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Original Article

Recombinant human fibroblast growth factor-2 promotes surgical-wound healing in the rat gingiva

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Abstract *Background/purpose:* Fibroblast growth factor-2 (FGF-2) has been shown to promote periodontal tissue regeneration in clinical settings. However, reports on the effects of FGF-2 on soft-tissue wound healing after periodontal surgery are scarce. This study aimed to investigate the effects of FGF-2 on gingival wound healing.
Materials and methods: Seven-week-old male Sprague–Dawley rats were divided into three groups. Each group included 15 rats, with five animals in each experimental period. Five untreated rats were used as the no-treated group. Incisions were made bilaterally in the palatal mucosa from the mesial corner of the maxillary first molar up to the incisor, full-thickness flaps were raised, and sutures were placed. In the control group, no additional treatment was performed, whereas in the FGF-2 and enamel matrix derivative (EMD) groups, FGF-2 and EMD were applied before suturing, respectively. Animals in all groups were euthanized 1, 3, and 7 days, and the maxilla was resected. The re-epithelialization, gingival thickness, inflammation, and angiogenesis were evaluated histopathologically.
Results: The FGF-2 group showed significantly greater re-epithelialization than the control group on day 1. The FGF-2 group showed significantly greater gingival thickness than the other two groups at all time points. The FGF-2 group showed significantly lesser inflammation than the control group on day 1. The FGF-2 group showed significantly higher angiogenesis than the control group on days 1 and 3.
Conclusion: FGF-2 promotes re-epithelialization, and increases angiogenesis and gingival thickness. Additionally, FGF-2 reduces the inflammatory response during wound healing.

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Introduction

Soft-tissue wound healing is comprises four overlapping stages — hemostasis, inflammation, granulation, and maturation.[1] Although scar formation frequently occurs, under favorable conditions, complete restoration of the normal tissue structure, that is, regeneration, can occur. Particularly in periodontal surgery, faster healing can lead to better outcomes by minimizing postoperative complications and scar formation, and early wound closure is essential for periodontal-tissue regeneration.

Fibroblast growth factor-2 (FGF-2) formulation (REGROTH, Kaken Pharmaceutical, Tokyo, Japan) has been applied for periodontal tissue-regeneration therapy in dentistry in Japan.[2] In a previous study, 0.3 % FGF-2 outperformed enamel matrix derivative (EMD, Emdogain, Straumann, Basel, Switzerland) in promoting bone regeneration and clinical attachment level gain after flap surgery for alveolar bone defects.[2]

FGF-2 facilitates angiogenesis, granulation tissue formation, and osteogenesis,[3,4] and can induce the proliferation and differentiation of periodontal ligament cells,[5] and periodontal-tissue regeneration. FGF-2 has been applied for the treatment of wounds such as bedsores and skin ulcers.[6] However, to date, evidence demonstrating the effect of FGF-2 on gingival wound healing, particularly wound closure after periodontal surgery, is lacking.

EMD, which is extracted and purified from porcine tooth embryos, has been used in periodontal tissue-regeneration therapy with favorable outcomes.[7,8] EMD mainly contains amelogenin, along with other components, such as enamel proteins and growth factors.[9] *In vitro*, EMD exhibits a

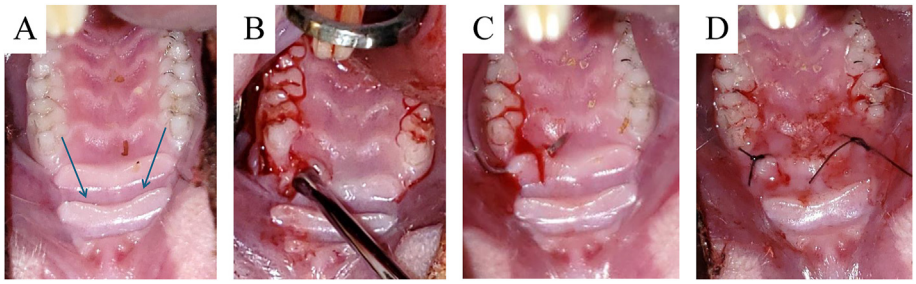

Figure 1 Surgical procedure. (A) Schematic of incision location. Arrows indicate incision lines. (B) After the incision is made, a full-thickness flap is elevated. (C) Suturing. D: One suture has been placed proximal to the first molar on each side.

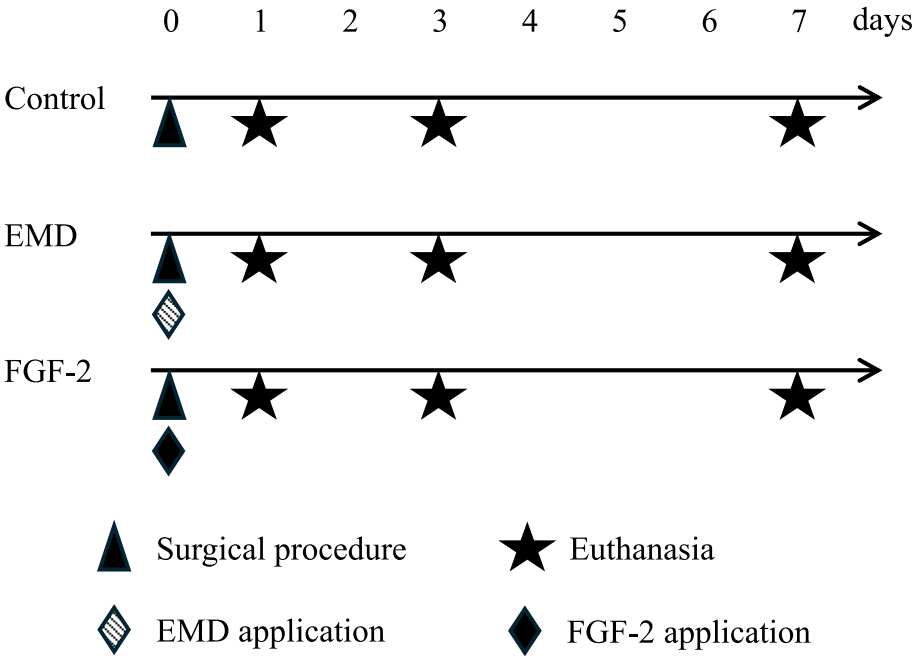

Figure 2 Study timeline. EMD: enamel matrix derivative. FGF-2: Fibroblast growth factor 2.

mitogenic effect on gingival fibroblasts[10,11] and promotes the synthesis of extracellular matrix components, including collagen.[10,12] Moreover, evidence from both *in vivo* and *in vitro* studies demonstrates that EMD enhances angiogenesis.[13,14] Through these actions, EMD is believed to facilitate periodontal-tissue regeneration and soft-tissue wound repair. In addition to being used to regenerate periodontal tissue, EMD has been used to promote healing of open palatal gingival wounds after free-gingival graft harvesting[15] and has been applied with connective-tissue grafts for root-surface coverage.[16]

Rodent models are attractive because they exhibit patterns of wound healing common to mammals,[17] are easy to handle, readily available, provide large sample sizes.[18] Rodent models have been widely used in wound-healing research, to facilitate in-depth and comparative analyses of cellular and molecular mechanisms.[18] Another advantage of using rats is their accelerated wound healing, which allows the process to be studied in days rather than in weeks as in humans.[18,19]

Therefore, in this study, a rat model of healing of incisional wounds in the oral mucosa was used. This study aimed to compare the effects of FGF-2 on early-stage wound healing after full-thickness flap surgery in the oral mucosa of rats with those of EMD.

Materials and methods

Animals

This study adhered to the Ethical Principles and Guidelines for Animal Experimentation and was approved by the Animal Care Committee of Fukuoka Dental College (approval number No. 21008, September 1, 2021). Seven-week-old male Sprague—Dawley rats, weighing between 250 and 270 g, were procured from Oriental Yeast Co., Ltd. and maintained under specific pathogen-free conditions within the Animal Center at Fukuoka Dental College, Japan.

Study design

Each group included 15 rats, with five animals in each experimental period (1, 3, and 7 days). Five untreated rats were assigned to the no-treatment group.

The surgical procedures and experimental protocols for the rat wound healing model used in this study were conducted with modifications to the animal experimental model performed by Villa et al.[20] Briefly, under general anesthesia using isoflurane, bilateral incisions reaching the periosteum were made in the palatal mucosa from the mesial corner of the maxillary first molar up to the incisor using a No. 12 scalpel. A full-thickness mucoperiosteal flap was elevated using a periosteal debridement tool (Fig. 1A and B). After hemostasis, 0.03 % recombinant human FGF-2, and EMD were applied to the wound surfaces in the FGF-2, and EMD groups, respectively. Thereafter, simple sutures were placed using 6-0 nylon (Manny, Tochigi, Japan) (Fig. 1C and D). The rats were euthanized on days 1, 3, or 7 with an overdose of 4.9 % (v/v) isoflurane (Fig. 2).

Histology

After euthanasia, the left and right maxillae of the rats were excised and fixed in 4 % paraformaldehyde at 4 °C for 10 h. Thereafter, the samples were demineralized using 10 % ethylenediaminetetraacetic acid at 4 °C for 3 weeks. Following demineralization, the samples were embedded in paraffin using the AMeX method.[21] Serial sections of 4 μm thickness were prepared from the palatal portion proximal to the first molar.

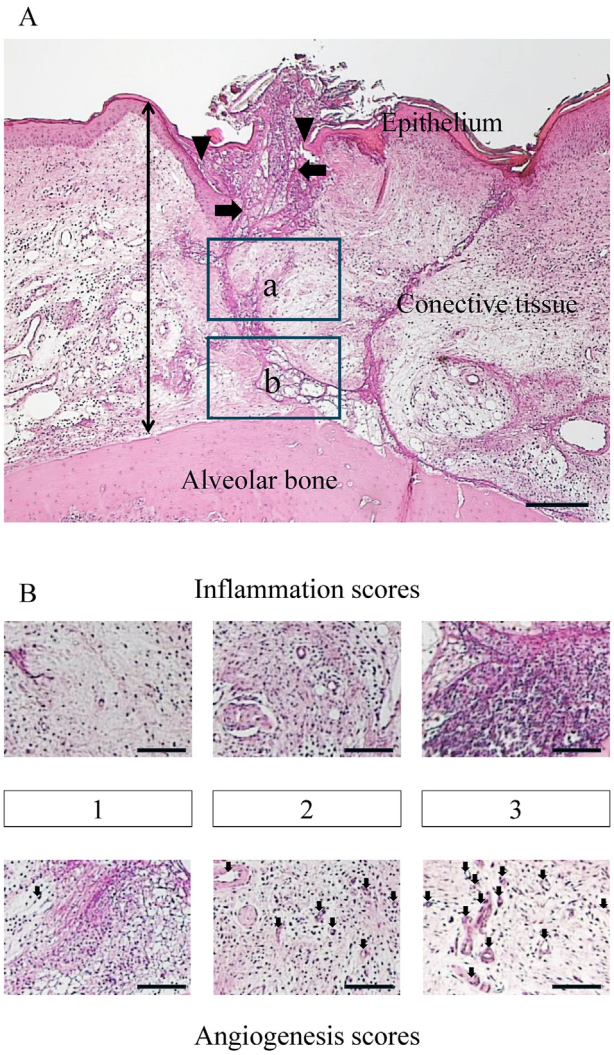

Figure 3 Evaluation sites. (A) Histology of the mid-distal cross-section of the wound site on day 1 in the control group. Epithelial migration was evaluated by comparing the position of the stratum corneum (arrowhead) to the edge of the wound surface and the position of the epithelial edge (arrow). Inflammation and angiogenesis were evaluated in the subepithelial connective tissue (a) and bone-surface connective tissue (b), respectively. The distance from the edge of the epithelial layer to the bone surface (↔) was measured as the gingival width. Hematoxylin and eosin staining. Scale bars: 200 μm. B: Representative tissue sections with different inflammation and angiogenesis scores. Arrows indicate capillaries. Hematoxylin and eosin staining. Scale bars: 100 μm.

Forty serial sections were prepared from each specimen, and every tenth section was selected for hematoxylin and eosin (H&E) staining. Histopathological evaluation was performed using an optical microscope.

Histomorphometric measurements

For histomorphometric analysis, H&E-stained sections were photographed using a camera attached to the optical microscope, and image analysis was performed using ImageJ (National Institutes of Health). Measurements were conducted according to the methodology outlined by Villa et al.[20] The demarcation between the damaged and intact tissue was determined, and the epithelial-edge advancement distance was determined. The extent of re-epithelialization was expressed as the ratio of newly formed epithelium to the total wound width calculated as follows: (new epithelial width/total wound width) × 100. This value was used as the degree of re-epithelialization, and 100 % re-epithelialization was defined as complete wound closure. The gingival width from the epithelial edge of the wound to the bone surface was measured to assess gingival swelling and proliferation. Additionally, the widths of the epithelium and connective tissue were separately measured (Fig. 3A).

Assessment of inflammation and angiogenesis in the connective tissue

To assess inflammation and angiogenesis, we evaluated two areas of connective tissue per section: an area of subepithelial connective tissue in the wound and an area of connective tissue proximal to the bone (Fig. 3A). Inflammatory responses were graded on a three-tier scale: 1 (mild), 2 (moderate), and 3 (marked), according to an index previously established by Kirchner et al.[22] This index indicates the degree of inflammatory-cell infiltration in the wound granulation tissue. Angiogenesis was evaluated

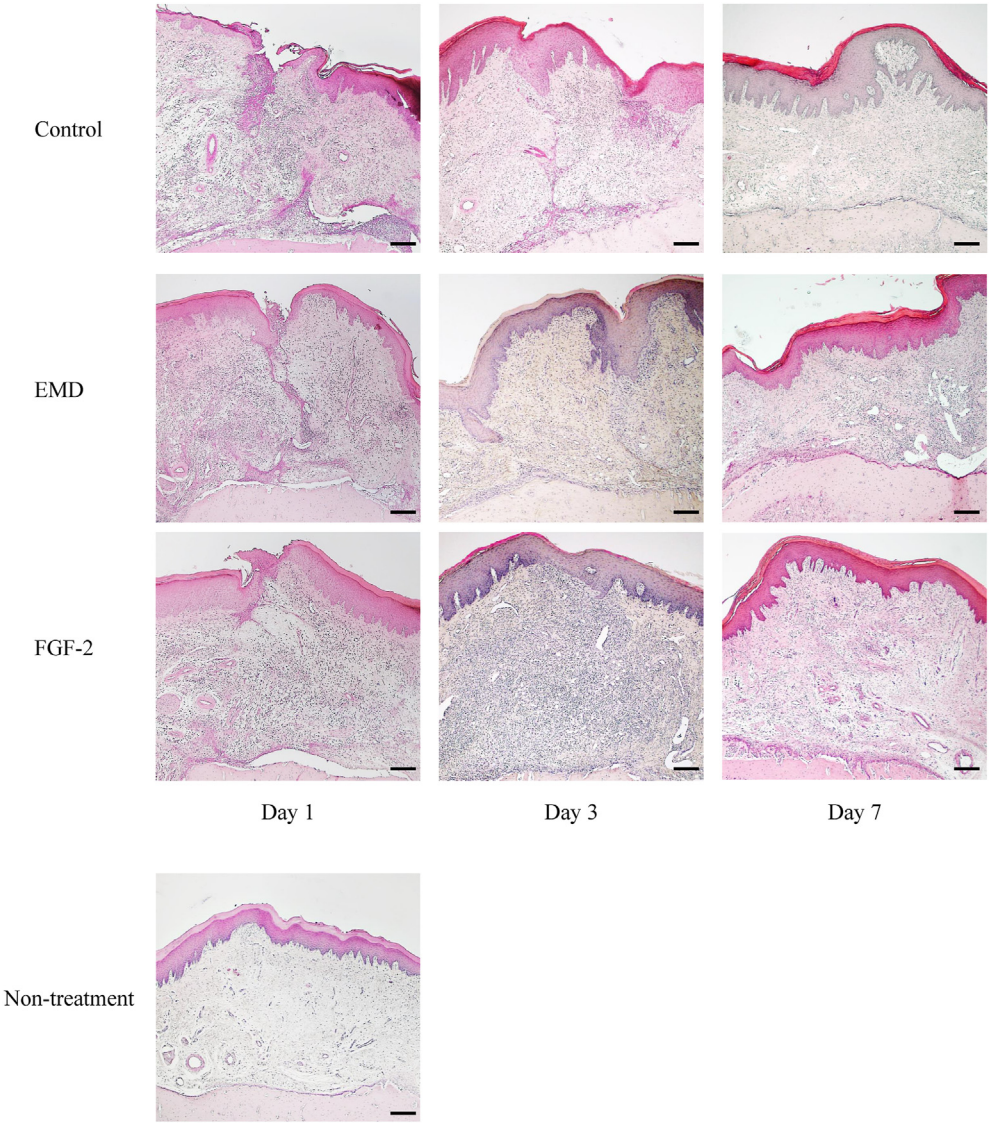

Figure 4 Histological images showing the healing pattern for each group on days 1, 3, and 7. Hematoxylin and eosin staining. Scale bars: 200 μm. EMD: enamel matrix derivative. FGF-2: Fibroblast growth factor 2.

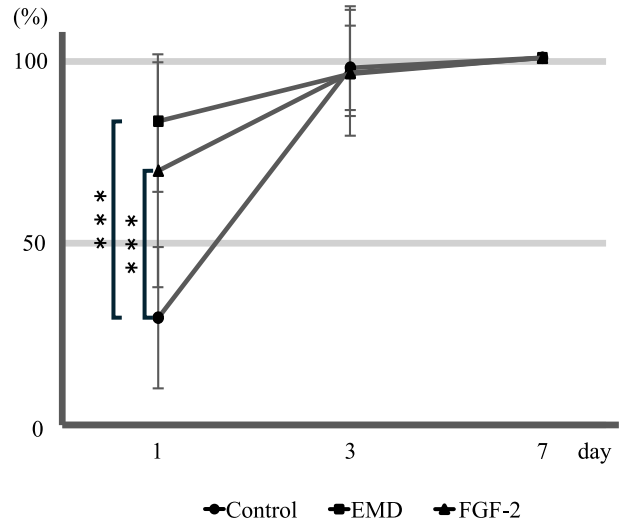

Figure 5 Re-epithelialization rate. Wound closure at each time period was evaluated by calculating the percentage of re-epithelialization relative to the non-wound oral mucosal margins. Re-epithelialization was determined based on histomorphometric analyses of the wound sections. Values are expressed as SEM. EMD, enamel matrix derivative; FGF-2, Fibroblast growth factor 2. The significance of differences between groups was determined by one-way analysis of variance, with post hoc Tukey's honestly significant difference test. Comparisons with the Control group at the same time-point. ***$P < 0.001$.

according to the index established by Altavilla et al.[23] and graded on a three-point scale: 1 = severely compromised, with one to two vessels per site, marked by significant edema, hemorrhage, and occasional vascular blockage; 2 = limited neovascularization, with three to eight capillaries per site, accompanied by moderate edema and congestion in surrounding tissues; and 3 = robust angiogenesis, with nine or more well-organized capillaries per site, oriented towards the epithelium and wound edge, exhibiting minimal edema and congestion (Fig. 3B).

Statistical analysis

One-way analysis of variance was used to analyze the amount of re-epithelialization and gingival thickness, and Tukey's honestly significant difference test was used for post hoc comparisons. Significant differences in the inflammatory response and angiogenesis were determined using the Kruskal–Wallis test, and group differences were assessed using Bonferroni post hoc tests. Statistical analyses were conducted using SPSS version 29.0 (IBM Corp., Armonk, NY, USA), with the level of significance established at $P < 0.05$.

Results

Histopathological findings

Photographs of H&E-stained sections of the healing tissue in each group are shown in Fig. 4. On postoperative day (POD) 1, marked inflammatory-cell infiltration in the connective tissue was observed in the control group, whereas the EMD and FGF-2 groups showed lesser inflammatory-cell infiltration. Epithelial laceration of the wound surface was observed in all sections in the control group. In contrast, in the EMD and FGF-2 groups, progressive epithelial migration and closure were observed in some sections, and few fibroblasts were observed in the wounded areas. In addition, dilated blood vessels were observed in the wound area, accompanied by edema, hemorrhage, and red blood cell infiltration. More capillary-like structures were observed, particularly in the FGF-2 group.

On POD 3, the number of polymorphonuclear leukocytes decreased in all groups. Mononuclear leukocyte infiltration, especially of monocytes/macrophages, was more prominent in the FGF-2 group. The number of fibroblast-like cells also increased. In the control group, areas of edema, red blood cell infiltration, and exudation decreased. However, the reduction was greater in the EMD and FGF-2 groups than in the control group. Furthermore, the epithelium was fused with few lacerations observed in all groups. In addition, the gingival thickness increased in the FGF-2 group.

On POD 7, all groups showed decreased inflammatory-cell infiltration, and no strong infiltrates were observed. The EMD and FGF-2 groups exhibited increased vascularity. The FGF-2 group maintained increased gingival thickness and showed the greatest increase in gingival thickness among all groups.

Quantitative assessment of wound healing

On POD 1, almost all samples in the control group showed epithelial cleavage, whereas samples in the EMD and FGF-2 groups did not, indicating a significant increase in re-epithelialization (Fig. 5). On POD 3, most sections did not show epithelial lacerations, and no differences between the groups were observed. On POD 7, no lacerations were observed in any of the sections.

Connective tissue assessment

On POD 1, the FGF-2 and EMD groups exhibited significantly reduced connective tissue inflammation compared to the control group. Furthermore, the number of vessels was significantly larger in the FGF-2 and EMD groups (Fig. 6A and B).

On POD 3, a trend towards a decrease in connective-tissue inflammation was observed in the FGF-2 and EMD groups compared to that in the control group; however, no significant difference was observed. A significant increase in the blood vessel count was observed in the FGF-2 group (Fig. 6C and D).

On POD 7, inflammatory-cell infiltration and vessel count were not significantly different between the groups (Fig. 6E and F).

Quantitative evaluation of gingival thickness

The thickness of the entire gingiva, epithelium, and connective tissue was assessed. Throughout the experimental period, the FGF-2 group exhibited a statistically significant increase in overall gingival thickness compared to the other

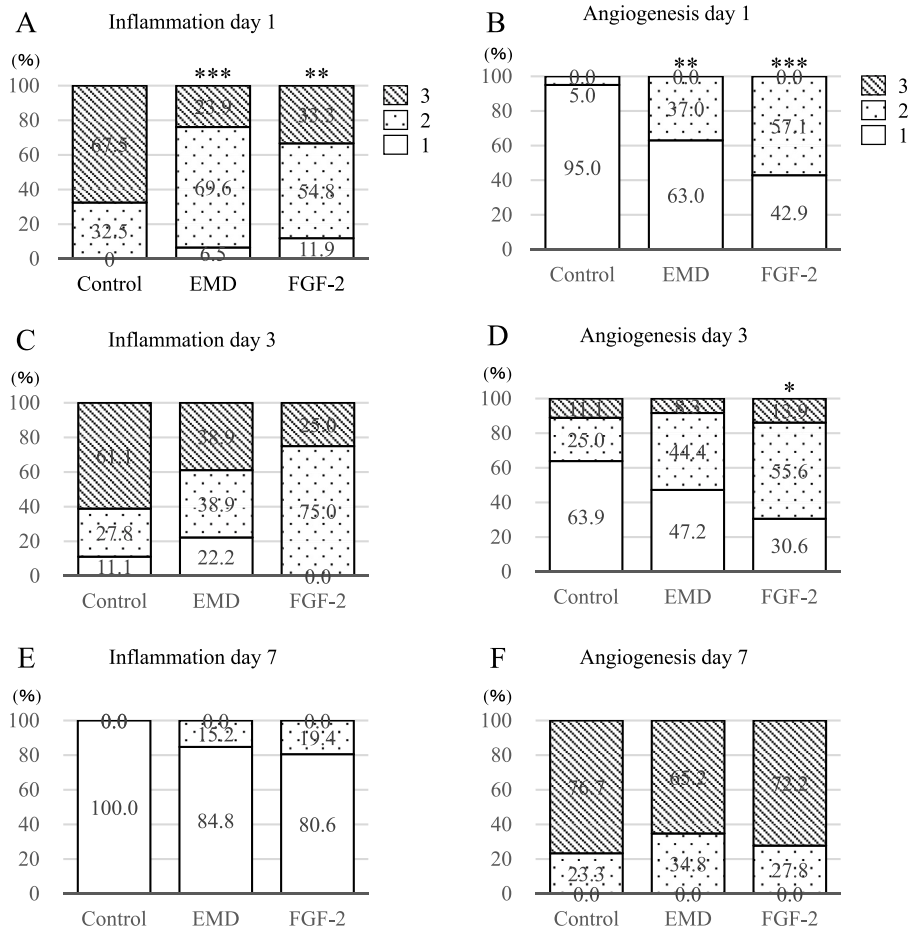

Figure 6 Inflammation and angiogenesis in the connective tissue. A–F) Histologic score frequency distribution. The degree of inflammatory-cell infiltration in the connective tissue at the wound site was evaluated by the inflammation score (A, C, E). The degree of neovascularization in the wound's connective tissue is evaluated by the angiogenesis score (B, D, F). EMD: enamel matrix derivative. FGF-2: Fibroblast growth factor 2. The Kruskal–Wallis test was used for significant differences in inflammatory response and angiogenesis, and differences between groups were determined using the post hoc Bonferroni test. Comparison with control. $^{**}P < 0.01$, $^{***}P < 0.001$.

groups (Fig. 7A–D, G). On POD 3, both the control and EMD groups showed a significant increase in the overall gingival thickness compared to that in the no-treatment group (Fig. 7D). On POD 7, the EMD group showed a significant increase in the overall gingival thickness compared to that in the no-treatment group (Fig. 7G).

Compared with the other groups, a significant increase in epithelial thickness was observed in the FGF-2 group on POD 1 and 7 (Fig. 7B and H). On POD 3, the FGF-2 and EMD groups showed a significant greater epithelial thickness compared to the no-treatment group (Fig. 7E).

Connective-tissue thickness was significantly greater in the FGF-2 group than in the other groups throughout the experimental period (Fig. 7C–F, and I). On POD 3, the control and EMD groups had significantly thicker connective tissue than the no-treatment group (Fig. 7F).

Discussion

This study used a rat model to histomorphometrically analyze the effects of FGF-2 and EMD on the early wound healing processes, encompassing re-epithelialization, inflammatory response, and angiogenesis. The results showed that FGF-2 and EMD significantly enhanced gingival wound healing. The FGF-2 and EMD groups showed significantly higher rates of gingival wound closure compared to the control group on POD 1. The outcomes in the EMD group in this study are consistent with those reported by Villa et al.[21] Additionally, FGF-2 promoted wound healing in a model of palatal mucosal defects, which is consistent with the findings of Ayvazyan et al.[24]

FGF-2 promotes wound healing by stimulating the proliferation of vascular endothelial cells and fibroblasts in various tissues, including the skin and mucosa.[25–28] *In vivo* studies have reported that FGF-2 promotes epithelialization of experimentally created wounds on the backs of mice and wound healing in the palatal mucosa.[24,29] Furthermore, FGF-2 induces fibroblast proliferation[28,30] and promotes epithelial cell migration.[31] We believe that these mechanisms enhanced gingival-wound re-epithelialization in our experiment.

Optimal gingival healing is essential for the restoration of vascular supply, tissue architecture, mechanical

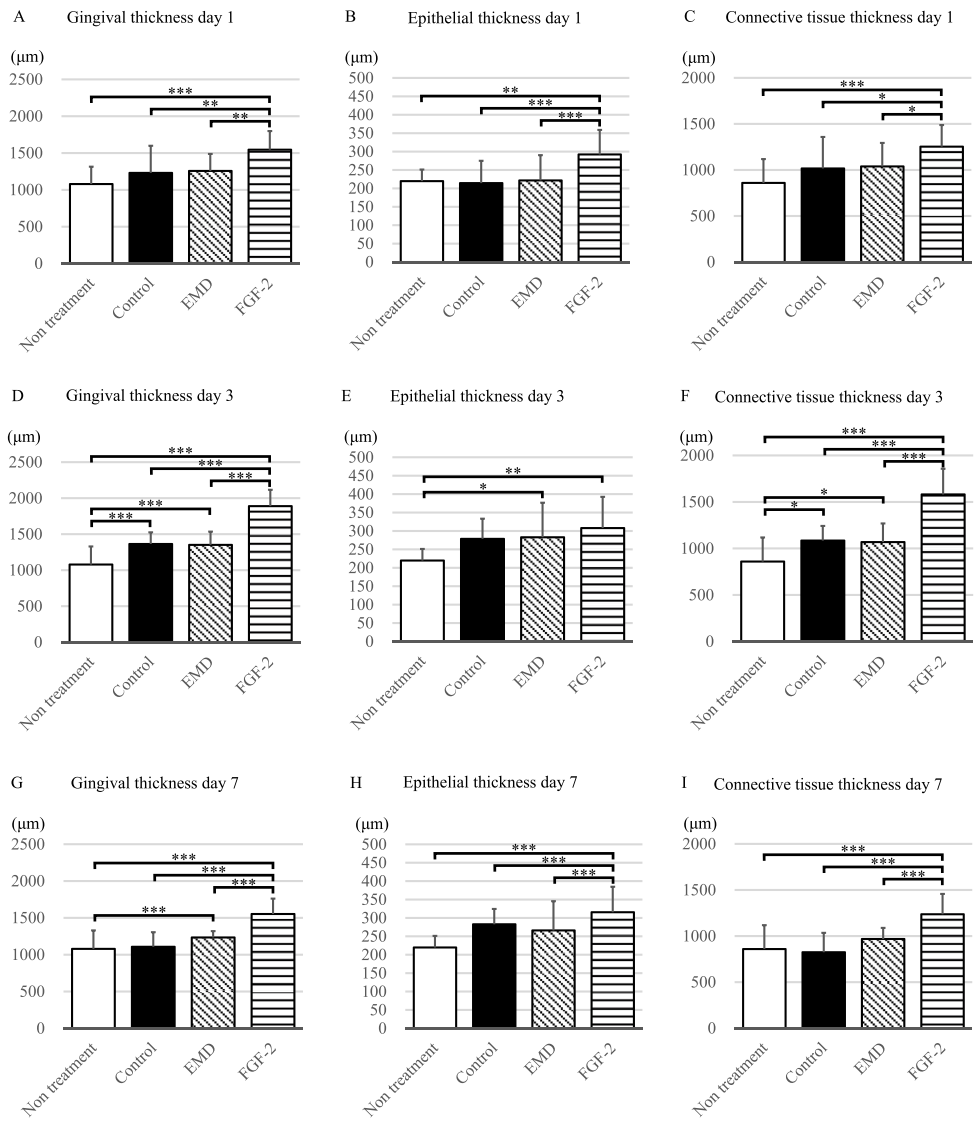

Figure 7 Gingival thickness. A, D, and G indicate gingival thickness. B, E and H indicate epithelial thickness. C, F and I indicate connective-tissue thickness. Values are expressed as SEM. EMD: enamel matrix derivative. FGF-2: Fibroblast growth factor 2. The significance of differences between groups was determined by one-way analysis of variance, with post hoc Tukey's honestly significant difference test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

resilience, and functional competence. Complete wound closure serves to shield the underlying connective tissues from external insults and pathogenic invasion, whereas angiogenesis reinstates appropriate partial pressure of oxygen and nutrient delivery. Additionally, the healing process restores the ability of the tissue to withstand mechanical stress through the regeneration of mechanosensitive elements within the extracellular matrix, which act as a defense against tearing forces.[1] In this study, FGF-2 and EMD promoted gingival wound healing after experimental periodontal surgery. This is clinically significant because soft-tissue healing and stability are essential for better outcomes in periodontal surgery, particularly in periodontal tissue-regeneration therapy.

In terms of inflammatory-cell infiltration, statistically significant attenuation of marked inflammatory responses was demonstrated in the FGF-2 group on POD 1 and 3. Regarding the anti-inflammatory properties of FGF-2, previous studies have reported that FGF-2 downregulates the expression of cluster of differentiation 40 (CD40) in periodontal ligament cells. Additionally, FGF-2 has been reported to suppress CD40 expression in mouse periodontal ligament cells and reduce the production of interleukin 6 and tumor necrosis factor α.[32] In this study, EMD-treated wounds showed reduced inflammatory-cell migration. These findings align with those of previous *in vitro* studies, which demonstrated that EMD reduces the gene expression and secretion of pro-inflammatory cytokines and increases the gene expression and production of growth factors associated with wound repair and regenerative processes.[33,34]

During angiogenesis, the FGF-2 group exhibited a greater number of vessels compared to the control group on POD 1, and this trend continued on POD 3. However, on POD 7, the number of vessels was similar between the groups. Animal studies using palatal-defect models have reported that FGF-2 increases the number and area of new blood vessels during

palatal tissue healing.[24] Furthermore, treatment of endothelial cells with FGF-2 promotes an angiogenic phenotype, characterized by increased proliferation and migration, and increased expression of specific integrins.[35] Another study indicated that FGF-2 is a potent inducer of angiogenesis,[36] which is consistent with the findings in this study.

The FGF-2 group exhibited a significantly larger increase in gingival thickness compared to both the control and EMD groups throughout the experimental period. This increase was mainly influenced by an increase in the connective tissue. FGF-2 has been reported to induce fibroblast proliferation,[28,30] and in a study using a palatal mucosal-defect model, FGF-2 increased the mucosal thickness.[24] In this study, the number of collagen fibers increased, which suggested that FGF-2 induced collagen-fiber formation. Furthermore, a significant increase in the epithelial-layer thickness was observed, which likely indicates induction of epithelial-cell proliferation. Further studies are required to determine the duration of increase in gingival thickness and the cells affected by this increase. Because FGF-2 induces an increase in gingival thickness, it can be potentially applied to procedures such as gingival graft harvesting for free-gingival transplantation. However, further research on this topic is required.

Previous studies have used models to assess the healing of extensive lesions, including circular or rectangular excisions in cutaneous or oral tissues.[24,37,38] However, these models do not replicate the smaller wounds produced by periodontal surgery, implant surgery, or regenerative therapy. In addition, owing to the limitations of this study, such as the small sample size and the fact that it is not a human study, we believe that further research is needed.

In summary, this study showed that FGF-2 and EMD promote wound healing in the gingival soft tissues. FGF-2 and EMD likely facilitated soft-tissue wound healing through their anti-inflammatory and angiogenic properties. FGF-2 increased gingival thickness, especially connective-tissue thickness. These results suggest potential clinical applications in periodontal plastic surgery, including connective-tissue grafting. However, further research is required to develop appropriate protocols to apply this technique in periodontal plastic surgery, such as connective-tissue grafting.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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