

Role of Fibroblast Growth Factor in Periodontal Regeneration

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ABSTRACT

Progression of periodontal disease results in destruction of the main supportive dental structures. To counteract this phenomenon, various periodontal regenerative procedures have been suggested. These procedures normally rely on growth factors-containing materials to enhance optimal regenerative outcomes. Several growth factors have shown significant results in periodontal regeneration. This review focuses on basic fibroblast growth factor 2 (FGF-2) and its documented role in periodontal regenerative procedures. Through an electronic search in the PubMed database and a manual search in relevant journals, evidence from in vitro experiments, animal studies, and human trials is presented and the best treatment conditions are discussed. A review of clinical trials indicates the ease of use and reduced costs of FGF-2 treatment in comparison with other growth factors. However, additional randomized clinical trials and long-term studies are required to fully confirm its clinical efficacy.

Keywords

Bone fill, Growth factors, Periodontal regeneration, Periodontics.

Introduction

Progression of periodontal disease tends to cause degradation of the main supportive dental structures, such as gingival tissue, periodontal ligaments, cementum, and bone [1]. Guided tissue regeneration (GTR) was proposed in 1986 for the management of tissue degeneration. GTR regulates cell migration through the use of a physical barrier and consequent space creation, with or without the addition of bone grafting material [2,3]. However, GTR failed to play a direct active role in stimulating undifferentiated mesenchymal cells' proliferation, migration, differentiation, or attachment within the periodontal ligament [1]. This led to the development of cytokine- and growth factor-based therapy capable of directly stimulating mesenchymal cells to differentiate and form the required cell types. This, in turn, improved regeneration quality, recovery time, and wound healing [4]. Growth factors are biologically active polypeptides with an immediate effect on immune response and function, cells proliferation, differentiation, and maturation [5]. Several growth factors have yielded significant results in periodontal regeneration. They include platelet-derived

growth factor [6], platelet-rich plasma [7], transforming growth factor beta [8], fibroblast growth factor (FGF) [9], insulin-like growth factor [10], plasma-rich fibrin [11], bone morphogenic protein (BMP-2) [12], and epidermal growth factor [13]. FGF encompasses a family of polypeptides that act as potent regulators of cell growth and differentiation. FGF was discovered in 1974 as a strong inducer of proliferation in fibroblasts from the pituitary gland of cows [14]. The FGF family includes 22 genes classified into seven subfamilies based on sequence similarity [15,16]. The most common types of FGF are FGF-1 or acidic FGF (aFGF) and FGF-2 or basic FGF (bFGF). FGF-2 is secreted mainly by fibroblasts of the periodontal ligament and is trapped in the extracellular matrix between gingival epithelium cells where it binds to heparin sulfate proteoglycan. FGF-2 receptors have been documented in periodontal ligament cells. FGF-2 is also capable of enhancing angiogenesis, cell migration, and wound healing. Recent experimental studies have reported the ability of FGF-2 to regenerate periodontal intrabony defects [17]. The aim of this review is to assess the effect of FGF-2 in periodontal regeneration based on findings from in vitro, animal, and human studies.

Methods

An electronic search using the PubMed database of the U.S. National

Library of Medicine, National Institutes of Health (Bethesda, MD, USA) was performed in December 2018. An additional manual search was conducted through the following journals: Journal of Periodontology, Journal of Clinical Periodontology, The International Journal of Periodontics and Restorative Dentistry, The International Journal of Oral and Maxillofacial Implants, and Clinical Oral Implant Research. Key search terms included “fibroblast growth factor”, “recombinant human fibroblast growth factor”, “periodontal regeneration”, “intra-bony defects”, “intra-bony defects”, and “furcation involvement”.

All available publication years were searched, although papers published in the last ten years were given greater consideration.

In Vitro Evidence of FGF-2 Treatment of Intra-bony Defects and Furcation Involvements

A recent in vitro study assessed gene expression and cell proliferation during periodontal regeneration, following the topical application of beta-tricalcium phosphate (β -TCP) on conditioned bovine dentin surface. Dentin slices (1.0×0.5×0.3 cm) were sectioned from the roots of bovine incisors, weighted, and divided in three batches of 12 slices each. The first batch contained six slices conditioned first with 25% citric acid and then 10 ng FGF-2, as well as six slices that received 10 ng FGF-2 only. The second batch consisted of six slices conditioned first with 25% citric acid and then 50 ng FGF-2, as well as six slices treated topically with 50 ng FGF-2 only. The third batch comprised of 12 slices without any growth factor, which served as controls. FGF-2 release, gene expression, and DNA content were assessed. Results revealed significantly increased cell proliferation as a result of increased FGF-2 release from conditioned dentin slices; however, no effect was seen regarding the expression of targeted genes [18].

Animal Trials Showing the Effect of FGF-2 Treatment of Intra-bony Defects and Furcation Involvements

Multiple animal studies have evaluated the effect of applying FGF-2, either alone or in combination with other regenerative materials, such as BMP-2, enamel matrix derivate (EMD) or β -TCP, to optimize the regenerative enhancement of these factors. These treatments have shown significant periodontal regeneration in both intra-bony defects and furcation areas.

Intra-bony Defects

Two- and three-wall defects were surgically created in a beagle dog model and were filled with FGF-2 (50 g/site) associated with a fibrin carrier. Sites filled with fibrin alone served as controls. In three-wall defects, there was no clear difference between test and control groups after one week. However, bone formation appeared higher in FGF-2-containing sites after two weeks, and was accompanied by increased periodontal ligament formation along with small deposits of cementum. Interestingly, no ankyloses, root resorption or epithelial down growth was observed [19].

FGF-2 showed significant regenerative ability when applied in 18 surgically created three-wall defects located in edentulous areas mesial to canines (3×3×4 mm), bilaterally in both the maxilla and

mandible of nine beagle dogs. FGF-2 was incorporated within gelatin microspheres to control its release and sandwiched with collagen sponge, which served as a scaffold in one of the defects. The sponge alone served as a control group on the contralateral side. Active vascularization and osteogenesis were observed in the FGF-2-treated group after just four weeks, with 2.4 mm of new cementum having formed and functionally oriented periodontal ligaments, whereas no cementum had formed in controls ($p<0.01$) [20].

Two studies provided evidence of optimum periodontal regeneration when FGF-2 was combined with β -TCP particles and applied to surgically created two-wall bony defects in 15 young male dogs. Histometric analysis performed under a scanning electron microscope after four weeks showed increased new bone formation in the β -TCP/FGF-2 group compared to the FGF-2 and β -TCP groups (76.3% vs. 59.3% and 65.3%; $p<0.01\%$), as well as new cementum formation (81% vs. 61.8% and 68.3%; $p<0.01$) [21].

Another study documented the regenerative effect of FGF-2 following the addition of EMD. The combined EMD/ β -TCP/FGF-2 treatment was applied in 16 one-wall bony defects (5×5×5 mm) in the lower premolars and first molar extracted from four healthy dogs. The outcome was compared to treatment with β -TCP alone, EMD/ β -TCP, and FGF-2/ β -TCP. After ten weeks, the EMD/ β -TCP/FGF-2 group showed more new cementum formation (4.31 mm vs. 2.02 mm; $p<0.05$) and connective tissue attachment (0.32 mm vs. 1.79 mm; $p<0.05$) than the other three conditions [22].

Tanaka et al. suggested a possible mechanism of action for FGF-2 based on the application of 0.3% FGF-2 with 3% hydroxypropyl cellulose (HPC) in three-wall bony defects created in 16 beagle dogs. Results showed enhanced periodontal regeneration accompanied by an increased number of blood vessels in the first seven days; this was followed by new cementum, periodontal ligament formation, and increased BMP-2 expression. The latter, in particular, was suggested to induce mesenchymal stem cell differentiation into osteoblasts and new bone formation after 28 days [23].

In light of these findings, Lee et al. evaluated the synergistic effect of a serial application of FGF-2 and BMP-2 on periodontal regeneration in 12 one-wall defects mesial to the lower mandibular first molars with a split mouth design. BMP-2 alone served as the control. Histologic and histomorphometric analysis revealed a significant amount of regeneration when compared to BMP-2 alone, with new cementum (24.7% vs. 18.6%; $p<0.05$) and bone formation (54.4% vs. 44.4%; $p<0.05$) after eight weeks. Interestingly, after four weeks, teeth treated with BMP-2 alone exhibited greater bone formation than the combined FGF-2/BMP-2 treatment (40.1% vs. 34.9; $p<0.05$) [24].

Similarly, six two-wall defects in a mandibular canine model were created in six healthy beagle dogs to evaluate the effect of FGF-2 immobilized in porous β -TCP. Three dimensional analysis was

performed two to eight weeks post application of FGF-2/porous β -TCP and compared results to treatment with porous β -TCP alone. The combination therapy resulted in a significantly higher bone density mineral content in a relative short period of time, beginning at two weeks after application and through the eighth week. These results indicated that FGF-2 might play a vital role in reducing the time required for periodontal regeneration; however further studies should be performed to confirm this hypothesis [25].

Furcation Involvements

A study on beagle dogs evaluated the effect of FGF-2 on periodontal healing in furcation areas. Grade II furcation defects (4 mm vertical and 3 mm horizontal) were surgically created in mandibular premolars and molars. Polyvinyl siloxane impression material was placed in the furcation to induce inflammation. One month later, defects were surgically accessed, degranulated, root planed, and filled with FGF-2 (30 g/site) combined with a gelatinous carrier. The samples were compared with furcations in which the same carrier was applied alone. Six weeks later, histomorphometric analysis revealed significant new bone formation compared with the control (79.6% vs. 42.8%; $p < 0.01$), new trabecular bone formation (41.4% vs. 21.8%; $p < 0.01$), and new cementum formation (75.8% vs. 34.3%; $p < 0.01$) [19].

A similar model with a split mouth design was set by surgically creating 12 grade II furcation defects (4 mm vertical and 3 mm horizontal) in the lower first molars in six female dogs. Polyvinyl impression material was placed in the defects. After four weeks, the flap was raised and 0.1% FGF-2 (30 g/site) was applied along with a gelatinous carrier, while the carrier alone was placed in the contralateral side. Histomorphometric analysis showed significant new bone formation (83.6% vs. 35.4%; $p < 0.01$), trabecular bone formation (44.1% vs. 16.6%; $p < 0.01$), and deposits of cementum (97% vs. 37.2%; $p < 0.01$) after six weeks [26].

Comparable results were achieved in grade II furcation defects accrued naturally due to periodontal disease seven weeks post application of 0.4% FGF-2 in combination with a gelatinous carrier. The percentage of new bone, new trabecular bone, and new cementum formation was significantly higher when compared to carrier alone (54.7% vs. 6.2%, 68.3% vs. 24%, and 69.2% vs. 31.7%; $p < 0.05$). Notably, FGF-2-treated sites exhibited also enhanced angiogenesis and regeneration of peripheral nerve fibers [10].

Application of 0.3% FGF-2 mixed with β -TCP was evaluated in 30 grade III furcation defects created in the lower premolars in five beagle dogs. This was compared to 0.3% FGF-2 alone or no material application. Histometric analysis eight weeks post surgery revealed significantly greater connective tissue attachment and massive bone regeneration, reaching the fornix of the furcation, in the β -TCP/FGF-2 group than in the other two groups. New bone formation in β -TCP/FGF-2, FGF-2, and control samples was as follows: 75.8%, 51.5%, and 24.3%, respectively ($p < 0.01$); whereas new cementum was recorded in 84.1%, 71.8%, and 23.4% ($p < 0.01$)

of samples, respectively. Epithelial down growth amounted to 2%, 12.9%, and 48.1%, respectively ($p < 0.05$). Neither root resorption nor ankyloses were observed in these groups [27].

To evaluate the synergistic effect of FGF-2 in periodontal regeneration, Murakami et al. conducted a trial on primates with six grade II furcation areas. Histomorphometric analysis eight weeks post surgery revealed significant cementum (71.2% vs. 38.9%; $p < 0.01$), new bone (71.3% vs. 54.3%; $p < 0.01$), as well as new trabecular bone formation (48.7% vs. 31.6%; $p < 0.01$) following topic application of FGF-2 and a gelatinous carrier, as opposed to gelatinous vehicle alone [19].

A second primate model was developed to test two different concentrations of FGF-2 and thus establish optimum regeneration results. Accordingly, 0.1% or 0.4% FGF-2 was applied with a gelatinous carrier and the mixture was compared to carrier alone in 32 grade II furcation involvements in four male primates. After eight weeks, histometric analysis showed significant periodontal regeneration as indicated by new bone growth in animals treated with 0.4% and 0.1% FGF-2 vs. carrier alone (71.3% and 58% vs. 54.3%, respectively; $p < 0.05$), as well as new trabecular bone growth (48.7% and 36.8% vs. 31.6%; $p < 0.01$) and new cementum formation (72.2% and 79.1% vs. 38.8%; $p < 0.01$). Thus, as suggested by these findings, the regenerative ability was clearly dose-dependent [28].

Human Studies Showing the Effect of FGF-2 Treatment of Intrabony Defects and Furcation Involvements

Following the promising results about FGF-2 in animal trials, FGF-2 has been tested further in several human studies. Kitamura et al. investigated its use on 74 patients, who were diagnosed with two- and three-wall intrabony defects 3 mm apical to the crestal bone. Different concentrations of freeze-dried FGF-2 (0.3%, 0.1%, and 0.03%) were used along with HPC, which served as a vehicle and control. A significant difference was found between 0.3% FGF-2/HPC and the control group (0% FGF-2/3% HPC) after 36 weeks, as determined by increased alveolar bone height (0.95 mm vs. 1.85 mm; $p = 0.021$). In contrast, no significant difference was found in terms of clinical attachment gain [29]. The same authors performed a multi-centered randomized clinical trial on 253 patients with periodontitis and intrabony defects (two- and three-wall defects, > 3 mm or deeper, apical to the remaining bone crest). Each participant contributed with one defect. Inclusion criteria for this study were: degree II or less tooth mobility and sufficient attached gingival tissue. Different concentrations of FGF-2 (0.2%, 0.3%, and 0.4%) mixed with HPC were applied. Results indicated that all concentrations were superior to HPC alone. Interestingly, when comparing the effect of different concentrations of FGF-2 for 36 weeks, the highest percentage of bone fill (50.58%) was obtained with 0.3% FGF-2 ($p < 0.01$). Two mechanisms of action of FGF-2 were proposed. According to the first, FGF-2 led to direct stimulation and proliferation of mesenchymal cells in periodontal ligaments, which then differentiated into either cementoblasts or osteoblasts. The second involved stimulation of angiogenesis and production of different types of extracellular matrix, such as

hyaluronic acid (HA) and osteopontin [30]. In 2016, Kitamura et al. confirmed that bone fill was significantly more pronounced with 0.3% FGF-2/HPC than HPC alone (37.1% vs. 21.5%; $p < 0.001$) after 36 weeks in a randomized controlled trial. However, they found no significant difference between the two groups in terms of clinical attachment re-gain. Consequently, a second part of the study compared the use of FGF-2, EMD, and flap surgery to treat the same defects. After 36 weeks, linear bone growth was 1.9 mm in the FGF-2 group and 1.3 mm in the EMD group ($p < 0.05$) [31]. Regarding the use of different carriers for FGF-2, Santana and Santana suggested HA was optimal due to its good bidirectional mechanism. The authors compared open flap debridement (OFD) alone to OFD with FGF-2/HA to treat intrabony defects in 30 patients with at least two such contralateral defects. A significant difference was found between the FGF-2 group and the OFD group in terms of probing depth (5.5 mm vs. 2.9 mm; $p < 0.05$) and probing attachment level gain (4.8 mm vs. 2.2 mm; $p < 0.05$), but not probing bone level and recession [32].

FGF-2 alone has been used also for treating aggressive periodontitis. A case report revealed an 86.9% increase in alveolar bone height following a 36-week treatment of intrabony defects with 0.3% of FGF-2, as well as good crestal bone stability after a six-year follow-up period [33]. Finally, the use of FGF-2 has been investigated also in combination with bone graft substitute. A comparison between different concentrations of FGF-2 (0.4%, 0.3%, and 0.17%) and β -TCP bone graft for the treatment of intrabony defects revealed that three weeks after surgery bone fill was 75% in the group receiving 0.3% FGF-2 and 63% in the control group. However, this difference was not considered significant ($p < 0.05$) [34].

Conclusion

Based on evidence discussed in this review, FGF-2 has been found to have a potential beneficial effect on tissue healing and periodontal regeneration. Specifically, it seems to increase the percentage of bone fill, reduce probing depth, and increase periodontal attachment level, particularly if mixed with controlled release systems (specific types of vehicles). By comparing the effect of different concentrations of FGF-2, the highest percentage of bone fill was achieved with 0.3% FGF-2. The use of FGF-2 to treat periodontal regeneration is promising due to ease of use and reduced costs when compared to other growth factors. Nevertheless, more randomized clinical trials with larger sample sizes are required to confirm previous findings and to understand the growth factor's precise effect. Finally, a longer evaluation period is required to fully confirm the clinical efficacy of FGF-2.

References

1. Murakami S. Periodontal tissue regeneration by signaling molecules. what role does basic fibroblast growth factor (FGF-2) have in periodontal therapy? *Periodontol.* 2000; 2011; 56: 188-208.
2. Murphy KG, Gunsolley JC. Guided tissue regeneration for the treatment of periodontal intrabony and furcation defects. A systematic review. *Ann Periodontol.* 2003; 8, 266-302.
3. Reynolds MA, Aichelmann-Reidy, ME, Branch-Mays GL, et al. The efficacy of bone replacement grafts in the treatment of periodontal osseous defects. A systematic review. *Ann Periodontol.* 2003; 8: 227-265.
4. Langer R, Vacanti JP. *Tissue engineering.* Science 1993; 260: 920-926.
5. Bartold PM, Walsh LJ, Narayanan AS. Molecular and cell biology of the gingiva. *Periodontol.* 2000; 24: 28-55.
6. Hollinger JO, Hart CE, Hirsch SN, et al. Recombinant human platelet-derived growth factor: biology and clinical applications. *J Bone Joint Surg Am.* 2008; 90: 48-54.
7. Aiba-Kojima E, Tsuno NH, Inoue K, et al. Characterization of wound drainage fluids as a source of soluble factors associated with wound healing: comparison with platelet-rich plasma and potential use in cell culture *Wound. Repair Regen.* 2007; 15: 511-520.
8. Dereka XE, Markopoulou CE, Vrotsos IA. Role of growth factors on periodontal repair. *Growth Factors.* 2006; 24: 260-267.
9. Galzie Z, Kinsella AR, Smith JA. Fibroblast growth factors and their receptors. *Biochem Cell Biol.* 1997; 75: 669-685.
10. Zhao G, Monier-Faugere MC, Langub MC, et al. Targeted overexpression of insulin-like growth factor I to osteoblasts of transgenic mice: increased trabecular bone volume without increased osteoblast proliferation. *Endocrinology.* 2000; 141: 2674-2682.
11. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part II: platelet-related biologic features. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2006; 101: 45-50.
12. Marie PJ, Debais F, Haÿ E. Regulation of human cranial osteoblast phenotype by FGF-2, FGFR-2 and BMP-2 signaling. *Histol Histopathol.* 2002; 17: 877-885.
13. Nordlund L, Hormia M, Saxen L, et al. Immunohistochemical localization of epidermal growth factor receptors in human gingival epithelia. *J Periodont Res.* 1991; 26: 333-338.
14. Gospodarowicz D, Bialecki H, Thakral TK. The angiogenic activity of the fibroblast and epidermal growth factor. *Exp Eye Res.* 1979; 28; 501-514.
15. Itoh N, Ornitz DM. Evolution of the Fgf and Fgfr gene families. *Trends Genet.* 2004; 20: 563-569.
16. Ornitz DM, Itoh N. Fibroblast growth factors. *Genome. Biol.* 2001; 2: 3005.
17. Parkar MH, Kuru L, Giouzei M, et al. Expression of growth-factor receptors in normal and regenerating human periodontal cells. *Arch Oral Biol.* 2001; 46: 275-284.
18. Rocha FRG, Souza JAC, Guimarães-Stabili MR, et al. Topical application of bFGF on acid-conditioned and non-conditioned dentin: effect on cell proliferation and gene expression in cells relevant for periodontal regeneration. *J Appl. Oral. Sci.* 2017; 25: 689-699.
19. Murakami S, Takayama S, Ikezawa K, et al. Regeneration of periodontal tissues by basic fibroblast growth factor. *J Periodont Res.* 1999, 34, 425-430.
20. Nakahara T, Nakamura T, Kobayashi E, et al. Novel approach to regeneration of periodontal tissues based on in situ tissue

- engineering: effects of controlled release of basic fibroblast growth factor from a sandwich membrane. *Tissue Eng.* 2003; 9: 153-162.
21. Oi Y, Ota M, Yamamoto S, et al. β -tricalcium phosphate and basic fibroblast growth factor combination enhances periodontal regeneration in intrabony defects in dogs. *Dent Mater J* 2009; 28: 162-169.
 22. Shirakata Y, Takeuchi N, Yoshimoto T, et al. Effects of enamel matrix derivative and basic fibroblast growth factor with β -tricalcium phosphate on periodontal regeneration in one-wall intrabony defects: An experimental study in dogs. *Int J Periodontics Restorative Dent.* 2013; 33: 641-649.
 23. Nagayasu-Tanaka T, Anzai J, Takaki S, et al. Action mechanism of fibroblast growth factor-2 (FGF-2) in the promotion of periodontal regeneration in beagle dogs. *PLoS One.* 2015; 10: e0131870.
 24. Lee A.R, Choi H, Kim J.H, et al. Effect of serial use of bone morphogenetic protein 2 and fibroblast growth factor 2 on periodontal tissue regeneration. *Implant Dent.* 2017; 26: 664-673.
 25. Matsuse K, Hashimoto Y, Kakinoki S, et al. Periodontal regeneration induced by porous alpha-tricalcium phosphate with immobilized basic fibroblast growth factor in a canine model of 2-wall periodontal defects. *Med Mol Morphol.* 2018; 51: 48-56.
 26. Murakami S, Takayama S, Kitamura M, et al. Recombinant human basic fibroblast growth factor (bFGF) stimulates periodontal regeneration in class II furcation defects created in beagle dogs. *J Periodontal Res.* 2003; 38: 97-103.
 27. Saito A, Saito E, Kuboki Y, et al. Periodontal regeneration following application of basic fibroblast growth factor-2 in combination with beta tricalcium phosphate in class III furcation defects in dogs. *Dent Mater J.* 2013; 32: 256-262.
 28. Takayama S, Murakami S, Shimabukuro Y, et al. Periodontal regeneration by FGF-2 (bFGF) in primate models. *J Dent Res.* 2001; 80: 2075-2079.
 29. Kitamura M, Nakashima K, Kowashi Y, et al. Periodontal tissue regeneration using fibroblast growth factor-2: randomized controlled phase II clinical trial. *PLoS One.* 2008; 3: e2611.
 30. Kitamura M, Akamatsu M, Machigashira M, et al. FGF-2 stimulates periodontal regeneration: results of a multi-center randomized clinical trial. *J Dent Res.* 2011; 90: 35-40.
 31. Kitamura M, Akamatsu M, Kawanami M, et al. Randomized placebo-controlled and controlled non-inferiority phase III trials comparing trafermin, a recombinant human fibroblast growth factor 2, and enamel matrix derivative in periodontal regeneration in intrabony defects. *J Bone Miner. Res.* 2016; 31: 806-814.
 32. de Santana RB, de Santana CM. Human intrabony defect regeneration with rhFGF-2 and hyaluronic acid—a randomized controlled clinical trial. *J Clin Periodontol.* 2015; 42: 658-665.
 33. Yoshinuma N, Koshi R, Kawamoto K, et al. Periodontal regeneration with 0.3% basic fibroblast growth factor (FGF-2) for a patient with aggressive periodontitis: a case report. *J Oral Sci.* 2016, 58, 137–140.
 34. Cochran DL, Oh TJ, Mills MP, et al. A randomized clinical trial evaluating rh-FGF-2/ β -TCP in periodontal defects. *J Dent Res.* 2016; 95: 523-530.