



Mini Review

Periodontal regeneration and FGF-2

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Periodontal diseases are highly prevalent inflammatory diseases that lead to a destruction of tooth-supporting tissues, such as the periodontal ligament, alveolar bone and cementum, and ultimately result in tooth loss. Conventional treatment for periodontal diseases is the mechanical removal of the periodontal biofilm, which although effective in reducing inflammation in lesion sites, does not allow regeneration of lost tissues. Therefore, it is important that new therapeutic procedures that encourage the regeneration of periodontal tissues destroyed by periodontal disease will be established. In recent years, the efficacy of topical application of recombinant cytokines for periodontal regeneration has been investigated. This review focuses on the biological activities of FGF-2 in promoting periodontal tissue regeneration.

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Introduction

Periodontal diseases have been characterized as chronic infectious diseases caused by bacterial biofilm (known as dental plaque) adhering to the teeth and are highly prevalent across the world. These diseases cause the destruction of the periodontal tissues that support teeth, such as periodontal ligament, alveolar bone, cementum, and gingiva, and finally lead to the loss of affected teeth. Conventional periodontal therapies are scaling and root planing, which remove mechanically periodontal biofilm. Although these treatments are effective at diminishing inflammation in affected lesions, the lost periodontal tissues cannot be regenerated^{1,2}. To regenerate damaged periodontal tis-

ues, stimulation of cementoblast and osteoblast proliferation and differentiation is required at the site of periodontal tissue defects to induce neogenesis of the tooth support structures. Recent studies have shown that multipotent mesenchymal stem cells exist in periodontal ligament that can differentiate into cementoblasts and osteoblasts³. The differentiation can be effectively stimulated by local application of recombinant cytokines, several of which have already been investigated for their ability to induce periodontal tissue regeneration. Direct local application of platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF)-I⁴, bone morphogenetic protein (BMP)-2^{5,6}, osteogenic protein (OP)-1⁷, transforming growth factor (TGF)- β 1⁸,

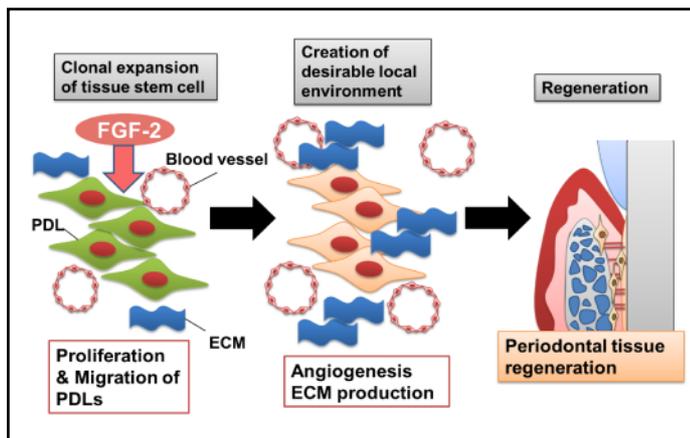


Fig.1 Schematic illustration of FGF-2-induced periodontal regeneration

FGF-2 stimulates the proliferation and migration of periodontal ligament cells (PDL) with maintaining their potent nature to differentiate into hard tissue-forming cells like osteoblasts and cementoblasts in the early stage of periodontal tissue regeneration. Further, FGF-2 induces angiogenesis and ECM production from PDL, leading to local environment suitable for the periodontal tissue regeneration.

brain-derived neurotropic factor (BDNF)⁹, and growth and differentiation factor-5 (GDF-5)¹⁰ to animal periodontal bone defects models stimulate and promote periodontal regeneration. We have been engaged in investigating the efficacy of human recombinant basic fibroblast growth factor (FGF-2) for periodontal regeneration. FGF-2 induces various biological responses including angiogenesis, mitogenesis of mesenchymal cells, and tissue repair¹¹. In this minireview, we present our various *in vivo* and *in vitro* studies on FGF-2-induced periodontal regeneration and the molecular mechanisms responsible (Fig.1).

In vivo effects of FGF-2 on periodontal regeneration

In our laboratory, a series of preclinical animal studies using beagle dogs and non-human primates (*Macaca fascicularis*) had been performed to evaluate the effects of topical application of FGF-2 on periodontal tissue regeneration^{12, 13}. As shown in Figure 2, topical application of FGF-2 significantly promotes periodontal regeneration compared to control site. Interestingly, histological observation also revealed neogenesis of cementum and alveolar bone, augmentation of angiogenesis and regeneration of peripheral nerve fibers at the FGF-2-applied sites^{14, 27} (Fig.3).

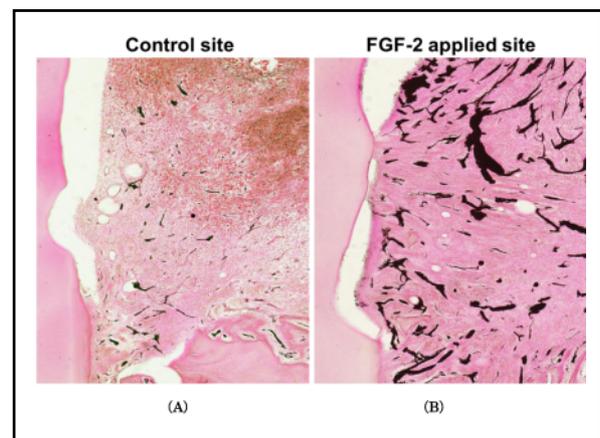


Fig.2 FGF-2-promoted angiogenesis at the defect sites of periodontal tissue

Topical application of 0.3% FGF-2 to artificially created three-walled bony defects in dogs promoted newly formed blood vessels. Blood vessels are shown by perfusion with Indian ink at the sites of bone defects. Representative histological figures of (A) control sites with no treatment and (B) FGF-2-applied sites are shown. Reproduced from ref.23).

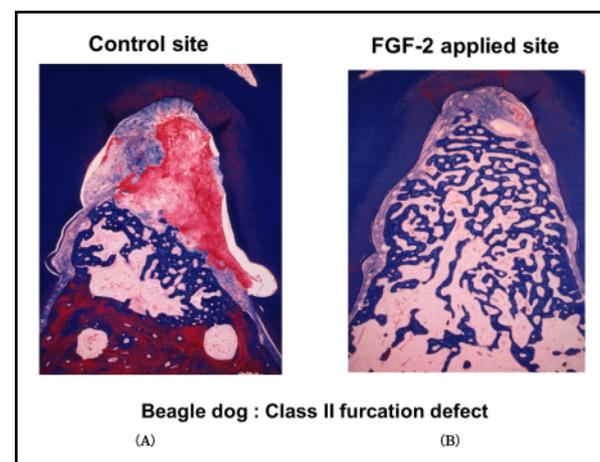


Fig.3 FGF-2-promoted regeneration of periodontal tissue

Topical application of FGF-2 to artificially-created furcation class II bone defects in beagle dogs promoted periodontal tissue regeneration. Representative histologic overviews of furcation defects in carrier-applied sites (A) and FGF-2 —applied sites (B) are shown. Reproduced from ref.27).

Clinical trial of FGF-2 for periodontal regeneration

Several animal studies have demonstrated the efficacy of FGF-2 for periodontal tissue regeneration. We have thus performed a randomized controlled clinical trial to evaluate the safety and efficacy of FGF-2 application. A Phase



Table Rate of bone fill in cases of vertical alveolar bone defects of human

Efficacy Endpoints											
	Vehicle alone		0.2% FGF-2			0.3% FGF-2			0.4% FGF-2		
	mean	SD	mean	SD	P value	mean	SD	P value	mean	SD	P value
% bone fill											
36 wks	15.11	21.90	33.24	33.15	0.003	50.58	31.46	<0.001	46.56	36.09	<0.001

P value :compared with vehicle alone

We measured rates of bone fill in 2- or 3-walled alveolar bone defects at 36 weeks after FGF-2 administration among vehicle (HPC) alone, 0.2% FGF-2, 0.3% FGF-2 and 0.4% FGF-2. This table shows mean bone fill rates (%) and standard deviations. All FGF-2 groups showed significantly increased ($p < 0.001$) bone fill, compared to vehicle alone. The difference between 0.1% and 0.3% FGF-2 groups was also significant ($p < 0.02$).

II clinical trial demonstrated that hydroxypropylcellulose (HPC)-based FGF-2 was effective at promoting the regeneration of periodontal tissue^{15, 16}. HPC is a derivative of cellulose with biodegradability. In Phase IIA trial, patients were randomly divided into four groups (HPC alone, 0.03% FGF-2, 0.1% FGF-2, and 0.3% FGF-2). We found that 0.3% FGF-2 caused a significantly larger increase in alveolar bone height than HPC alone. In Phase IIB trial, patients were randomly divided into four groups comprising four FGF-2 groups (0, 0.2, 0.3 and 0.4% FGF-2). Each dose of FGF-2 showed a significant increase in alveolar bone height at 36 weeks after administration compared to HPC alone, with the most significant increase being observed the 0.3% FGF-2 group (Table). Additionally, no serious adverse effects attributable to the FGF-2 drug were identified in either trial.

Effects of FGF-2 on periodontal ligament cells

FGF-2 has multiple functions on various cells, including promotion of proliferation of mesenchymal cells and enhancement of angiogenesis, both of which could be related to periodontal regeneration. In the process of periodontal tissue regeneration, periodontal ligament cells play important roles^{3, 17}. To clarify the cellular and molecular mechanisms by which FGF-2 promotes periodontal tissue regeneration, we have performed a series of *in vitro* studies to reveal the effects of FGF-2 on periodontal ligament cells. As a result, we revealed that FGF-2 dose-dependently promoted periodontal ligament cell proliferation, and that co-stimulation with fetal calf serum synergistically enhanced FGF-2-induced proliferation¹⁸. We confirmed by RT-PCR

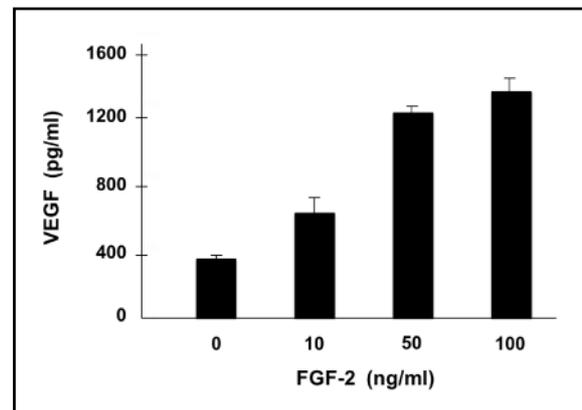


Fig.4 Effects of FGF-2 on VEGF production in human periodontal ligament cells

Human periodontal ligament cells were incubated for 48 hours with various doses of FGF-2, and their supernatants tested by ELISA for VEGF secretion. Data are expressed as mean \pm SD of three assay determinations.

that periodontal ligament cells express FGFR1 and FGFR2 mRNA¹⁹. We then investigated alkaline phosphatase (ALPase) activity and calcified nodule formation in periodontal ligament cells and found that both were significantly suppressed in a dose-dependent manner by FGF-2. However, the suppressive effects of FGF-2 on the cytodifferentiation of periodontal ligament cells into hard-tissue forming cells was reversible, and mineralized nodule formations returned when FGF-2-stimulated periodontal ligament cells were re-cultured in the absence of FGF-2. We speculate that FGF-2 cause periodontal ligament cell proliferation by suppressing their cytodifferentiation, while maintaining their multipotent nature.

Cell migration is an essential event for embryogenesis,

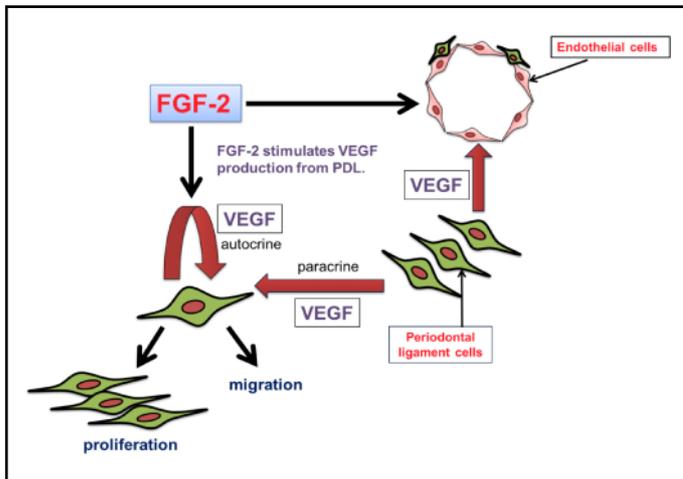


Fig.5 Schematic illustration: Possible mechanisms of FGF-induced periodontal tissue regeneration

Topical application of FGF-2 contributes to periodontal tissue regeneration by regulating the proliferation, migration and extracellular matrix production of periodontal ligament cells (thin arrow). Furthermore, FGF-2-induced VEGF production from periodontal ligament cells co-operates with FGF-2 to promote migration of periodontal ligament cells and tube formation by endothelial cells.

tissue development, wound healing, and tissue regeneration. Because it is important for multipotent cells to migrate to the site where tissue regeneration is needed, we investigated the migratory effect of FGF-2 on periodontal ligament cells and observed significant migration²⁰. The process of cell migration requires multiple coordinated mechanisms such as activation of signaling pathways, membrane-linked cytoskeleton reorganization, and interaction with the extracellular matrix. We described how FGF-2 regulates the biosynthesis of CD44 and hyaluronan, and that the interaction between these molecules leads to migration of periodontal ligament cells²⁰. Furthermore, extracellular matrix, which is important for the support and anchorage of cells modulates various cellular functions. We also found that FGF-2 inhibits collagen biosynthesis but induces the expression of osteopontin and hyaluronan in periodontal ligament cells^{18, 20, 21}.

Another important mechanism by which FGF-2 induces periodontal tissue regeneration is by enhancing angiogenesis, the process of new blood vessel formation that plays important roles in various physiological and pathological conditions such as embryonic development, tumor growth, wound repair and inflammation²². Our previous *in vivo* study demonstrated that FGF-2 application increased blood vessel formation in 3-walled bony defects in the periodontal tissues of beagle dogs²³(Fig.1). Angiogenesis is an indispensable mechanism for tissue regeneration and one of the representative functions of FGF-2. It is well known that FGF-2 directly activates endothelial cells. To investigate our hypothesis that periodontal ligament cells stimulated with FGF-2 were involved in angiogenesis at the site of

tissue regeneration, we studied the effects of FGF-2 on the interaction between periodontal ligament cells and endothelial cells. We found that FGF-2 treatment increased the release of vascular endothelial growth factor (VEGF) expression from human and mouse periodontal ligament cells in a dose-dependent manner (Fig.2). Co-administration of FGF-2 and VEGF synergistically promotes further proliferation and migration of periodontal ligament cells. We observed that FGF-2-stimulated periodontal ligament cells differentiated into cells displaying many characteristics of pericytes (mural cells embedded within the vascular basement membrane of blood microvessels)²⁴, and helped to support vessel tube formation (unpublished data). Based on these results, we now speculate that FGF-2 induces local secretion of VEGF, which binds to VEGF receptors on periodontal ligament cells in an autocrine/paracrine manner and induces their proliferation and migration. In addition, periodontal ligament cells stimulated with FGF-2 (and/or VEGF) differentiate (or at least change phenotypic characteristics) into pericyte-like cells, which could be an important step in the regeneration of periodontal tissue after FGF-2 application (Fig.3).

Stem Cell therapy

The majority of current periodontal regeneration therapies that involves the application of cytokines depend on endogenous tissue stem cells that exist in periodontal ligament tissue. However, aging negatively influences the number and biological properties of these periodontal ligament stem cells²⁵. The potential use of stem cells isolated from other tissues to supply cells for regeneration of alveolar



bony defects is highly desirable. Many researchers are attempting to utilize somatic mesenchymal stem cells isolated from tissues such as bone marrow and adipose tissue to enhance periodontal regeneration. Some groups, including ours, have demonstrated that transplantation of mesenchymal stem cells obtained from adipose tissues enhances periodontal regeneration at applied sites²⁶⁾(ref.26 and unpublished data), a cell therapy that could be employed in improving periodontal treatment. It is necessary to assess further any potential synergistic effects of “cytokine therapy” and “stem cell therapy” that may optimize our ability to engineer periodontal tissue regeneration in the future.

Conclusions

Since the early 1990s, many studies have investigated the effects of various cytokines on periodontal tissue regeneration. However, current regenerative procedures are limited to certain indications such as intrabony defects. The ultimate goal of periodontal therapy is to accomplish the full functional and anatomical regeneration of periodontal tissues disrupted by the progress of periodontal disease. Our research will continue to investigate and develop novel therapeutic procedures for periodontal tissue regeneration.

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Conflict of interest

None

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