The ultimate goal of periodontal treatments is the regeneration of all lost periodontal tissues including bone, cementum and the periodontal ligament (PDL). Until now, the clinical methods for periodontal regeneration have been associated with significant failure or incomplete success. Various studies have reported the promising effects of growth factors/cytokines on periodontal regeneration. Growth factors/cytokines include proteins or steroid hormones that bind to cellular receptors, known as signalling molecules, and that trigger cellular responses that eventually stimulate cell proliferation and differentiation. The present review aims to provide an overview of the main growth factors that play an important role in and have been used in the regeneration of periodontal components.

Key words: growth factors, cementogenesis, osteogenesis, periodontal regeneration, stem cells


The supporting structure of the teeth, i.e., the periodontium, is composed of alveolar bone, root cementum, the periodontal ligament (PDL) and the gingiva which cover other components. After completion of tooth crown development, root formation begins and dental follicle stem cells (DFSCs) differentiate to fibroblasts, cementoblasts and osteoblasts to form the PDL, cementum and alveolar bone, respectively. These structures function as a unit in which the principal PDL fibre connects the cementum to the alveolar bone and provides an apparatus to control all forces and support the dentition. Destructive chronic inflammation of these supporting tooth structures, known as periodontitis, eventually results in tooth loss. The ultimate goal of periodontal treatments is the regeneration of all lost periodontal tissue. Until now, the clinical methods for periodontal regeneration have been associated with significant failure or incomplete success, and most have been technique sensitive.

Growth factors/cytokines include proteins or steroid hormones that bind to cellular receptors, known as signalling molecules, and result in cellular responses that eventually stimulate proliferation and differentiation. Various studies have reported the promising effects of these signalling molecules on periodontal regeneration. The present review aimed to provide an overview of the main growth factors that play an important role in and have been used in the regeneration of periodontal components.

Table 1 summarises the included studies and Fig 1 illustrates the main growth factors that play an important role in the regeneration of each periodontal component.
Growth factors used in alveolar bone regeneration

Bone morphogenetic proteins (BMPs)

Bone morphogenetic proteins (BMPs) are multifunctional growth factors that are so-called due to their osteoinductive properties and are part of the transforming growth factor-beta (TGF-β) superfamily which enhances mineralisation in several tissues. BMPs can also recruit endogenous MSCs and osteoblasts to defect sites. Over 20 types of BMPs have been shown to differentiate MSCs into osteoblasts; however, the most potent factors among them include BMP-2, 4, 6, 7, 9 and 13. In a study by Cheng et al. on different types of BMPs on the osteogenic activity of MSCs and osteoblastic cells, it was shown that BMP-2, 6 and 9 may play an important role in inducing osteoblast differentiation of MSCs. BMPs have been found to regulate the differentiation of MSCs mainly through Smad proteins. Moreover, BMPs can function in the form of heterodimers, including BMP-4/7, 2/7 and 2/6/9.

Açil et al. found that BMP-7 induces osteoblast/cementoblast differentiation of PDL stem cells (PDLSCs) and DFSCs in a dose- and time-dependent manner. BMP-2, 6 and 7 increase biomineralisation of hPDLSCs. The most pronounced induction of biomineralisation of hPDLSCs occurs in BMP-6.

BMP-7 increases the expression of osteoblastic genes in human gingiva-derived MSCs. BMP-9 promotes osteogenesis in DFSCs. BMP-2, 6 and 7 increase biomineralisation of hPDLSCs. The most pronounced induction of biomineralisation of hPDLSCs occurs in BMP-6.

PTH regulates the differentiation of DFSCs into osteoblasts. BMP-7 mediates cementogenesis of PDLSCs and DFSCs in a dose- and time-dependent manner. BMP-3 inhibits BMP-2 mediated osteoblastic differentiation and enables maintenance of the PDL and root cementum.

EMD affects both proliferation and differentiation of PDLSCs. Induces DFSCs towards the cementoblast phenotype. Induces the formation of cementum-like structures on teeth affected by periodontal disease.

Table 1: Growth factors used in periodontal complex regeneration.

<table>
<thead>
<tr>
<th>Location</th>
<th>Growth factor</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone</td>
<td>BMPs</td>
<td>BMP-7 induces PDLSCs and DFSCs towards the osteoblast/cementoblast in a dose- and time-dependent manner. BMP-7 increases the expression of osteoblastic genes in human gingiva-derived MSCs. BMP-9 promotes osteogenesis in DFSCs. BMP-2, 6 and 7 increase biomineralisation of hPDLSCs. The most pronounced induction of biomineralisation of hPDLSCs occurs in BMP-6.</td>
</tr>
<tr>
<td>PTH</td>
<td></td>
<td>Regulates the differentiation of DFSCs into osteoblasts.</td>
</tr>
<tr>
<td>IGF</td>
<td></td>
<td>Increases the volume of newly formed bone following tooth extraction. Promotes osteogenic differentiation and osteogenesis but decreases the odontogenic differentiation and dentinogenesis capacity.</td>
</tr>
<tr>
<td>Vit D</td>
<td></td>
<td>Stimulates osteoblastic differentiation with the subsequent increase of bone mineral matrix deposition.</td>
</tr>
<tr>
<td>Cementum</td>
<td>EMD</td>
<td>Affects both proliferation and differentiation of PDLSCs. Induces DFSCs towards the cementoblast phenotype. Induces the formation of cementum-like structures on teeth affected by periodontal disease.</td>
</tr>
<tr>
<td>PTH</td>
<td></td>
<td>Is essential for cementoblast differentiation. Improves the stability of tooth movement by promoting periodontal regeneration.</td>
</tr>
<tr>
<td>CP-23</td>
<td></td>
<td>Differenitates both DFSCs and PDLSCs to the cementoblast lineage.</td>
</tr>
<tr>
<td>BMP</td>
<td></td>
<td>BMP-7 mediates cementogenesis of PDLSCs and DFSCs in a dose- and time-dependent manner. BMP-3 inhibits BMP-2 mediated osteoblastic differentiation and enables maintenance of the PDL and root cementum.</td>
</tr>
<tr>
<td>PDL</td>
<td>FGF2</td>
<td>Decreases the expression of osteo/cementogenic markers in hPDLCs. Increases the expression of teno/ligamentogenic markers in PDLSCs. Induces PDLSCs towards fibroblastic differentiation and inhibits mineralisation. Stimulates and maintains the fibroblastic feature in hPDLSCs.</td>
</tr>
<tr>
<td></td>
<td>TGF-β</td>
<td>TGF-β1 suppresses the proliferation of PDL cells and contributes to fibroblastic differentiation. Increases tenomodulin but decreases scleraxis. TGF-β3 enhances periodontal tissue regeneration significantly.</td>
</tr>
<tr>
<td></td>
<td>PDGF</td>
<td>PDGF-BB enhances mitogenesis and matrix biosynthesis in PDLSCs and enhances alveolar bone formation and cementogenesis in large periodontal bone defects.</td>
</tr>
</tbody>
</table>

BMP, bone morphogenic protein; CP-23, human cementum protein 1 (also CEMP-1); DFSCs, dental follicle stem cells; EMD, enamel matrix derivative; FGF2, fibroblast growth factor 2; hPDLSCs, human PDL stem cells; IGF, insulin-like growth factor; MSCs, mesenchymal stem cells; PDGF, platelet-derived growth factor; PDLSCs, PDL stem cells; PTH, parathyroid hormone; TGF-β, transforming growth factor β; Vit D, vitamin D.
associated genes in human PDLSCs in a time and dose-dependent manner. They suggested that BMP-2, 6 and 7 are potent regulators for gene expression and biomineralisation of human PDLSCs; however, the effect of BMP-6 on mediating the mineralisation of human PDLSCs was superior to BMP-2 and 718.

Parathyroid hormone (PTH)

Both parathyroid hormone (PTH) and PTH receptor (PTHrp) are important signals that regulate osteoblastic differentiation8. PTHrp is required for several different regulations of enchondral bone development, differentiation of bone precursor cells and development of craniofacial tissues.

The dental follicle, which is involved in tooth eruption and alveolar bone regeneration, has been found to express a large amount of PTH19. Klingelhöffer et al19 showed that PTH participates in the early phase of osteogenic differentiation in DFSCs. The PTH-PTHrp autocrine signal maintains the physiological cell fates of DFSCs to establish the functional periodontal attachment apparatus and orchestrates tooth eruption20. Moreover, Pieles et al21 reported that PTH supports the expression of BMP-2, which is strongly involved in the osteogenic differentiation of DFSCs.

Insulin-like growth factor (IGF)

Insulin-like growth factor (IGF) is signalling through type 1 receptor that stimulates cell proliferation, function and survival of osteoblasts22. IGF-1 can regulate osteogenic differentiation in an endocrine, paracrine or autocrine manner which is regulated by a family of six IGF binding proteins (IGFBPs). IGFBP-3 and 5 have been shown to stimulate the actions of IGF-1, whereas IGFBP-1, 2, 4 and 6 are known inhibitors of IGF 1 in bone23.

In a study by Wang et al24, stem cells from the apical papilla (SCAPs) treated with IGF-1 showed an increase in osteogenic differentiation; however, the odontogenic differentiation and dentinogenesis capacity of SCAPs was reduced significantly. A similar study by Feng et al25 showed that IGF-1 triggers early-stage osteogenic differentiation and maintains the later-stage osteogenic differentiation of dental pulp stem cells (DPSCs). IGF-1 was also administrated following tooth extraction in a diabetic rat model, and the results showed that IGF-1 not only increased new bone formation but also normalised the expression of glucose transporter 1 in diabetic rats26.

Vitamin D (Vit D)

Vitamin D (Vit D) is crucial for bone mineralisation as well as maintenance of calcium homeostasis. It has also been shown to play an important role in the proliferation and differentiation of MSCs to osteoblasts27,28. Vit D is an important regulator of Runt-related transcription factor 2 (RUNX2), with which it cooperates in inducing the expression of osteocalcin, which is also a key protein that regulates osteoblastic differentiation29. Posa et al30 showed that Vit D treatment increases osteogenic differentiation of tooth bud stem cells (DBSCs).
**Growth factors used in cementum regeneration**

**Enamel matrix derivative (EMD)**

Enamel matrix derivative (EMD) is an enamel matrix extract that mainly contains amelogenins, which have been shown to be involved not only in formation of enamel but also in that of the periodontal attachment. Several studies have illustrated the effect of EMD on the proliferation and differentiation of PDLSCs. Davenport et al. showed that in the presence of EMD, human PDL fibroblasts differentiate to cells more similar to cementoblasts than fibroblasts. The amelogenins can form an insoluble extracellular matrix that has a high affinity for collagens and hydroxyapatite, and histological evaluation has shown that it can form acellular cementum which is essential for PDL fibre attachment. Kenmou et al. found that EMD induced human DFSCs towards the cementoblastic phenotype through BMP-dependent pathways. Moreover, two in vivo studies confirmed that new cementum was formed following administration of EMD. Bosshardt et al. showed that EMD can induce the formation of cementum-like structures on teeth affected by periodontal disease.

**BMPs**

BMPs are best known for their potential in osteoblastic differentiation; however, several studies have indicated the effect of BMP-7 and BMP-3 on cementoblastic differentiation. Bozic et al. showed the BMP-7 mechanism induces differentiation and mineralisation of cementoblasts, and does so via inducing procollagen COOH-terminal proteinase enhancer 1 (PCPE1) and BMP-1. Torii et al. showed that BMP-7 mediates cementogenesis of PDLSCs via activation of protein tyrosine phosphatase-like, member A/cementum attachment protein (PTPLA/CAP) and cementum-derived protein (CEMP1). Similarly, it has been shown that BMP-7 can induce cementoblast differentiation of PDLSCs and DFSCs in a dose- and time-dependent manner. In the analysis of tooth development by Aberg et al., it was reported that BMP-3 is involved in cementum development. Another study showed that BMP-3 inhibits BMP-2-mediated osteoblastic differentiation. The negative regulation of BMP-3 in mineralisation enables the maintenance of the PDL between bone and root cementum.

**Cementum-derived protein (CEMP1)**

CEMP1, also known as CP23, is well-known as a key marker for cementoblast differentiation; however, it has been shown to be expressed not only in cementoblasts, but also in PDLSCs. An immunohistological evaluation by Alvarez-Pérez et al. showed the distribution of CEMP1 throughout the entire root surface, including acellular and cellular cementum, cementocytes and cells located near the blood vessels in the PDL. Further studies showed that the application of CEMP1 on both DFSCs and PDLSCs differentiate them to the cementoblast lineage, indicating that CEMP1 is a key protein in cementoblast differentiation.

**PTH**

PTH, which is involved in mediating several important biological actions, such as endochondral bone development, promotes cementogenesis in a protein kinase A (PKA) and extracellular signal-regulated mitogen-activated protein kinase 1/2 (ERK1/2)-dependent manner. It has been speculated that PTH could promote cementoblastic differentiation and cementogenesis. Most of the cells expressing PTH are in the dental follicle and on the root surface. The deletion of this peptide receptor (PPR) in these progenitors leads to failure of eruption and significantly truncated roots lacking PDLs. PPR is likely to orchestrate cementoblast differentiation of the progenitors, as the PPR-deficient cells fail to form the acellular cementum, and rather form irregular cellular cementum on the root surface. This phenotype can be interpreted as accelerated and disordered differentiation. Li et al. showed that intermittent PTH administration could promote cementogenesis and regeneration of the tooth root caused by resorption.

**Growth factors used in PDL regeneration**

**Fibroblast growth factor 2 (FGF2)**

Fibroblast growth factor 2 (FGF2), also known as the basic FGF, is a heparin-binding cytokine that plays a role in the inflammatory phase as an anti-inflammatory cytokine, and the proliferative phase of wound healing. Its angiogenic and fibrous tissue forming activity, along with its ability to stimulate proliferation and differentiation of MSCs, make it suitable to be used in wound healing and periodontal regeneration.

Hyun et al. showed have shown that FGF2 acts as a signalling molecule and increases the expression of scleraxis and tenomodulin, early and late teno/ligamentogenic markers, respectively, in human PDLSCs and that FGF2 decreases the expression of osteo/cementogenic markers on hPDLCs and has an antagonist effect on BMPs. A similar observation was made by...
Murakami et al\textsuperscript{60}, who found that FGF2 decreased collagen 1 (COL1) expression and calcification. It therefore appears that FGF2 guides human PDLSCs towards fibroblastic differentiation and inhibits mineralisation; however, administration of FGF2 in combination with BMP-2 was found to enhance bone regeneration both in vitro and in vivo\textsuperscript{61,62}.

Nagayasu-Tanaka et al\textsuperscript{63}, in a dog model, showed that periodontal regeneration in the presence of FGF2 was revealed to promote disappearance of blood clots and granulation tissue formation, which are replaced rapidly with new bone; thus, bone formation is more accelerated. Besides, the vascularised connective tissue with tight collagen fibres on the root surface extends coronally from the existing PDL and forms new cementum and PDL with Sharpey’s fibres. Rapid dense connective tissue formation in the presence of FGF2 maintains gingival tissue at higher levels and consequently creates a regenerative space and inhibits further periodontal collapse. The clinical application of FGF2 shows its effectiveness in bone regeneration of periodontal defects; however, there is still a lack of clinical attachment\textsuperscript{64,65}.

**TGF-β**

TGF-β is a superfamily of growth factors with multifunctional effects. Its role in wound healing occurs through its effect on cell proliferation, differentiation and migration. Three isoforms, i.e., TGF-β1, 2 and 3, with significant homology have been detected for TGF-β. It has been reported that the isoforms 1 and 3 which signal through the same receptor complex, TGF-β receptor type II (TβRII)\textsuperscript{66}, play roles in periodontal regeneration\textsuperscript{67,68}.

TGF-β1 has long been recognised as a prerequisite for the differentiation of myofibroblasts that play a key role in the remodelling and reconstruction of connective tissue by the secretion and organisation of the extracellular matrix and by endowing tissue with contractile forces\textsuperscript{69}. Moreover, Fujii et al\textsuperscript{70} showed the exclusive distribution of TGF-β1 throughout the PDL tissues and proved that the amount of TGF-β1 in PDL tissues is greater than that in pulp tissues or alveolar bone tissues, indicating the important physiological role played by TGF-β1 in PDL cells.

TGF-β1 has been shown to suppress the proliferation of PDL cells, while its upregulation of actin alpha 2 (ACTA2), COL1 and fibrillin-1 encoding gene (FBN1) contributes to their fibroblastic differentiation\textsuperscript{71}. Besides, TGF-β1 has been shown to increase tenomodulin but decrease scleraxis\textsuperscript{59}. Hence, subsequent administration of TGF-β1 after FGF2 on the differentiation of PDLSCs into fibroblastic cells has been suggested to accelerate the regeneration of functional periodontium\textsuperscript{71}. TGF-β3 is of particular interest due to its association with dermal wound and tendon healing promotion without fibrotic scar formation\textsuperscript{72}. TGF-β3 has been shown to enhance the proliferation and early differentiation of MSCs into osteoblasts, chondrocytes, adipocytes and tendon cells\textsuperscript{66}. In a study by Moshaverinia et al\textsuperscript{72}, in a mouse model, PDLSCs and gingival stem cells encapsulated in TGF-β3–loaded RGD-modified alginate microspheres showed the successful differentiation of given cells into tendon-like tissue.

**Platelet-derived growth factor (PDGF)**

Platelets can produce and release growth factors and cytokines involved in angiogenesis, inflammation and immune response which eventually enhance tissue repair. PDGF is one of the growth factors stored in platelets\textsuperscript{73} that are actively involved in tissue regeneration and wound healing\textsuperscript{74}. Various studies have confirmed the effect of PDGF on the proliferation of PDL fibroblasts\textsuperscript{75-77}. Three different forms of PDGF, i.e., PDGF-AA, PDGF-AB and PDGF-BB, have been identified\textsuperscript{74,78}. Of these, the efficacy of PDGF-BB in both soft and hard tissue regeneration of the periodontium has been demonstrated most clearly. Studies have shown that PDGF-BB is the most effective form, enhancing PDL cell mitogenesis and matrix biosynthesis\textsuperscript{74,77}. Jin et al\textsuperscript{75} also demonstrated enhanced alveolar bone formation and cementogenesis in large periodontal bone defects using gene therapy with a mode of PDGF-BB delivery in vivo. Furthermore, the use of PDGF-BB in combination with IGF\textsuperscript{79} or FGF\textsuperscript{74} has been shown to improve periodontal regeneration.

**Conclusion**

Optimal periodontal regeneration includes restoration of all periodontium components, i.e., alveolar bone, cementum and PDL. Knowledge regarding specific growth factors and cytokines involved in regeneration of each of them may serve as a basis for development of the therapeutic methods targeting all periodontium components. Future studies in this field should be dedicated to designing a combination of these factors in a single smart delivery system and evaluating the effectiveness of their periodontal complex regeneration.
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Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Drs Fazele ATARBASHI-MOGHADAM and Maryam REZAI RAD contributed to the data analysis; Drs Soran SIJANIV ANDI, Pouya KHODAYARI and Masoumeh MAHMOUM were involved in the data gathering. All authors contributed to the writing and editing of the manuscript.

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