The Promising Clinical Applications of Growth Factors in Periodontal Regeneration: A Literature Review

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Abstract

Periodontitis results in devastation of periodontium, including gingiva, periodontal ligament, cementum, and alveolar bone and eventually lead to tooth loss if the disease is left untreated. The main goals of periodontal therapy are to eradicate the inflammation and to regenerate the lost periodontal structures. Although the biological and biochemical properties of various growth factors have been shown, the predictability and clinical success of periodontal regenerative therapy is not fully established. Growth factors are naturally occurring polypeptide molecules which have the potential to stimulate or regulate cellular events in wound healing process. Ongoing research into the use of growth factors and delivery systems offers the potential to directly influence a future strategy of periodontal therapy.

The purpose of this review is to discuss current evidences of the following biological agents: platelet-derived growth factor, transforming growth factor-β, bone morphogenetic protein and fibroblast growth factor for the clinical applications in periodontal regenerative treatment.

Review (J Int Dent Med Res 2017; 10(3): pp. 1021-1028)

Keywords: Growth Factors, Regeneration, Periodontitis.

Received date: 25 August 2017 **Accept date:** 11 September 2017

Introduction

Periodontitis is characterized by an inflammatory destruction of periodontal ligament, cementum and adjacent bone, resulting in the eventual loss of the teeth. The important goals of periodontal therapy are the elimination of the infections and the resolution of chronic inflammation in order to establish a healthy periodontium and, if possible, restore lost tissues to their original form and function. Various treatment approaches have been suggested for promoting periodontal tissue regeneration (GTR), using different barrier membranes and bone graft materials that have gained clinical acceptance in the treatment on certain types of periodontal defects. Based on the understanding of the biological function, growth factors (GFs) have been evaluated for their potential to promote periodontal wound healing and regeneration.[1,2]

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Such biologically active substances, used either alone or in combination with other GTR materials, have also been tested for their efficacy to improve regenerative outcomes in infrabony defects[3] or furcation lesions.[4] The predictability of using GFs to treat periodontal defects may be limited due to instability and quick dilution in the target site, using GFs with controlled release delivery vehicles, such as scaffolds, may represent a new and promising treatment approach.

The purpose of current review is therefore to analyze the current evidence supporting the potential of GFs in periodontal regeneration.

Literature Review

Growth factors (GFs) are a diverse group of hormone-like polypeptides that modulate importance cellular responses such as cell adhesion, proliferation, chemotaxis, differentiation and matrix synthesis via binding to specific cell surface receptors. They are generally represented by homologous families containing several members with distinct overlapping receptor interactions and hence, mediate different cellular responses.

Similarly, their receptors are also clustered in family groups of sequence-related proteins.

It becomes logical to use these molecules to achieve the desired cell population which result in the reconstruction damaged periodontium through biomimetic processes that occur during the generation of periodontal tissues in the developing embryo and postnatal life.[5]

These well-orchestrated physiological events suggests that creating the optimal environment for regeneration requires a combination of several GFs as found in natural repair processes. The use of a single recombinant GF may also induce several cascades of molecular, biochemical and morphological signals that will result in tissue regeneration.[6]

GFs share several common features. They are naturally occurring cell products that are released or activated when cell division is needed. GFs exert their biological effects via an interaction with a specific receptor present at the target cell surface. The production of GFs in normal cells is a highly regulated process. In contrast, abnormal production of GF is thought to be a key component of cancer and other proliferative disorders. GFs are multifunctional proteins, meaning that they may stimulate a wide variety of cellular activities. GF can stimulate the same cell that secrete them or can affect nearby cells. Tissue regeneration *in vivo* probably reflects the combined effect of several different GFs.

GFs	PDGF	TGF-β	BMP	FGF
Source	Degranulating platelets	Degranulating platelets	Osteoblasts	Macrophage and osteoblasts
Mechanism of action	Mainly chemotaxis and mitogenesis	- Differentiation of mesenchymal cells into osteoblasts - Fibroblast accumulation - ECM production	- Proliferation of osteoblasts - Differentiation of osteoblasts - Increased mineralization - Expression of ALP and OC	- Proliferation of PDL cells - Migration of PDL cells - Differentiation of PDL cells - ECM production
Indications	1) Intrabony defects 2) Furcation defects 3) Gingival recession	1) Intrabony defects 2) Furcation defects	Systemic or anatomic condition where successful bone regeneration cannot be achieved with conventional grafts	1) Peri-implant defects 2) Intrabony defects
Commercially available	GEM21S (rhPDGF-BB; Osteohealth)	Not yet available	- Infuse bone graft (BMP-2; Medtronic) - Osigraft (BMP-7; Stryker Biotech)	Not yet available

PDL: periodontal ligament, ECM: extracellular metrix, ALP: alkaline phosphatase, OC: osteocalcin

Table 1. Summary of growth factors.

Advances in gene cloning technology have made large amounts of recombinant growth factors for applications in tissue regeneration. Recombinant growth factors known to promote periodontal hard and soft tissue healing, such as platelet-derived growth factor, transforming growth factor beta, bone morphogenetic proteins and fibroblast growth factor (Table 1) have been used in pre-clinical and clinical trials for the treatment of large periodontal or infra-bony defects, as well as around dental implants.

Platelet-derived growth factor (PDGF)

The PDGFs are a family of dimeric disulfide-bound GFs that exert their biologic effects by activating 2 tyrosine kinase receptors, the PDGF-α and PDGF-β receptors, which dimerize upon ligand binding. The PDGF family is encoded by four different genes namely PDGF-A, -B, and the most recently discovered PDGF-C and -D. In nature, PDGF can exist as a homodimer (PDGF-AA, PDGF-BB, PDGF-CC and PDGF-DD) or heterodimer (PDGF-AB).

During blood clotting, PDGF is released from platelets after degranulation. It is released from macrophages, endothelial cells smooth muscle cells and fibroblasts cells at the site of wound healing. Therefore, it has been proposed that this GF stimulates early healing events in the wound repair process. PDFG stimulates cell proliferation at physiological concentrations ranging between 0.1 and 1 ng/ ml and promotes cell chemotaxis in various cell types, including monocytes, gingival fibroblasts and periodontal ligament cells.[7,8] Level of several matrix molecules, like proteoglycan and collagen were also increased on cultures of periodontal fibroblasts stimulated by PDGF.[9] Up to now, PDGF-BB is the most effective form for stimulating the potential for regeneration of the periodontal tissues.

Role of PDGF in periodontal regeneration

PDGF is the first GF to be evaluated in periodontal regenerative studies. It was discovered by Lynch and coworkers to promote regeneration of periodontal ligament, cementum and alveolar bone in the late 1980s. After that, its ability to stimulate periodontal regeneration has been studied extensively both preclinical and clinical experiments. Thereby, recombinant human PDGF-BB (rhPDGF-BB) has been approved by the US Food and Drug

Administration (FDA) for treatment of periodontal related defects (e.g., intra-bony defects, furcations, and gingival recessions defect). The commercially available product of rhPDGF-BB is GEM 21S, (Osteohealth, Boston, MA) which uses β-tricalciumphosphate (β-TCP) as a carrier. This product has been extensively tested in preclinical and clinical studies including both animal and human participants.

Animal studies have investigated the role of rhPDGF in bone defects, showing interesting results in which this growth factor facilitates tissue repair. Using lateral ridge defects in dogs, Schwartz and coworkers observed that rhPDGF-BB, in combination with biphasic calcium phosphate as a carrier, stimulated neovascularization and bone regeneration.[10]

Clinical studies have corroborated the promising potential shown in both *in vitro* and preclinical studies. Camelo and coworkers revealed that rhPDGF-BB, in association with insulin growth factor, demonstrated a significant increase in bone fill at 9 months postoperatively.[11] In the studies of Nevins and coworkers, the authors documented that rhPDGF-BB, in combination with allogenic bone, may stimulate periodontal regeneration in class II furcation defects and interproximal intra-bony lesions.[12,13] Recently, systematic reviews have concluded that treatment with PDGF-BB demonstrated significantly more defect fill and clinical attachment level (CAL) gain than carriers alone in intra-bony defects.[14]

The therapeutic potential of this GF for the treatment of gingival recessions have been evaluated in clinical trials. McGuire and coworkers compared rhPDGF in combination with beta-tricalcium phosphate and a collagen membrane with connective tissue grafts[15] and coronally advanced flaps.[16] The results showed that PDGF appear to lead to stable, clinically effective results in reduction of gingival recession, increased root coverage, and increased height of keratinized tissue.

rhPDGF-BB has also shown interesting results in implant related therapies such as vertical and horizontal bone augmentation[17], sinus augmentation procedures[18], and ridge preservation procedures[19]. Overall, results have demonstrated faster and greater new bone formation, reduced healing times, and enhance tissue regeneration when compared to control groups.

These studies demonstrated that PDGF has potential in stimulating bone formation and periodontal regeneration and indicate that it holds the potential as an adjunct to periodontal surgery. However, the clinical efficacy of this material promoting guided bone regeneration (GBR) and treatment of peri-implant defects and its long-term effect remain to be investigated.

Transforming growth factor beta (TGF-β)

TGF-β is a member of a large family of cell regulatory proteins that are structurally related, but differ markedly in their function. Three closely related isoforms of TGF-β exist in mammals (TGF-β1, TGF-β2, and TGF-β3). These proteins exert their biological activities by binding to a heteromeric complex containing the type I and type II TGF-β receptors. It was found that both type I and type II TGF-β receptors are up-regulated during wound healing in periodontal tissues.[20] TGF-β1, the most abundant isoform of the TGF-β family and mainly produced by gingival epithelial cells and macrophage, plays important roles in the regulation of periodontal inflammation and wound healing.

The three major activities of TGF-β include biphasic effects on cell proliferation, regulation of extracellular matrix deposition and exhibition of complex immune regulatory properties. It can also modulate other GFs like PDGF, epidermal growth factor (EGF) and FGF. The cell responses to TGF-β are critically depends on the stage of differentiation of the cell and the tissue microenvironment. In this scenario, it has been reported that granulation tissue fibroblasts responded to TGF-β1 through an increase in the production of urokinase, a proteolytic enzyme critical for tissue remodeling. On the other hand, TGF-β1 inhibited urokinase production or did not respond to this GF in healthy gingival fibroblasts.[21]

TGF-β has a role in recruitment of osteoprogenitor cells and their subsequent proliferation, suggesting that this GF support periodontal wound healing and regeneration.[22] In addition, TGF-β generally acts as a weak mitogen for human osteoblastic cells and increases bone matrix formation by cells of the osteoblastic lineage.[23] Experimental evidence suggests that TGF-β may be involved in coupling bone formation by osteoblast to bone resorption by by osteoclast. Thus, this GF plays a critical role in bone remodeling and maintaining

postnatal bone mass.

In periodontal research, Teare and coworkers, binary applications of rhOP-1 and rhTGF-β3 in Matrigel matrix were implanted in furcation defects of *P. ursinus* to induce periodontal tissue regeneration. Sixty days after implantation, histological and histomorphometric studies led the authors to conclude that rhOP-1 and rhTGF-β3 synergized to induce substantial periodontal regeneration and cementogenesis.[24] According to the recently published data, TGF- β is a cost-effective clinical strategy for induction of bone formation.[25]

In a more recent pilot study, Ripamonti and coworkers evaluated cementogenesis and alveolar bone induction during *in vivo* periodontal tissue regeneration upon implantation of rhTGF-β3 in furcation defects of *P ursinus*. The authers found that rhTGF-β3 induced cementogenesis and osteogenesis with TGF-β3, cementum protein-1 and osteocalcin up-regulation.[26]

Evidence presented in these studies points that TGF-β has therapeutic potential for periodontal tissue regeneration; however, future studies should be performed especially *in vivo* to understand the regulation of this GF in various clinical conditions of periodontal diseases.

Bone morphogenetic protein (BMP)

The name BMP was given in 1965 by Urist and colleagues to the active components in demineralizing bone and bone extracts. BMPs are members of TGF-*β* superfamily which over 20 BMPs with various functions have been identified in humans to date. BMP is a dimeric molecules critically dependent on the single intermolecular disulfide bond for biological activity. Based on their sequence similarity and known functions, BMPs are typically divided into at least four distinct subfamilies: BMP-2 (osteogenic protein (OP)-2)/BMP-4; BMP-5, BMP-6, BMP-7 (OP-1), BMP-8a and BMP-8b; BMP-9 and BMP-10; and BMP-12, BMP-13 and BMP-14 and growth differentiation factor-5. BMP binds to specific BMP type I and BMP type II receptors, triggering specific intracellular pathways that promote the differentiation of mesenchymal cells into chondroblasts and osteoblasts, which inducing new bone formation. Moreover, BMP regulates the expression of a wide array of target genes involved in bone physiology, including alkaline phosphatase, osteocalcin, osteopontin and osterix.[27]

Recombinant human BMP-2 (rhBMP-2: Infuse Bone Graft, Medtronic Spinal and Biologics, Memphis, TN, USA) and recombinant human BMP-7 (rhBMP-7: Osigraft, Stryker Biotech, Malmö, Sweden) are currently the only proteins in the group to been approved by the US Food and Drug Administration (FDA) for clinical use in humans, which explains why they are clearly the most extensively evaluated BMPs in the periodontal field. Several in vitro experiments have demonstrated that BMP stimulates PDL cell differentiation into osteoblasts and increase expression of mineralized tissue markers.[28] Induction and maintenance of bone formation by the BMPs occur in a synergistic and synchronous way with a wide variety of different proteins modulating in the process.[29]

Both preclinical and clinical trials have investigated the therapeutic potential BMPs applied locally into damaged tissues. Miyaji and coworkers used beagle dog model to evaluate the effect of application of recombinant human BMP-2 (rhBMP-2) to exposed roots, the formation of cementum-like tissue was frequently observed.[30] Using BMP-2 expressed in mesenchymal stem cells, Chung and coworkers demonstrated that periodontal regeneration was occur on periodontal defects created over the mandibular premolar areas in Beagle dogs. They observed formation of newly synthesized cementum, bone and Sharpey fibers in BMP-treated defects.[31] Lee and coworkers evaluated a bio-absorbable membrane loaded with rhBMP-2 in a rabbit calvarial defect, more bone formation was found after loading with rhBMP-2 compared with control unloading membranes.[32]

In histological studies in baboons, Ripamonti and coworker assessed the efficacy of recombinant human osteogenic protein-1 (rhOP-1) or rhBMP-7 when implanted in furcation defects exposed surgically or by inflammatory processes in *Papio ursinus*.[33] Long-term study after rhBMP-7 implantation showed a well-organized periodontal ligament space with periodontal ligament fibers cursing from the new mineralized cementum to the new generated alveolar bone, with abundant of supporting capillaries. In the same animal model, a combination of rhOP-1 and rhTGF-β have been conducted in animal studies regarding the periodontal regeneration properties of these GFs.[24]

Human clinical studies utilizing combined two BMPs or in combination with other growth factors have provided insight as to their potential use. Crude preparations of BMP-2 and BMP-3 applied in surgically created furcation defects appeared to stimulate periodontal regeneration.[28] Using the dual delivery BMP-2 and insulin-like growth factor-1 (IGF-1) has been shown to improve bone formation at the defect margins in a rabbit femoral osteotomy defect over BMP-2 alone at 8 weeks post-implantation.[34]

Saito and coworkers utilized rhBMP to determine their potential for treating intrabony, supra-alveolar, furcation, and fenestration defects. Histologic analysis revealed periodontal regeneration with areas of ankylosis. Healing through ankylosis and root resorption have been a concern, so most of the recent research utilizing rhBMPs has involved in the preparation of implant site for ossteointeration or for implant site development (i.e., wound healing, ridge preservation and sinus augmentation).[35] Using combined BMP-2 gene therapy with allograft enhanced the defect healing and improved the strength of implant fixation with osseointegration in a bone defect at the bone-implant interface.[36]

Misch conducted human case series for bone augmentation of atrophic posterior mandible prior to implant placement, using rhBMP-2/absorbable collagen sponge and titanium mesh. After a 6-month healing period, dental implants were placed in grafted sites without the need for further bone augmentation. All 10 implants became integrated and were restored with single crowns.[37]

Up until now, BMP-2 represent a very promising alternative to autogenous bone grafts for alveolar ridge/maxillary sinus augmentation. However, more studies are still needed to figure out the best dose and carrier as well as to compare its effectiveness to the popular allogenic bone grafts for clinical application.

Fibroblast Growth Factor (FGF)

The FGFs are membranes of the heparin binding growth factor family. This GF was discovered in 1970s as a protein inducing proliferative activity in fibroblasts. In human, more than 20 members of the FGF family that have similar characteristics have been identified. Among these members, FGF-2 or basic FGF (bFGF) has been studied extensively in regenerative medicine and periodontal tissue regeneration. FGF-2 exert their biologic effects by activating its cognate receptor, FGF receptor (FGFR)-2 and FGFR-3.

FGF-2 stimulated proliferation and differentiation of numerous cell types involved in periodontal wound healing including fibroblasts, osteoblasts and endothelial cells.[38] In addition, FGF-2 possesses a potent angiogenic and mitogenic activity on mesenchymal cells within periodontal ligament space.[39]

Role of FGF-2 in periodontal regeneration

Several animal studies have shown that FGF-2 is effective in enhancing the periodontal regeneration process.

Takayama and coworkers surgically created furcation class II bone defects in non-human primates and examined the periodontal regeneration efficiency of topical application of FGF-2 in the bony defects. They concluded that a local application of FGF-2 can significantly enhance periodontal regeneration.[40]

Sato and coworkers examined the effects of bFGF in a collagen gel on the regeneration of periodontal tissue in experimentally induced partial defects in a beagle dog. They observed the formation of dense fibers bound to alveolar bone and new cementum in bFGF treated-defects. The results suggested that bFGF in a collagen gel is a suitable therapy for damaged PDL and could lead to achievable methods of treatment for periodontal defects.[41]

Ishii and coworkers investigated the regenerative potential of basic FGF-2 in combination with beta tricalcium phosphate (β-TCP) on surgically created gingival recession in beagle dogs. They reported that FGF-2/β-TCP enhanced new bone and cementum formation without any significant root resorption in root coverage in this dog model.[42]

Oortgiesen and coworkers investigated the effect of injectable macroporous calcium phosphate cement (CaP) combined with BMP-2 or FGF-2 on the regenerative potential using an established intra-bony periodontal defects in Wister rats. The histology and histomorphometry results presented a good response in terms of bone healing was seen with CaP/BMP-2 and CaP/FGF-2 treatment, while only the CaP treatment revealed limited effects on PDL and bone healing after 12 weeks.

The best results were observed with the combined treatment of CaP and FGF-2,

suggesting that these topical combinations might be a promising treatment for periodontal regeneration.[43]

A recent animal study by Anzai and coworkers evaluated the long-term observation of periodontal tissue regenerated by FGF-2 treatment compared with normal physiological healing tissue controls in artificial 2-wall bony defects by assessing tissue histology and three-dimensional microstructure. At 13 months after treatment, the lengths and height of the regenerated periodontal ligament and cementum as well as area of the newly formed bone in the FGF-2 group were larger than those in the control group. These results demonstrated that the periodontal tissue regenerated by FGF-2 was maintained for 13 months after treatment and was qualitatively equivalent to that generated through the physiological healing process.[44]

For periodontal regeneration in humans, Kitamura and coworkers undertook a multi-center, randomized, double-blind study on the potential of local applications of FGF-2 in periodontal regeneration. 0.2%, 0.3%, or 0.4%FGF-2 or placebo were administered to vertical bone defects during flap surgery. The results showed that percentage of bone fill was significantly greater in the 0.3%FGF-2 group than it was in the placebo group but the clinical attachment level regained was not significantly different between groups at 36 weeks. These results support the efficacy and safety of topical FGF-2 applications for regeneration of periodontal tissue in periodontitis patients.[45-47]

Recently, systematic reviews on treating periodontal intra-bony defects have concluded that although limited available evidence suggests that FGF-2 improves defect fill but it does not have a significantly effect on CAL gain.[14] Therefore, FGF-2 might be another promising candidate for bone augmentation but more clinical studies are required to prove their therapeutic effectiveness.

Currently, FGF-2 has not been extensively studied yet in the field of periodontics. Further studies should continue focusing on exploring its efficacy, safety, and proper dosage for FGF2 to be effective in different clinical situations.

Delivery systems

One limitation of GFs application *in vivo* seems to be the unpredictable nature of the resulting tissue regeneration. It has been suggested that the clinical efficacy of rhBMPs depends on the carrier system used to ensure an effective delivery of adequate protein concentrations to the site being treated and to control its release pattern.[48] Thus, the development of new delivery devices has improved the efficacy of GFs delivery for clinical use. Two common types of carrier materials used in GF delivery are natural-origin polymers (i.e., collagen matrix,[49,50] extracellular matrix-based hydrogel[32,47]) and synthetic biodegradable polymers (i.e., poly[lactide-co-glycolide]). Bioabsorbable controlled-release vehicles have been fabricated to sequester GFs and release them at optimal doses in a timely manner depending on the biological demand of the target tissue. The potential applications of gene therapy have recently expanded to include the treatment of periodontal or peri-implant defects. This approach addresses potential to genetically modify the target cells to sustainability express of the required GFs with in periodontal wounds which may provide better periodontal tissue regeneration.[51] Few studies have investigated gene delivery via adenoviral vectors or nonviral vectors such as synthetic liposomes carrying genetic information for encoding rhGFs combined with a collagen matrix in animal models.[52,53] The results of these study showed a significantly positive effect of growth factor gene delivery on autologous bone formation and osseointegration in bone defects. However, patient safety must be take into account when specific vectors and delivery methods, such as a natural or synthetic carriers were used.

Conclusions

Several pre-clinical and clinical studies have showed significant regeneration of periodontal tissues with the application of GFs. According to this present review, PDGF and BMPs have demonstrated promising results, mainly in animal studies. Although these biological agents proved to be effective as an adjuvant to various approaches in periodontal regeneration, basic principles of periodontal surgery, appropriate patient and/or site selection remain important to achieve optimal treatment outcomes.

More research is needed to further understand these biologics, providing more

information with regard to its efficacy, optimal condition, long term effects and safety. Moreover, future studies should focus on developing a carrier for the delivery of GFs to increase its overall efficacy for periodontal regeneration.

Acknowledgements

This study was supported by Naresuan University Research Grant (R2561B012: fiscal year 2017).

Declaration of Interest

The authors confirm that this article content has no conflict of interest.

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