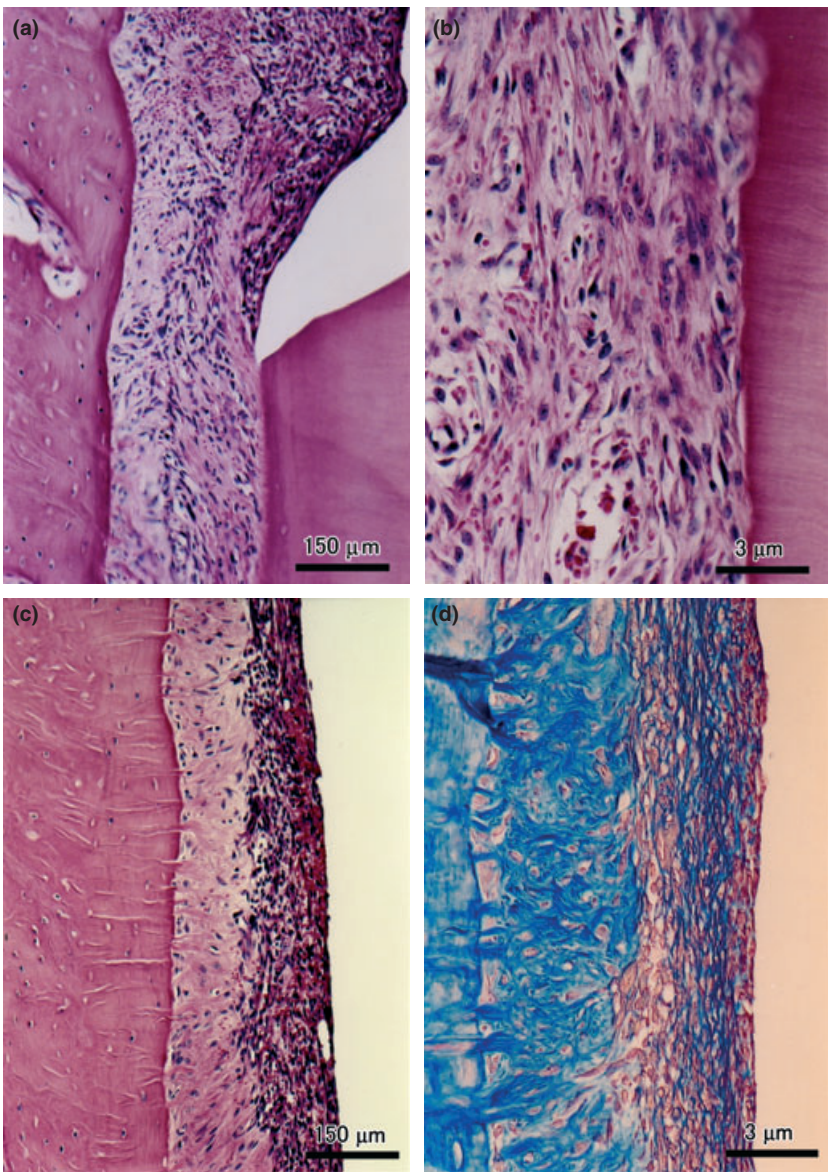

Fig. 3. Representative photomicrographs of experimental (platelet-derived growth factor-BB) group: week 2. (a) periodontal ligament (PDL) in the notch area shows dense distribution of connective tissue, which appeared to be continuously extending from the PDL in the area apical to the notch. (b) In the area coronal to the notch, newly formed connective tissue composed of spindle-shaped cells and capillaries is observed near the root surface. (c, d) In the area coronal to the notch, collagen bundles of PDL had been inserted into the adjacent alveolar bone. (a, b, and c: H & E staining, d: Azan-Mallory's staining).

At 8 weeks after reimplantation, alveolar bone was detected at the periphery of the reimplanted teeth (Fig. 5c). The cells were lined tightly along the new cementum and bone (Fig. 5d).The PDL collagen fibers were inserted into the new cementum and adjacent bone, thereby reestablishing an oriented attachment apparatus. The replacement resorption was rarely observed. In areas with ankylosis, the resorbed root surface was in close proximity or in direct contact with the alveolar bone.

Control group: At 4 weeks after reimplantation, in the area coronal to the notch, replacement resorption at the root surface and discrete bone formation in close proximity to the root surface were observed, showing signs of dental ankylosis (Fig. 6a). Formation of new bone in close contact with the PDL was observed in the coronal portion, indicating the potential for dental ankylosis to develop to a more advanced stage.

At 8 weeks after reimplantation, in the area coronal to the notch, all roots examined showed multiple areas of resorption. Replacement resorption was a frequent feature. In areas of ankylosis (Fig. 6b), the resorbed dentin surface was in direct contact with bone.

Apical portion of root

In our preliminary experiment, we performed toluidine blue staining on the root after extraction and after the root surface debridement of coronal area. PDL was

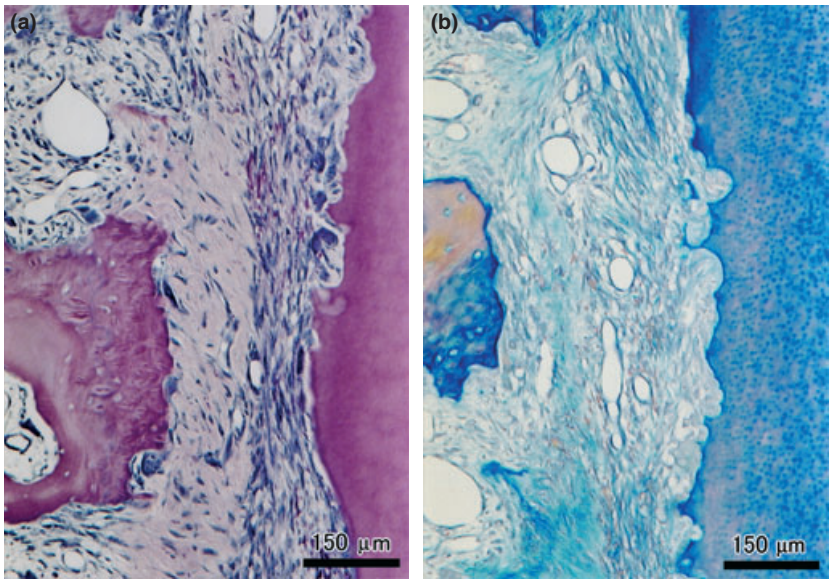

Fig. 4. Representative photomicrographs of control group: week 2. (a) In the area coronal to the notch, small resorption cavities and osteoclast-like cells are observed at the root surface. Cell-rich fibrous connective tissues from the adjacent bone marrow invaded periodontal ligament. (b) Collagen fibers were sparsely and randomly distributed throughout periodontal tissue. (a: H & E staining, b: Azan-Mallory's staining).

uniformly observed in the apical portion of root, prior to reimplantation (data not shown).

Specimens from the experimental and control groups showed similar patterns of healing throughout the observation time points. By the 8th week, alveolar bone had increased to surround the entire root of the reimplanted tooth, and new PDL was evident between the root surface and bone. Cementoblast-like cells were aligned along the surface of the cementum. Root resorption was rarely observed.

Immunohistochemical observation

In the experimental group, the PCNA-positive cells were evident in the connective tissue area near the alveolar bone (Fig. 7a). In the control group, few PCNA-positive cells were observed (Fig. 7b). The number of PCNA-positive cells was statistically significantly greater in the experimental group than in the control group ($P < 0.01$) (Table 1).

Histomorphometric assessment

The results of histomorphometric measurement in the coronal portion of the root at 4 and 8 weeks after reimplantation are presented in Table 2. Root resorptions extending to the dentin area were already observed after 4 weeks. At both observation periods, the occurrence of dentin or replacement resorption in the experimental group was significantly lower than that in the control group ($P < 0.01$).

Discussion

In vitro (28) and animal or human clinical (20, 21, 29, 30) studies on PDGF-BB demonstrated its effects on the regeneration of periodontal tissue. PDGF-BB possesses a potent ability to stimulate proliferation and chemotaxis of PDL cells (18, 31) and plays a pivotal role in the periodontal wound healing. This prompted us to investigate the effect of PDGF-BB on periodontal tissue healing in tooth reimplantation.

In the present study, the coronal portion of the reimplanted root was scaled and planed to remove PDL and cementum during the extra-alveolar period, as a model for root surface damage. The results revealed that the reimplanted root surface achieved favorable and predictable healing in the experimental group. The application of PDGF-BB induced formation of new cementum on the root surface and new PDL in the previously denuded coronal area, thus restoring periodontal integrity following reimplantation. In contrast, in the control group, the healing was frequently characterized by ankylosis. This finding is in agreement with the study by Ninomiya et al. (23). The favorable healing process and reduction in replacement resorption seen with the application of PDGF-BB suggest that it is effective and important in the promotion of normal PDL reconstruction in avulsed teeth.

Newly formed cementum was observed in the area coronal to the notch prepared. This may indicate that the previously exposed dentin surface was covered with cells that would constitute intact PDL. The healing outcome of periodontal therapy depends on the nature of the cells that migrate to the wound (32). In the present study, connective tissue fibers near the notch area appeared to be derived, at least in part, from PDL remnants in the apical portion of the root. In the apical portion of the root, no obvious difference in periodontal healing was observed between the experimental and control groups. However, PDL cells remaining in the apical portion were considered to be stimulated by PDGF-BB and appeared

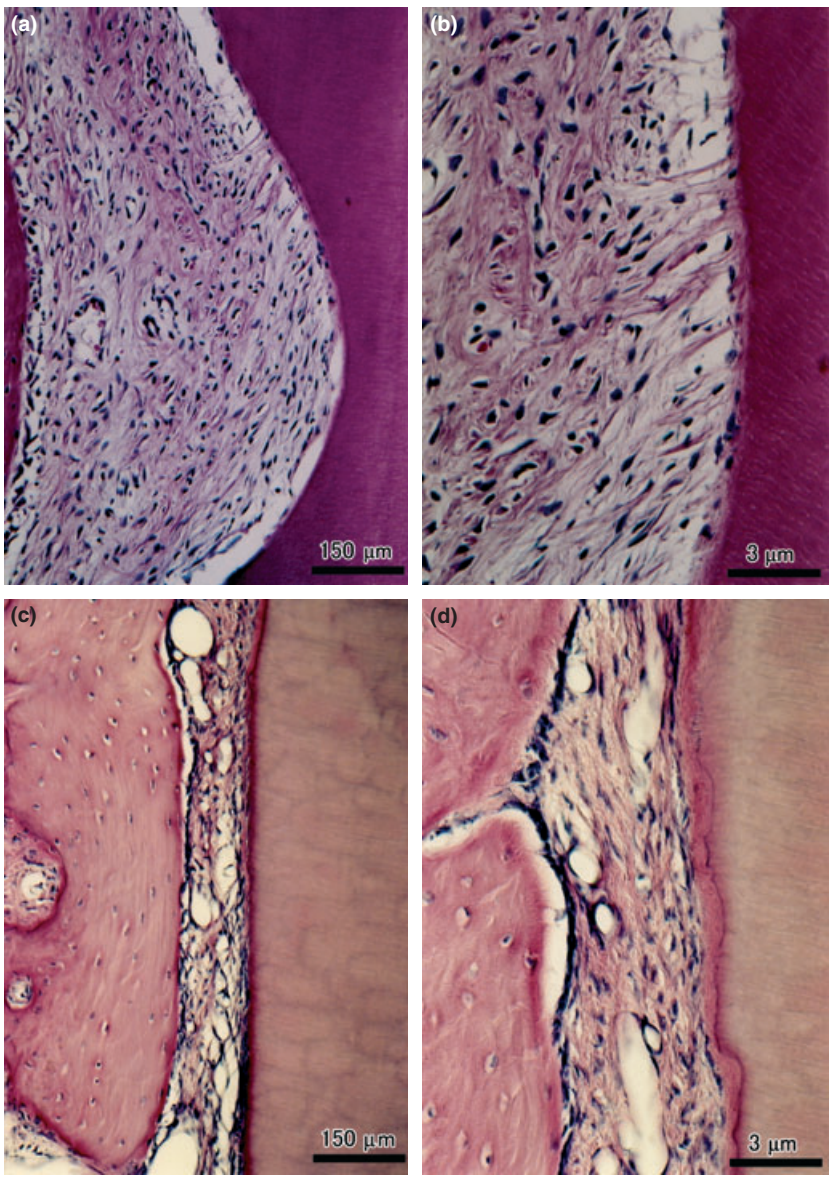

Fig. 5. Representative photomicrographs of experimental (platelet-derived growth factor-BB) group: week 4 (a, b) and week 8 (c, d). (a) Newly formed periodontal ligament (PDL)-like tissue is observed between root surface and new bone in the notch area. New cementum is observed on the root surface. (b) In the area coronal to the notch, cementoblast-like cells are observed at newly formed cementum surface. PDL fibers are inserted into newly formed cementum. (c) In the area coronal to the notch, intact PDL is observed at root surface of reimplanted tooth. (d) Newly formed cementum is observed at dentin surface. (a, b, c, and d: H & E staining).

to proliferate into the coronal area of the root during the process of wound healing. In contrast, PDL cells in the control group appeared only to migrate rather than proliferate.

In the present study, we did not use a carrier or vehicle in topical application of PDGF-BB, mainly because of the limitation of space in relation to reimplantation. Although exact data regarding the pharmacologic clearance of PDGF-BB in the periodontal lesion are not available, in a dermal wound bed, the half-life of PDGF-BB applied in aqueous buffer is reported to be approximately 1 h (33) to 4 h (34), with only negligible activity retained after 24 h (33). Thus, it is speculated that PDGF-BB's effect on cell differentiation is inhibited transiently and proliferation enhancement is emphasized, which could result in an increase in fibroblast number in PDL space during early stage of reimplantation.

Despite the relatively short pharmacologic half-life, its biological effect on tissue repair could last up to 20 days postwounding (33, 34). PDGF-BB has been shown to promote proliferation of macrophages, synthesis of extracellular matrices, and neoangiogenesis (34). It is possible that the stimulated macrophages at the healing site may lead to the secondly production of growth factors including PDGF, transforming growth factor-β, and FGF-2 (23, 35), contributing to the continued biological effect on the healing site.

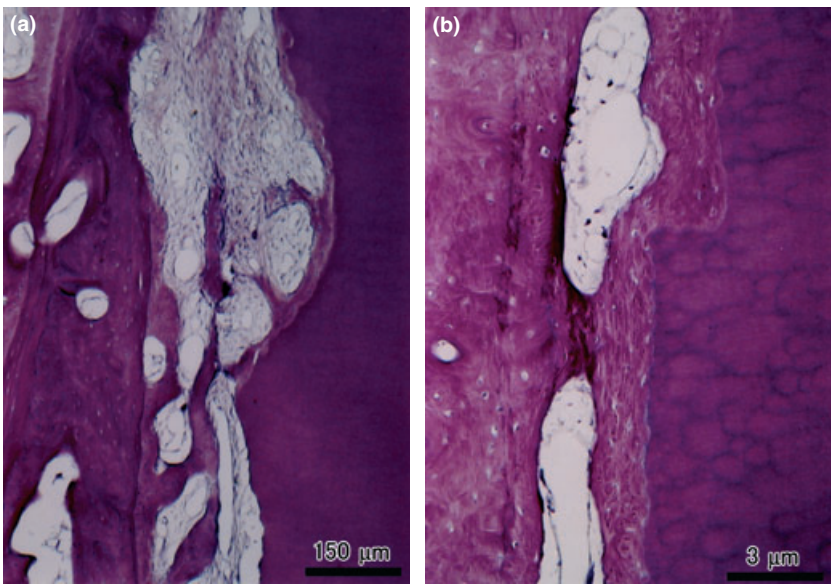

Fig. 6. Representative photomicrographs of control group: week 4 (a) and week 8 (b). (a) In the area coronal to the notch, newly formed bone tissue is observed at the root surface, showing signs of dental ankylosis. (b) Substantial ankylosis covering areas of root resorption is observed.

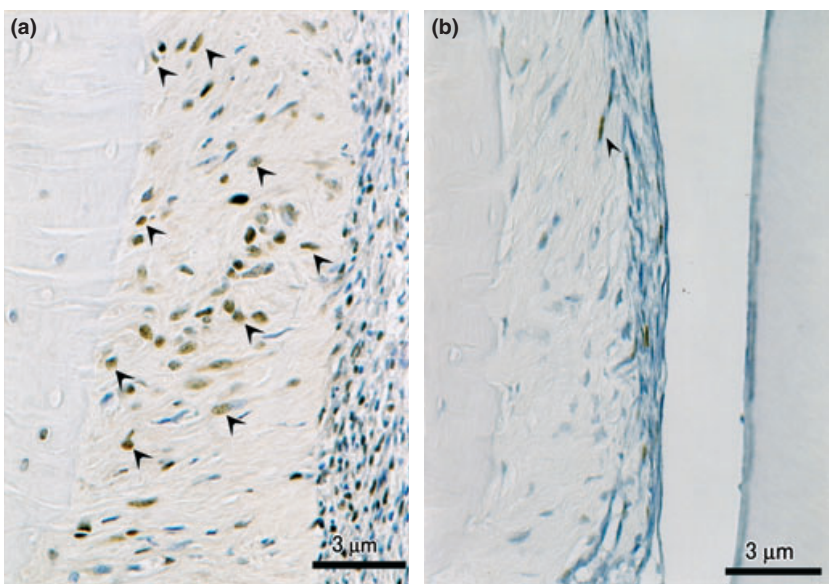

Fig. 7. Representative photomicrographs of immunohistochemical staining of proliferating cell nuclear antigen (PCNA) at week 2. (a) Experimental (platelet-derived growth factor-BB treated) group; PCNA-positive cells (arrow) are evident in the connective tissue area near the alveolar side. (b) control group; very few PCNA-positive cells are observed.

In our preliminary experiment, PDL on the alveolar bone surface in the control group appeared to be necrotic at 1 week after reimplantation (data not shown). At week 2, very few PCNA-positive cells were observed, and osteocyte-like cells accumulated around residual PDL on the alveolar bone side. Signs of replacement resorption and ankylosis were frequently observed in the coronal region during the healing process. These findings are consistent with data from an earlier *in vivo* reimplantation study (11). The formation of mineralized tissue on the root surface with necrotic PDL follows proliferation of cell-rich fibrous connective tissue from the adjacent bone marrow space (1, 2). Replacement resorption occurs after proliferation of cell-rich fibrous connective tissue from the adjacent bone marrow to the dentin surface (36). The findings from the control group indicated that PDL on the alveolar bone side, which could prevent attachment of bone-derived osteocytes and contact with the root surface, was inactive, thus threatening the survival of the reimplanted tooth. PDGFs exert multiple biological responses, including mitogenesis and chemotaxis of PDL fibroblasts (18), cementoblasts (37),

Table 1. PCNA-positive cells in the connective tissue area coronal to the notch, at week 2

	Experimental	Control
Number of positive cells	149.7 ± 10.9**	53.0 ± 8.9

PCNA, proliferating cell nuclear antigen; PDGF, platelet-derived growth factor.
Experimental; PDGF-BB treated.
Values given as mean (%) ± standard deviations (n = 15).
**P < 0.001, Student's t-test.

Table 2. Histomorphometric assessment of the area coronal to the notch at weeks 4 and 8

	Dentin resorption		Replacement resorption	
	Experimental	Control	Experimental	Control
4 weeks	3.1 ± 1.8	8.8 ± 4.6	0.6 ± 0.8*	16.4 ± 6.1
8 weeks	2.1 ± 0.78	26.0 ± 6.4	2.0 ± 0.8*	20.8 ± 0.8

PDGF, platelet-derived growth factor.
Experimental; PDGF-BB treated.
Values given as means (%) ± standard deviations (n = 5).
*P < 0.01, by Student's t-test.

and osteoblasts (38). Among these cells, Hock and Canalis (39) showed that the PDGF's effect on proliferation was most pronounced on fibroblast. In the experimental group, PDGF-BB treatment was considered to activate fibroblast-like cells on the alveolar bone side as well, because a number of PCNA-positive cells were observed near the bone. This may have prevented migration of cell-rich fibrous connective tissue from the adjacent bone marrow to the wound.

As an alternative strategy to limit the root resorption associated with late reimplantation, Lustosa-Pereira et al. (5) used alendronate, a substance that inhibits bone resorption through direct or indirect effects on the clasts, and found that it reduced the root resorption in rat. Although the exact effect of PDGF-BB on the clasts is still unclear, Kubota et al. (40) reported that osteoclasts/osteoclast precursors stimulated by receptor activator of nuclear factor-κB (NF-κB) ligand (RANKL) directly regulate osteoblastic differentiation with PDGF-BB and indicated that PDGF-BB is an important factor in bone remodeling. PDGF-BB may be a complex combination, including not only direct regulation but also indirect influences of growth factors or cytokines induced by PDGF from other types of cells (40). These complex properties of PDGF-BB may have contributed to the suppression of ankylosis, leading to periodontal healing, possibly regeneration.

In conclusion, the results of the present study suggest that PDGF-BB promotes healing of periodontal tissue and reduces occurrence of replacement resorption in reimplanted teeth. The use of PDGF-BB may improve clinical outcome in tooth reimplantation.

Acknowledgement

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