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# Regenerative effect of basic fibroblast growth factor on periodontal healing in two-wall intrabony defects in dogs

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***Abstract***

**Aim:** The aim of the present study was to evaluate the effect of a basic fibroblast growth factor (bFGF) candidate treatment on periodontal healing in two-wall intrabony defects in dogs.

**Materials and Methods:** Two-wall intrabony defects ($5 \times 5 \times 5$ mm) were created surgically on the distal and mesial sides of bilateral mandibular second and fourth premolars in four Beagle dogs. bFGF, enamel matrix derivative (EMD) and platelet-derived growth factor with $\beta$-tricalcium phosphate (PDGF/$\beta$-TCP) treatments, and sham-surgery (OFD) were rotated among the four defects in each animal, EMD and PDGF/$\beta$-TCP serving as benchmark controls. The animals were euthanized for radiographic and histologic evaluation at 8 weeks.

**Results:** Bone formation was significantly greater in the bFGF group ($4.11 \pm 0.77$ mm) than in the EMD ($3.32 \pm 0.71$ mm; $p < 0.05$) and OFD ($3.09 \pm 0.52$ mm; $p < 0.01$) groups. The EMD ($4.59 \pm 1.19$ mm) and PDGF/$\beta$-TCP ($4.66 \pm 0.7$ mm) groups exhibited significantly greater cementum regeneration with periodontal ligament-like tissue than the OFD group ($2.96 \pm 0.69$ mm; $p < 0.01$). No significant differences were observed between the bFGF and the PDGF/$\beta$-TCP groups in any of the histometric parameters.

**Conclusions:** The candidate bFGF treatment supported periodontal regeneration comparable with that of established benchmarks: EMD and PDGF/$\beta$-TCP.

Key words: basic fibroblast growth factor; enamel matrix derivative; intrabony defects; periodontal regeneration; platelet-derived growth factor; tricalcium phosphate

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**Conflict of interest and source of funding statement**

The authors report no conflicts of interest related to this study.
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Recently, on the basis of new understanding of the cellular and molecular biology of periodontal development and regeneration, tissue engineering technologies using scaffolds, growth factors, and cells or their combination have been developed (Taba et al. 2005). It has been demonstrated that amelogenin-like factors [e.g., enamel matrix derivative (EMD)] and growth factors [e.g., bone morphogenetic proteins (BMPs), insulin-like growth factor-1 (IGF-1), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), etc.] exert pleiotropic effects, stimulating key events required for periodontal regeneration, such as DNA synthesis, chemotaxis, differentiation, and matrix synthesis (Taba et al. 2005, Trombelli & Farina 2008).

bFGF is a cytokine found in various tissues, and is a member of the heparin-binding growth factor family. bFGF performs broad mitogenic and cell survival activities, and is involved in a variety of biological processes, including angiogenesis, wound healing, and embryonic development. Topical application of recombinant human bFGF (rhbFGF) appears to be a powerful



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pharmacological tool in dermal wound healing in humans (Klagsbrun & Moses 1999). Furthermore, bFGF has been reported to support periodontal regeneration effectively without ankylosis or epithelial down-growth in intrabony and furcation defects in preclinical models (Murakami et al. 1999, 2003, Takayama et al. 2001), and for clinical attachment gain and radiographic alveolar bone gain in humans (Kitamura et al. 2008).

The aim of the present study was to evaluate the effect of a bFGF candidate treatment on periodontal healing using a two-wall intrabony defect model in dogs.

## Materials and Methods

### Animals

Four healthy male Beagle dogs, 13–18 months old, weighing 12.0–14.0 kg, were used. The animals exhibited intact dentition with healthy periodontium. The procedures and protocol design (in part established by Kim et al. 2004) described here were approved by the ethical committee of the Animal Research Center of Kagoshima University, Japan (H19 no. 147).

### EMD

Emdogain®-Gel (Straumann, Basel, Switzerland), a premixed ready-to-use heat-treated Emdogain® dissolved in a propylene glycol alginate solution, was used.

### rhbFGF (bFGF)

Freeze-dried rhFGF-2 (Kaken Pharmaceutical Co. Ltd.) in a 3% hydroxypropyl cellulose 1000–4000 cP (HPC) (Wako Pure Chemical Industries Ltd., Osaka, Japan) solution prepared as a gel-like material containing 0.3% rhbFGF was used.

### rhPDGF-BB mixed with β-TCP (PDGF/β-TCP)

PDGF/β-TCP (GEM 21S®, BioMimetic Therapeutics, Franklin, TN, USA), each kit including 0.5 $cm^3$ of β-TCP particles (0.25–1.0 mm) and 0.5 ml of a 0.3 mg/ml PDGF/β-TCP solution, was used.

### Surgical protocol

One surgeon (Y. S.) performed all surgical procedures under general and local anaesthesia using aseptic routines. Medetomidine hydrochloride (0.05 ml/kg IM; Dormitor, Orion Corporation, Espoo, Finland) was administered for pre-medication. General anaesthesia was achieved using sodium thiopental (0.005 ml/kg IV; Ravonal, Tanabe Inc., Osaka, Japan) maintaining spontaneous breathing. Local anaesthesia was performed using lidocaine HCl/epinephrine (2%, 1:80,000; Xylocaine, Fujisawa Inc., Osaka, Japan). The mandibular third premolars were extracted to provide space for developing the two-wall intrabony defects and for flap management to allow wound closure for primary intention healing. Penicillin G (200,000 U IM) was administered for infection control. Two-wall intrabony defects were prepared on the mesial aspect of the mandibular fourth premolars and the distal aspect of the second premolars after a 10-week healing interval (Fig. 1). Briefly, sulcular incisions were performed along the distal aspect of the first molar and the mesial aspect of the first premolar, and a semi-lunar incision was performed from the distal angle of the fourth premolar to the mesial angle of the second premolar beyond the mucogingival junction. Following elevation of the mucoperiosteal flap, 5 × 5 × 5 mm (width × height × depth) two-wall intrabony defects were created using round and fissure burs with a sterile saline coolant. Cementum was removed using Gracey curettes and a chisel. Reference notches were made using a #1 round bur on the root surface at the base of the defects, at the cemento-enamel junction (CEJ) for histometric analysis, and on the crown surface to indicate the precise centre plane of the two-wall intrabony defects and to aid in optimal histologic processing.

### Wound management

Bilateral mandibular two-wall intrabony defects (16 in total), four defects per dog, received one of the following treatments each: EMD, bFGF, PDGF/β-TCP, or open flap debridement (OFD) as control. The experimental conditions were rotated between defect sites in subsequent animals (Table 1, Fig. 1). The root surfaces at defects to receive EMD were conditioned with a 35% phosphate acid gel (Ultra-Etch®, Ultradent Products Inc., South Jordan, UT, USA) for 15 s and then, along with the adjacent mucoperiosteal flaps, thoroughly rinsed with sterile saline. The EMD gel was then applied to the root surfaces, and the defects were filled up to the adjacent alveolar crest. The defects in the bFGF group were treated in the same fashion, but without root conditioning. Before the placement of PDGF/β-TCP, the β-TCP particles were

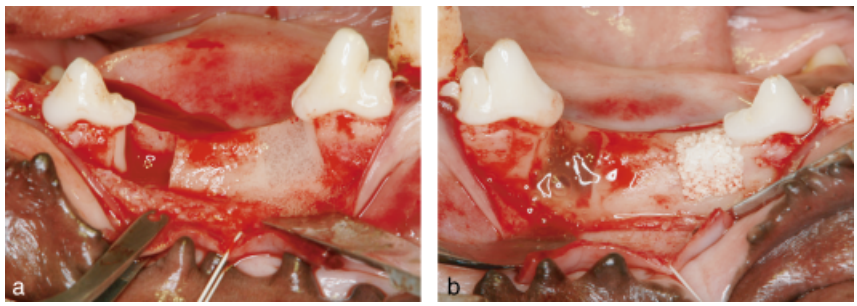


*Fig. 1.* Clinical photographs showing the surgically created and treated 2-wall intrabony defects. (a) Left: open flap debridement (OFD), right: basic fibroblast growth factor (bFGF). (b) Left: enamel matrix derivative (EMD), right: platelet-derived growth factor with β-tricalcium phosphate (PDGF/β-TCP).

*Table 1.* Experimental design scheme for four dogs

| Dogs | Tooth | | | |
|---|---|---|---|---|
| | LP2 | LP4 | RP2 | RP4 |
| 1 | PDGF/β-TCP | EMD | bFGF | OFD |
| 2 | EMD | PDGF/β-TCP | OFD | bFGF |
| 3 | EMD | OFD | bFGF | PDGF/β-TCP |
| 4 | OFD | bFGF | PDGF/β-TCP | EMD |

LP2, left second premolars; LP4, left fourth premolars; RP2, right second premolars; RP4, right fourth premolars; OFD, open flap debridement; EMD, enamel matrix derivative; bFGF, basic fibroblast growth factor; PDGF/β-TCP, platelet-derived growth factor with β-tricalcium phosphate.

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fully saturated with the rhPDGF solution and the PDGF/$\beta$-TCP construct was allowed to rest for approximately 10 min. The PDGF/$\beta$-TCP construct was then filled in from the bottom of the defect close to the residual bone crest with moderate pressure so as not to crush the particles. Care was taken during surgery to prevent mixing of the various gel-like materials investigated. The maxillary second, third, and fourth premolars were then reduced in height using a diamond disc bar to alleviate potential occlusal trauma from the maxillary teeth to the experimental sites postsurgery. A periosteal releasing incision was made to allow coronal repositioning of the flap, followed by suturing (Gore-Tex CV-5 Suture, W. L., Gore and Associates Inc., Flagstaff, AZ, USA), slightly coronal to the CEJ.

#### Postsurgical protocol

The animals were fed a soft diet (DKM, Oriental Yeast Co. Ltd., Tokyo, Japan) for 2 weeks. An analgesic of buprenorphine HCl (0.05 ml/kg IM; Lepetan, Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan) and an antibiotic (penicillin G, 200,000 U IM) were administered daily for 3 days. For plaque control, a 2% solution of chlorhexidine gluconate (Hibitane concentrate, Sumitomo Inc., Osaka, Japan) was used three times a week for 8 weeks postsurgery. The sutures were removed 2 weeks after surgery.

#### Radiographic recordings and histological processing

Intraoral radiographs were taken using dental films (Ultra-speed DF-57, Kodak Carestream Health Inc., Rochester, NY, USA) immediately and at weeks 4 and 8 postsurgery. Then, 8 weeks after surgery, the animals were euthanized by an overdose injection of sodium thiopental. All the defects, including the experimental and control sites, were then dissected along with the surrounding soft and hard tissues. The tissue blocks were fixed in 10% buffered formalin, trimmed according to the intraoral radiographs and the reference notches on the crown, and rinsed in phosphate-buffered saline. These samples were then analysed using a micro-computed tomography system (Scan Xmate-E080®, Comscantecno Corporation, Kanagawa, Japan) and software (TRI/3D VIEWER, Ratoc System Engineering Co. Ltd, Tokyo, Japan) for observing the bucco-lingual appearance (including the defect margin and root location in the alveolar bone) and for discerning the central portion of the defects to obtain the optimal histological sections. The samples were decalcified in a Plank–Rychlo solution for 3 weeks, dehydrated, and embedded in paraffin. Step serial sections of 6 $\mu$m thickness were then prepared along the mesio-distal plane, stained with haematoxylin/eosin at intervals of 90 $\mu$m, and examined under a light microscope (Eclipse E800, Nikon Inc., Tokyo, Japan).

#### Histometric analysis

All the specimens were analysed histometrically under a light microscope equipped with a computerized image system (Image-pro Plus Media Cybernetics, Silver Spring, MD, USA). For histometric analysis, three sections, approximately 90 $\mu$m apart, were selected from the most central area of each two-wall defect, identified by the length of the root canal and the reference notches as mentioned above. The mean value of each histometric parameter was then calculated for each site. The following parameters were measured by two masked examiners. Sixteen sections from all sites were read by one expert examiner (T. Y.) without calibration before the measurements. Forty-eight hours later, the same examiner read all 16 sections again to evaluate intra-examiner reproducibility. The same 16 sections were then read by the second examiner (K.T.) to assess the inter-examiner reproducibility. Intra- and inter- calibration of examiners was accepted at the 90% level.

1. Defect height (DH): distance between the apical extent of root planing and CEJ.
2. Apical extension of the junctional epithelium (JE): distance between the apical extension of the junctional epithelium and CEJ.
3. Connective tissue adhesion (CT; without cementum): distance between the apical extent of the junctional epithelium and the coronal extent of newly formed cementum.
4. New bone formation (NB): distance between the apical extent of root planing and the coronal extent of newly formed alveolar bone along the root surface.
5. New cementum formation (NC): distance between the apical extent of root planing and the coronal extent of newly formed cementum on the denuded root surface.

#### Statistical analysis

The means and standard deviations of each parameter were calculated for each treatment group using the values obtained from the subject animals ($n = 4$). Intergroup comparisons were made using the non-parametric Friedman test. A comparison of two groups was performed using Wilcoxon's signed rank test when the Friedman test showed significant differences. Following Wilcoxon's test, the Bonferroni method was applied for post hoc testing. A *p value* of $<0.05$ was considered as statistically significant. All calculations were performed using a statistical software program (STATA version 9.2, Stata Corp, College Station, TX, USA).

## Results

#### Clinical and radiographic observations

After surgical treatment, clinical healing was uneventful at all 16 sites, with limited signs of inflammation. No visible adverse reactions, including material exposure, increased tooth mobility, infection, and suppuration, were observed throughout the experimental period. There was an apparent increase of the radiopaque area and the trabecular structure in the bFGF-treated sites. The PDGF/$\beta$-TCP-treated sites showed the most apparent radiopacity immediately after surgery, resolving by week 8 (data not shown).

#### Histologic observations

*OFD group*

The healing pattern in the OFD group was characterized by extensive collapse of the flap, resorption of the host bone crest, and limited periodontal regeneration. The amount of new cementum was minimal and a small amount of narrow bone formed above the apical notch (Fig. 2a). Connective tissue fibres were observed aligned parallel to or detached from the denuded root surface (Fig. 2b). The area of connective tissue adjacent to the root surface without cementum formation was broader in the OFD sites than in the other groups.

*EMD group*

In the EMD sites, only a slight collapse of the flap was observed, and new bone

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formation with narrow bone growth along the root surface occurred to a varying extent. Apical extension of the junctional epithelium was restrained at the CEJ (Fig. 3a). A greater amount of new cementum was observed in the EMD group than in the OFD group. A thin continuous layer of new acellular extrinsic fibre cementum was predominantly observed on the denuded root surface. Dense collagen fibres were seen inserting into the newly formed cementum, oriented oblique to the root surface (Fig. 3b and c).

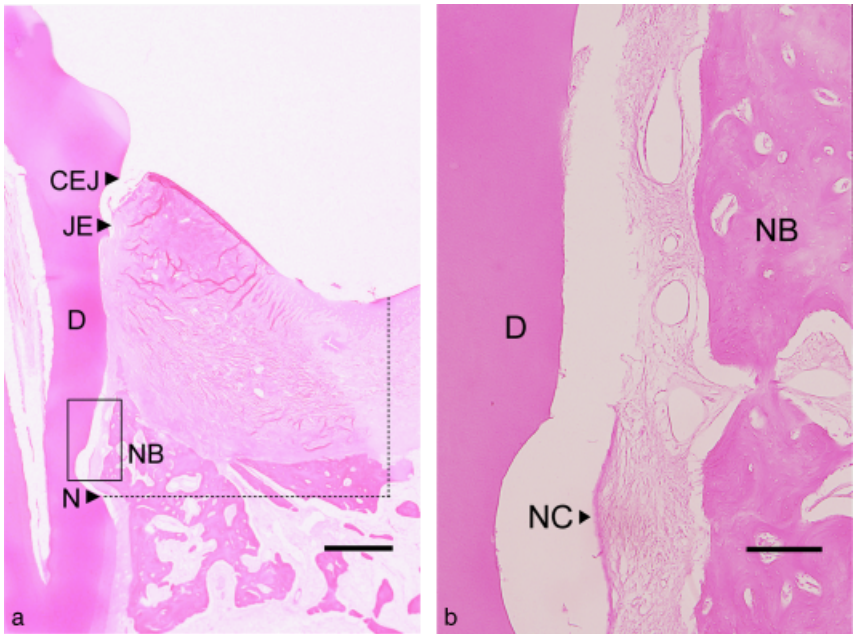


*Fig. 2*. Representative photomicrographs of a two-wall intrabony defect treated with open flap debridement (OFD). (a) Overview. Periodontal regeneration was restricted to the apical portion. The dotted lines show the original defect margin (scale bar: 1 mm; haematoxylin and eosin stain). (b) Higher magnification of the framed area in (a), showing loosely arranged collagen fibres near the root dentin (D) (scale bar: 200 $\mu$m; haematoxylin and eosin stain). CEJ, cemento–enamel junction; JE, junctional epithelium; NB, new bone; N, notch (apical extent of root planing).

*bFGF group*

In the bFGF sites, the flap was stable in position and no collapse was observed. A significant amount of new bone, along with new cementum, was observed close to the level of the junctional epithelium. New bone formation was also noted to be extending from the host bone crest towards the coronal region of the defect (Fig. 4a). New cementum, with or without collagen fibres obliquely oriented to the root surfaces, was observed (Fig. 4b and c). The collagen fibres appeared to be sparser than those observed in the EMD and PDGF/$\beta$-TCP groups. In the bFGF-treated sites, acellular and cellular cementum were observed, with no clearly defined apicocoronal pattern of distribution. The presence of thick cellular intrinsic fibre cementum seemed to be more frequent in the bFGF-treated sites than in the EMD-treated sites.

*PDGF/β-TCP group*

In this group, soft tissue was almost unaltered without collapse of flap. Newly formed bone was observed along and around the root surface, but not on the $\beta$-TCP remnants (Fig. 5a). $\beta$-TCP remnants were sparse and appeared to be encapsulated in the newly formed bone or connective tissue but without direct contact. New bone also formed in the middle portion of the defect, presenting woven bone-like structures or a thin layer of bone-like tissue. New cemen-

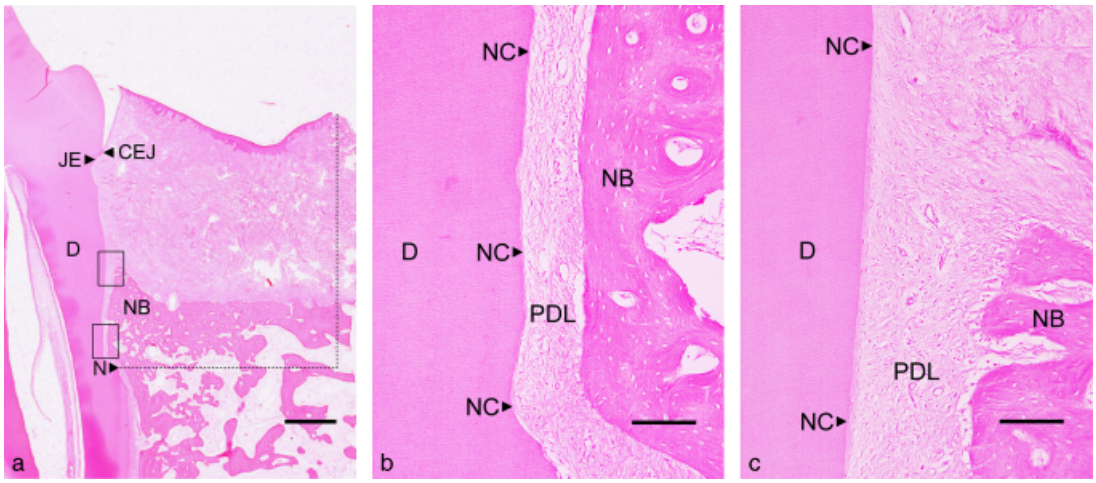


*Fig. 3*. Representative photomicrographs of a two-wall intrabony defect treated with an enamel matrix derivative (EMD). (a) Overview. new bone (NB) formation was rather narrow and developed along the root surface. The dotted lines show the original defect margin (scale bar: 1 mm; haematoxylin and eosin stain). (b) Higher magnification of the apical framed area in (a). (c) Higher magnification of the coronal framed area in (a). Obliquely oriented collagen fibres were observed between the bone and the predominantly acellular cementum. A thin continuous layer of new cementum was observed, both in the apical and in the coronal portions of the defect (scale bar: 100 $\mu$m; haematoxylin and eosin stain). CEJ, cemento–enamel junction; JE, junctional epithelium; NC, new cementum; PDL, periodontal ligament; N, notch (apical extent of root planing); D, root dentin.

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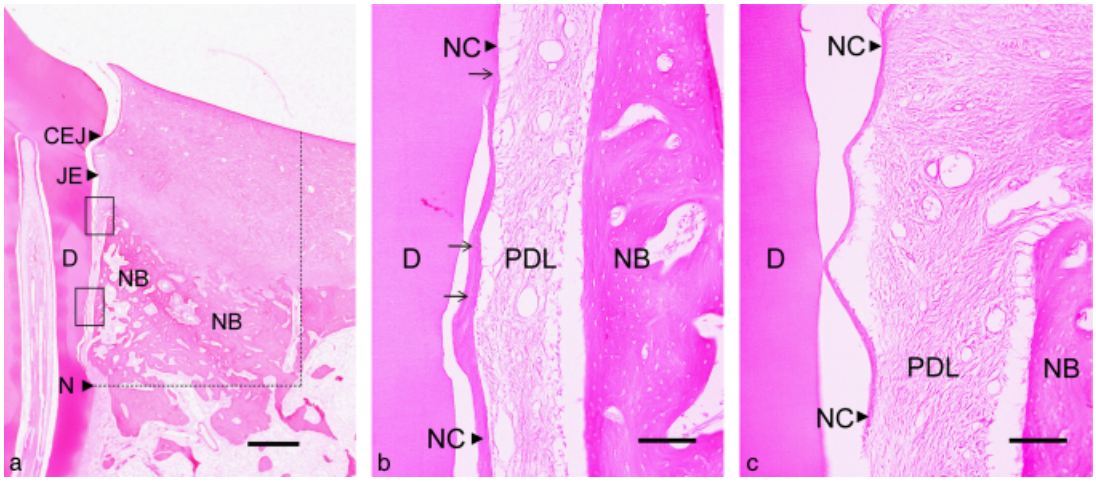


*Fig. 4*. Representative photomicrographs of a two-wall intrabony defect treated with basic fibroblast growth factor (bFGF). (a) Overview. Note the extensive new bone (NB) formation extending from the host bone crest towards the coronal region of the defect. The dotted lines show the original defect margin (scale bar: 1 mm; haematoxylin and eosin stain). (b) Higher magnification of the apical framed area in (a). (c) Higher magnification of the coronal framed area in (a). Thick new cementum (NC) with or without collagen fibres obliquely oriented to the root surface and several blood vessels were observed (scale bar: 100 $\mu$m; haematoxylin and eosin stain). CEJ, cemento–enamel junction; JE, junctional epithelium; PDL, periodontal ligament; N, notch (apical extent of root planing); D, root dentin; arrows, cementocytes.

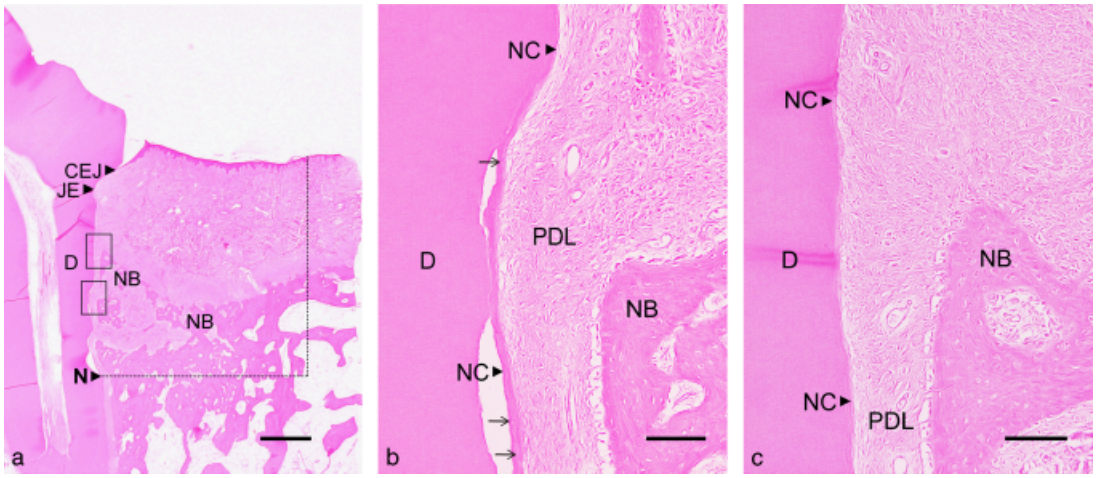


*Fig. 5*. Representative photomicrographs of a two-wall intrabony defect treated with PDGF/$\beta$-TCP. (a) Overview. New bone (NB) formation is visible extending coronally from the host bone with a slight concaved growth. A small amount of $\beta$-TCP and sparse new bone was observed in the middle portion of the defect. The dotted lines show the original defect margin (scale bar: 1 mm; haematoxylin and eosin stain). (b) Higher magnification of the apical framed area in (a). New cellular cementum with inserting collagen fibres was noted predominantly at the apical portion of the defect. (scale bar: 100 $\mu$m; haematoxylin and eosin stain). (c) Higher magnification of the coronal framed area in (a) showing functionally oriented collagen fibres between the new bone and predominantly acellular cementum (scale bar: 100 $\mu$m; haematoxylin and eosin stain). CEJ, cemento–enamel junction; JE, junctional epithelium; NC, new cementum; PDL, periodontal ligament; N, notch (apical extent of root planing); D, root dentin; arrows, cementocytes.

tum with inserting collagen fibres running perpendicular to the root surfaces was observed covering the entire defect. Cellular intrinsic fibre cementum was mostly found at the apical portion and tended to change to acellular extrinsic fibre cementum at the coronal portion on the denuded root surface (Fig. 5b and c). Highly vascularized new periodontal ligament-like tissue, tightly confined between the new cementum and new bone, maintained its width up to the coronal portion.

### Histometric analysis

The results of the histometric analysis are summarized in Table 2.

The length of junctional epithelium migration observed in the EMD group was significantly shorter than those in the OFD, bFGF, and PDGF/$\beta$-TCP groups. The amount of connective tissue adhesion (without cementum) in the OFD group was significantly greater than that in the bFGF and PDGF/$\beta$-TCP groups. Moreover, new bone formation was more extensive in the bFGF group than in the OFD and EMD groups. There was no significant difference in new bone formation between the OFD and the EMD groups. The groups treated with EMD and PDGF/$\beta$-TCP showed significantly

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*Table 2*. Histometric parameters (mean ± SD in mm; $n = 4$) for each experimental condition

| | Experimental condition | | | | |
|---|---|---|---|---|---|
| | 1<br>OFD | 2<br>EMD | 3<br>bFGF | 4<br>PDGF/$\beta$-TCP | statistically significant differences |
| DH | 6.29 ± 0.17 | 6.18 ± 0.38 | 6.46 ± 0.1 | 6.17 ± 0.42 | NS |
| JE | 0.84 ± 0.35 | 0.34 ± 0.15 | 0.71 ± 0.2 | 0.83 ± 0.18 | 1 *versus* 2 ($p<0.05$)<br>2 *versus* 3 ($p<0.05$)<br>2 *versus* 4 ($p<0.05$) |
| CT | 2.44 ± 0.75 | 1.40 ± 0.97 | 1.15 ± 0.74 | 0.58 ± 0.5 | 1 *versus* 3 ($p<0.01$)<br>1 *versus* 4 ($p<0.01$) |
| NB | 3.09 ± 0.52 | 3.32 ± 0.71 | 4.11 ± 0.77 | 3.66 ± 0.33 | 1 *versus* 3 ($p<0.01$)<br>2 *versus* 3 ($p<0.05$) |
| NC | 2.96 ± 0.69 | 4.59 ± 1.19 | 4.28 ± 1.3 | 4.66 ± 0.7 | 1 *versus* 2 ($p<0.01$)<br>1 *versus* 4 ($p<0.01$) |

OFD, open flap debridement; EMD, enamel matrix derivative; bFGF, basic fibroblast growth factor; PDGF/$\beta$-TCP, platelet-derived growth factor with $\beta$-tricalcium phosphate; NS, nonsignificant; DH, defect height; JE, junctional epithelium migration; CT, connective tissue attachment (without cementum); NB, new bone; NC, new cementum.

greater cementum formation than the OFD group. No significant differences were observed between the bFGF and the PDGF/$\beta$-TCP groups in any of the histometric parameters.

## Discussion

In intrabony defects, the number of residual bone walls has a strong influence on healing potential after periodontal regenerative therapy. It is well known that periodontal healing potentially results in complete regeneration even without regenerative therapy, especially in three-wall intrabony defects (Renvert et al. 1985, Kim et al. 2004). It has been demonstrated that periodontal regenerative therapy, without some grafting materials, is not reliable in one-wall intrabony defects (Tonetti et al. 1993, Lekovic et al. 2000, Cochran et al. 2003), although one-wall intrabony defects are a better model for evaluating regenerative periodontal therapies (Blumenthal et al. 2003, Kim et al. 2005). From the clinical point of view, two-wall intrabony defects are prevalent and various periodontal therapies with and without grafting materials are currently being conducted. For these reasons, two-wall intrabony defects were considered worth evaluating for periodontal healing after the application of EMD, bFGF, or PDGF/$\beta$-TCP. However, in terms of histometric evaluation, two-wall intrabony defects are not preferable because the coefficient variation of the histometric parameters is larger than that of typical one- and three-wall intrabony defects (Kim et al. 2004). In the present study, reference notches on the crown and micro CTs were used to discern the centre of the two-wall intrabony defects, which enabled an unbiased evaluation.

It has been documented that EMD leads to more bone regeneration compared with OFD (Hammarström et al. 1997, Heijl et al. 1997). However, there were no statistically significant differences regarding new bone formation between the OFD and the EMD groups in the present study. The discrepancy between the results may be explained by the reports that new bone formation occurs moderately along the root surface following predominantly acellular cementum and periodontal ligament formation (Gestrelius et al. 1997, Mellonig 1999, Windisch et al. 2002, Shirakata et al. 2007), and that EMD has no or less osteoinductive activity (Intini et al. 2008, Plachokova et al. 2008), depending on the type of the bony defects, with a limited space-making capacity (Lekovic et al. 2000, Cochran et al. 2003).

In the defects treated with bFGF, new, dominantly thick, cellular intrinsic fibre cementum and new bone formation were observed. Functionally oriented collagen fibres with many blood vessels in some parts of the denuded root surface were also observed, but they appeared to be sparser than those observed in the EMD and the PDGF/$\beta$-TCP groups. These findings were similar to previous animal studies (Murakami et al. 1999, 2003, Rossa et al. 2000, Takayama et al. 2001, Sato et al. 2004). In vitro studies have suggested that bFGF facilitates the proliferation of periodontal ligament cells while maintaining their differentiation (Takayama et al. 1997), promotes angiogenesis by inducing laminin (Nicosia et al. 1994, Takayama et al. 1997), or increases the production of extracellular matrix from periodontal ligament cells (Shimabukuro et al. 2005, Terashima et al. 2008). Interestingly, in the bFGF group, no collapse of the flap was found without carrier materials, and this group showed the greatest amount of new bone formation among the groups examined. This may be because the bFGF/HPC applied in the present study caused rapid and intensive osteogenic bone formation by stimulating the proliferation of osteoblasts rather than that of fibroblasts, vascular endothelial cells, or epithelial cells.

Although there were no significant differences in any of the histometric parameters between the bFGF and PDGF/$\beta$-TCP groups, PDGF/$\beta$-TCP induced bone regeneration less aggressively than bFGF. These findings regarding new bone formation can be partly attributed to the $\beta$-TCP property. Radiographic analysis revealed that most significant radiopacity immediately postsurgery resolved by week 8, indicating rapid resorption of the $\beta$-TCP particles used in this study. Only a small amount of unresorbed $\beta$-TCP was observed in the defects, exhibiting encapsulation by connective tissue or sparse new bone. This result agrees with the finding that $\beta$-TCP particles are embedded in surrounding connective tissues with minimal inflammatory infiltrate and appear to be inhibitory to bone formation (Baldock et al. 1985, Camelo et al. 2003, Nevins et al. 2003, Ridgway et al. 2008).

Within the limitations of the present study, it can be concluded that the candidate bFGF treatment supported periodontal regeneration comparable with that of established benchmarks:

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EMD and PDGF/β-TCP. Thus, the bFGF treatment appears to be a promising novel treatment. Further studies with short- and long-term observations are required to clarify the difference between clinical outcomes following the regenerative therapy in two-wall intrabony defects in a large number of humans under strictly standardized conditions.

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**Clinical Relevance**

*Scientific rationale for the study*: Tissue engineering technologies using scaffolds and growth factors have been developed for periodontal regeneration. The present study compared periodontal healing of two-wall intrabony defects treated with EMD, bFGF, and PDGF/$\beta$-TCP in dogs.

*Principal findings*: EMD promoted acellular cementum formation but failed to induce significant bone regeneration compared with open flap debridement. Significantly greater periodontal regeneration was observed in bFGF- and PDGF/$\beta$-TCP-treated sites. bFGF-treated sites showed the most aggressive bone formation.

*Practical implications*: The present histological findings provide a basis for understanding the different regenerative properties and effects of EMD, bFGF, and PDGF/$\beta$-TCP in two-wall intrabony defects.

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