GROWTH FACTORS - PERIODONTAL REGENERATION: CURRENT OPINIONS IN PERIODONTOLOGY

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Abstract

Periodontal tissue has the capacity for repair and regeneration. Periodontal regeneration definition implies the formation of new bone, new cementum and a functionally oriented periodontal ligament.

Introduction:

Periodontitis, evoked by the bacterial biofilm (dental plaque) that forms around teeth, progressively destroys the periodontal tissues supporting the teeth, including the periodontal ligament, cementum, alveolar bone and gingiva1. Thus, the rationale of periodontal therapy is to eradicate the inflammation of the periodontal tissues, to arrest the destruction of soft tissue and bone caused by periodontal disease, and regenerates the lost tissue2. Periodontal surgical procedures have focused on the removal of hard and soft tissue defects by regenerating new attachment3. Periodontal wound-healing studies, however, indicate that conventional periodontal therapy most commonly results in repair rather than regeneration4, 5.

For periodontal regeneration to occur, progenitor periodontal ligament cells need to migrate to denuded root surfaces and attach to it following proliferation and mature into an organized as well as functional fibrous attachment apparatus. Similarly, progenitor bone cells must also migrate, proliferate and mature in conjunction with the regenerating periodontal ligament. Significant advances have been made during the last decade in understanding the factors controlling the migration, attachment and proliferation of cells.

Periodontal repair implies healing after periodontal surgery which results in healing without restoration of the attachment apparatus6. The regeneration of the periodontal tissues is dependent on four basic components. The appropriate signals, cells, blood supply and scaffold needed to target the tissue at the defect site. All these elements play a fundamental role in the healing process and in the reconstruction of the lost tissue.

The cells provide the machinery for new tissue growth and differentiation whereas the growth factors or morphogens modulate the cellular activity and provide stimuli to the cells to differentiate and produce matrix for the developing tissue. The new vascular networks provide the nutritional base for tissue growth and homeostasis. Finally, scaffolds guide and create a template structure three-dimensionally to facilitate the above processes required for tissue regeneration7.

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The major cellular events in tissue repair are mitogenesis, migration and metabolism. In nature, the proteins responsible for co-ordinating these events are growth factors. The naturally occurring molecules acting with matrix proteins act as a key regulator for biological events and therefore, showing pleiotrophic effects in wound repair including the periodontium.

They are natural cell products that are released or activated when cell division is needed. If mesenchymal cells from periodontal ligament or perivascular region of the bone proliferate and colonize the root surface, regeneration occurs.

Naturally occurring molecules such as polypeptide growth factors in conjunction with certain matrix proteins are a key regulator for biological events. Of these, the fibroblast growth factors (FGFs), platelet-derived growth factor (PDGF), insulin-like growth factors (IGFs), epidermal growth factor (EGF) and certain attachment proteins appear to have an important role in periodontal wound healing. Growth factors are believed to have the potential to accelerate the healing process and, therefore, enhance tissue regeneration in challenging clinical scenarios. The hope is to discover how to use them to accelerate and direct the healing event into one that will produce periodontal regeneration.

Growth factors are polypeptide molecules released by cells in the inflamed areas that regulate events in wound healing these are naturally occurring proteins that regulates various aspects of cell growth and development acting locally or systemically to affect the growth and function of cells in several ways.

Exogenous growth factors are the proteins that bind to receptors on the cell surface, with the primary result of activating cellular proliferation and or differentiation. Many growth factors are quite versatile, stimulating cellular division in numerous difficult cell types while others are specific to particular cell type.

Growth factors comprises of molecules that function as growth stimulation but also as growth inhibitors (sometimes referred to as a negative growth factors), factors that stimulate cell migration or as chemotactic agents or inhibits cell migration or invasion of tumour cells, factors that modulate differentiated functions of cells, factors involved in apoptosis, factors involved in angiogenesis, or factors that promote survival of cells without influencing growth differentiation.

**History of growth factors:**
1. Stanley Cohen and Rita Levi-Montalcini in 1952 discovered the first growth factor, nerve growth factor.
2. Another nerve growth factor was purified from snake venom in 1959, the discovery of nerve growth factor (NGF) opened up a huge new area of cell biology, and won the Nobel Prize in 1986.
3. Salton and Daughaday 1957 described Insulin Growth Factors.
4. Urist in 1965 discovered demineralised, lyophilized segments of bone induced new bone formation when implanted in muscle pouches in rabbit. He proposed "Bone Morphogenetic Protein" in the scientific literature in the Journal of Dental Research, 1971.
5. Platelet Derived Growth Factor was discovered by Kohler, Lipton and Ross Eet al in 1974.
6. Vascular Endothelial Growth Factor was first identified in guinea pigs, hamsters, and mice by Senger et al. 1983. It was cloned by Ferrara and Henzel in 1989.
7. VEGF alternative splicing was discovered by Tischer et al 1991. Between 1996 and 1997, Christinger and De Vos obtained the crystal structure of VEGF. VEGF receptor discovered by Ferrara et al. 1992.
8. Fibroblast growth factor was found in pituitary extracts by Armelin 1983 and then was also found in a cow brain extract by Gospodarowiczet al., and tested in a bioassay that caused fibroblast to proliferate (first published report in 1974).
9. Epidermal Growth Factor was further isolated from the submaxilllary gland in a mouse in 1962.
10. Insulin Growth Factor-1 isoform referred to as Cementum Growth Factor which is larger than IGF -1 inmolecule size discovered by Ikezawa et al 1997.
11. Hepatocyte growth factor (HGF) was originally reported in 1984 to be a mitogenic protein for mature hepatocytes in primary culture. The primary structure of HGF became evident with cDNA cloning in 1989. It was purified from the rat platelets in 1986.
Description:
Growth factor is a general term to denote a class of polypeptide hormones that stimulate a wide variety of cellular events such as proliferation, chemotaxis, differentiation and production of extracellular matrix proteins. 

Terranova & Wikesjo 1987:
Growth factor is a general term used to denote a class of naturally occurring proteins that function in the body to promote the mitogenesis (proliferation) directed migration and metabolic activity of cells.

Lynch SE 1994:
Growth factors are natural proteins that regulate the main cellular events involved in tissue regeneration and its application has become an area of increasing interest in periodontal regenerative medicine.

Anitua E et al 2013:
Features of growth factors:
Natural cell products:
Growth factors are natural cell products released or activated when cell division is needed. This action typically occurs during such events as wound healing or tissue regeneration.

Local action:
With few exceptions, growth factors are locally acting.

Receptor activities:
Because growth factors cannot diffuse across the cell membrane, growth factors must exert their activity by first binding to high-affinity cell membrane receptors. The capacity of a cell to respond to a given factor is therefore dependent on the presence of these receptors.

Regulation:
The production of polypeptide growth factors is tightly regulated in normal cells.

Multifunctional activity:
Polypeptide growth factors are multifunctional, meaning that they may stimulate a wide variety of cellular activities, which include growth, migration, differentiation and production of extracellular matrix proteins.

Mechanism of action:
In some cases, growth factors can stimulate the same cell that synthesizes the molecule (autocrine stimulation) or can affect nearby cells (paracrine stimulation).

Regeneration:
Tissue regeneration in vivo probably reflects the combined effect of several different growth factors.

The cell cycle:
Cell division occurs in various stages comprising the cell cycle. The genetic material, cells must replicate their chromosomal DNA. During DNA replication, the cohesion proteins attach to replicated sister chromatids holding them together. This sister chromatid cohesion is critical for the subsequent alignment of each pair of sister chromatids on the mitotic spindle, and it is therefore essential for the subsequent segregation of one (and only one) chromatid of each pair into each of the two daughter cells.

The cell division cycle is divided into four stages such as, G1, S, G2, and M. DNA replication occurs in S ("synthesis") phase. DNA packaging, chromosome segregation and cell division (cytokinesis) occurs in M (mitosis) phase. S phase and M phase are segregated by Gap phase. G1 is the gap phase between M and S. Cell growth is an important event of G1 phase. The transition from G1 to S is called the critical control point in the cell cycle.
G2 is the gap between S and M, and provides time to ensure DNA is replicated and packaged prior to cell division. G0 or quiescence occurs when cells exit the cell cycle due to the absence of growth-promoting signals. Ordered progression through each phase is intricately regulated through both positive and negative regulatory signalling molecules. The G1, S, and G2 phases comprise interphase, which accounts for most of the time in each cell cycle. The M phase is short (approximately 1 hour of a 24 hour cell cycle).

**Cyclin Dependent Kinases: The core activators of cell cycle control system:**
Cell cycle progression is positively regulated by a family of protein kinases called cyclin-dependent kinases (Cdks), which function to turn specific proteins on and off at appropriate times in the cell cycle. Other protein kinases, Cdks turns proteins on or off by phosphorylating them.

Cyclins were first identified as key cell-cycle regulators for they undergo a cycle of synthesis and regulated destruction. There are several different Cdks and a number of cyclins.

**Brakes on the cell cycle: Cdk inhibitors:**
The Rb protein are seen as a “brake” on the cell cycle for it prevents transcription of the gene for cyclin E, by inhibiting E2F. Three other proteins act as “brakes” are the Cdk inhibitors p16, p21, and p27. They act by binding directly to Cdk-cyclin complex and blocking the protein kinase activity.

This class includes receptors linked to protein kinases, which fall into two subgroups: the receptor tyrosine kinases (RTKs) and the receptor serine/threonine kinases.
Transcription / Translation

Fig 2: Transcription/Translation.

Rationale for Growth Factors:
To Accelerate
1. Cell migration.
2. Cell adhesion.
3. Cell differentiation.
4. Cell proliferation.
5. Production of new tissue components/blood vessels. - Faster and more predictable healing

Growth Factors and their Functions

- PDGF, BMP, TGF-β, PGF
- BMP
- PGE
- PDGF, ILGF, PCP
- ILGF, TGF-β

BMF: Bone Morphogenetic Protein
PCGF: Fibroblast Growth Factor
ILGF: Insulin-like Growth Factor
PDGF: Platelet-derived Growth Factor
TGF-β: Transforming Growth Factor-β
Mechanism of Action:
There are a number of mechanisms which can regulate different growth factor pathways. Different signalling pathways can also interact with each other in order to fine tune the final observed outcome. These mechanisms are listed below.
1. The regulation of growth factor activity by the secretion of soluble antagonists which are often regulated in both autocrine and paracrine fashions.
2. The regulation of receptor expression on specific cells
3. The interaction between intracellular signalling mechanisms in both antagonistic and synergistic ways.

Endocrine:
Synthesis by specific cells, target (receptor) is far away. A growth factor produced by one cell and acting on another is described as paracrine regulation. A cell that recaptures its own product is known as autocrine regulation. Few growth factors act during embryogenesis. Interaction by membrane contact is Juxtacrine signalling. Hormones inside the cell regulate signals is Intracrine signalling.
Receptors:
Cell surface receptors:
G-protein linked, Receptor tyrosine kinases, Serine threonine –r-kinases. Intracellular receptor - Vit-D3, Estrogen, Corticosteroids

Fig 5: Receptors On Growth Factors.

Cell differentiation:
1. Osteoblastic pathway
2. Fibroblastic pathway
3. Adipocytic pathway
4. Muscle cell pathway

During the early stages of bone formation, the action of transforming growth factor-b is to recruit and stimulate osteoprogenitor cells to proliferate, providing a pool of early osteoblasts. In contrast, during later phases of osteoblast differentiation, transforming growth factor-b blocks differentiation and mineralization for these effects appears to be dependent on bone cell source and the local environment, resulting in the inhibition of DNA synthesis at high transforming growth factor-b concentrations. Additionally, transforming growth factor-b inhibits the expression of the Runx2 and osteocalcin genes, whose expression is controlled by Cbfa1/Runx2 in osteoblast-like cell lines, and this was found to be mediated by Smad3. Transfoming growth factor-b interacts with a range of other growth factors in bone with a resulting complex response. Further work is needed to clarify the role of transforming growth factor-b during periodontal regeneration and wound healing and to determine the inter-relationship between transforming growth factor-b and other growth factors having effects with different stages of osteoblastic differentiation.

Bone morphogenetic proteins are secreted signalling molecules which have a variety of functions during development and cell differentiation. They were discovered for their remarkable ability to induce cartilage and bone formation from non-skeletal mesenchymal cellsby recapitulating the entire sequence of events occurring during endochondral ossification. Among the BMP family, BMP-2, BMP-4 and BMP-7 have key roles during osteoblast commitment and differentiation. The primary effect of BMPs is on the pluripotent cells that are capable of differentiating into other mesenchymal cell types, and BMP-2 can direct these cells to commit to an osteoblastic pathway, as can BMP-4 and BMP-6. Bone morphogenetic proteins can also increase the differentiation of committed cells to the osteoblast lineage, with the formation of bone nodules and expression of markers of the mature osteoblast phenotype.
The fibroblast growth factors are a family of structurally related polypeptides that are known to play a critical role in angiogenesis and mesenchymal cell mitogenesis. Fibroblast growth factor-1 and fibroblast growth factor-2 in vitro stimulate osteoblast proliferation (in calvarial cells, ROS 17/2.8 and MC3T3-E1) but do not increase collagen production or alkaline phosphatase in differentiated osteoblasts\(^\text{20}\) although these effects may be differentiation stage-specific as constitutive fibroblast growth factor signaling inhibits osteoblastic differentiation\(^\text{21}\) and dramatically increases apoptosis when cells are exposed to differentiating conditions. Fibroblast growth factors are strongly mitogenic to bone marrow stromal cells and are able to maintain the self-renewal of these cells in culture and these growth factors may have potential as an adjunctive agent to increase bone formation.

Platelet-derived growth factor is a powerful mitogen for connective tissue cells, and although it can stimulate, and is synthesized by, mesenchymal cells and osteoblast like cells\(^\text{22}\), it does not have powerful bone-induction properties. Platelet-derived growth factor isoforms have a strong chemotactic effect on osteoblasts and other connective tissue cells\(^\text{23}\), and may act to recruit mesenchymal cells during bone development and remodeling.

Growth hormone and insulin-like growth factors play critical roles in skeletal development. The evidence suggests that the major effects of insulin-like growth factors are to promote the late-stage differentiation and activity of osteoblasts.

![Cell Differentiation](Fig 6:- Cell Differentiation.)
Cell signaling:

Interaction of growth factor intracellular signalling pathways:
There are a number of examples described where different growth factor pathways regulate each other during intracellular signalling. Of particular interest, studies have demonstrated the regulation of SMAD signalling following BMP stimulation by additional growth factor signalling pathways\(^\text{24}\). Recent results have demonstrated the down regulation of BMP-activated SMAD signalling by both transforming growth factor-\(\beta\), via stimulating the expression of inhibitory SMAD\(^{\text{25}}\), and by extracellular signal regulated protein kinase and PI3 kinase pathways, mediated by insulin-like growth factor-1 and other growth factor signals\(^{\text{26}}\). These observations suggest many mechanisms whereby different growth factors regulate responsiveness to other signalling pathways. In this way it is
possible that specific growth factor expression and signalling activity can be very delicately regulated to balance proliferation and differentiation events within cells of the osteoblast lineage.

Cell proliferation:

![Diagram of cell proliferation](image)

*Fig 8:* Cell Proliferation.
Cell death:

Fig 9: Apoptosis.

Receptor tyrosine kinases:
Activation of the Epidermal Growth Factor (EGF) receptor tyrosine kinase:

EGF binding to EGF receptor through an extracellular ligand binding domain. Dimerization causes one subunit to phosphorylate the other (transphosphorylation) on specific tyrosine residues. The SH2 domain of the Grb2 adaptor protein then binds to the region of the EGF receptor containing the phosphorylated tyrosine. Grb2 uses its second common protein domain called SH3, which binds to another protein called Sos. Grb2 is known as an adapter protein, since it functions to hold two other proteins together. Sos is a member of a large family of proteins that regulate G proteins (GTP-binding proteins) by causing the exchange of a tightly bound GDP molecule for GTP. Sos is a guanine nucleotide exchange protein (GEF) that activates the Ras protein.

Ras is a monomeric GTP-binding protein that is only active in its GTP-bound form. Ras is inactive in its GDP form. When Sos binds to Grb2 at the EGF receptor, it is brought close to membrane-bound Ras-GDP molecules, causing the Ras to release its GDP and bind a GTP in its place.

Growth factor signalling pathways mediating proliferation in multipotential stromal cells:

Binding of fibroblast growth factor (FGF) to fibroblast growth factor receptor (FGFR), binding of epidermal growth factor (EGF) and heparin-binding (HB)-EGF to epidermal growth factor receptor (EGFR) and binding of platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) to platelet-derived growth factor receptor (PDGFR) causes phosphorylation of the respective receptors, causes recruitment of the adaptor protein.
Grb2 and the nucleotide exchange factor SOS, which causes activation of downstream pathways, primarily phosphoinositide-3 kinase (PI3K)-Akt/protein kinase B (PKB) and the mitogen-activated protein kinase (MAPK) Erk.

Phosphorylated Erk enters the nucleus and activates transcription of cellular proliferation genes like c-myc, or it activates downstream receptors like Rsk that further activates proliferation genes. Akt similarly prevents the expression of proteins like Myt1 and Wee1, which are involved in inhibiting proliferation. Bone morphogenetic protein (BMP)-2 activates proliferation via the MAPK Erk pathway, unlike BMP-3 that activates Smad2 and Smad3 via Activin signaling. TGFβ3 is a potent transforming growth factor beta (TGFβ) mitogen which causes proliferation via activation of Smad2, Smad3 and Smad4.

Binding of Wnt3a to the Frizzled receptor causes activation of the protein Dishevelled and inactivation of the Axin–APC–Gsk3 complex, which leads to a nuclear influx of β-catenin, activating the cell cycle proteins cyclin D1 and c-myc. TGFβ also causes an influx of β-catenin in a Smad3-dependent manner. Binding of hepatocyte growth factor (HGF) to c-Met under low doses causes activation of Erk and Akt, but with a higher dose, it inhibits proliferation by the activation of p38 MAPK pathway, causing the expression of cell cycle progression inhibitors such as, p21Waf1 and p27Kip. APC, adenomatous polyposis coli protein; Gsk3, glycogen synthase kinase 3; RSK, ribosomal S6 kinase; Smad. Sma and Mad related proteins.

**Growth factor signaling pathways mediating in multipotential stromal cells:**

Vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) binds to PDGFR, and hepatocyte growth factor (HGF) binding to c-Met causes phosphoinositide-3 kinase (PI3K) to activate, further converting PIP2 to PIP3 and activating Akt/protein kinase B (PKB). This leads to the inhibition of the Fork head family of transcription factors Foxo1, Foxo3 and Foxo4, and also causes inhibition of pro-death proteins Bim, Bad and Caspase9.

Binding of epidermal growth factor (EGF) to epidermal growth factor receptor (EGFR), in addition to activating Akt, brings together the guanine nucleotide exchange factor SOS and the small adapter protein Grb2, which activates the mitogen-activated protein kinase (MAPK) pathway: Ras-Raf-Mek1/2-Erk1/2.

All these activated receptors, however, are quickly internalized by clathrin machinery or by alternate internalization mechanisms into the endosome where they continue to signal. The internalization of the EGF–EGF receptor complex continuing to signal in the cytosol, but onceinside the lysosome, the receptor along with the ligand completely degrades and the survival signal is lost. Both the Akt and Erk signals generated therefore are acute and transient.

Tethering of growth factors near to the membrane of EGF (Tegf) causes a more sustained signaling of Erk and Akt leading to multipotential stromal cell (MSC) survival for a more prolonged time period. Bcl2, B-cell lymphoma 2; DAG, diacyl glycerol; Erk, extracellular signal related kinase; eNOS, endothelial nitric oxide synthase; HSP90, heat shock protein 90, IP3, inositol triphosphate; NF, nuclear factor; PDGFR, platelet-derived growth factor receptor; PLCγ, phospholipase C gamma; PIP2, phosphatidylinositol-4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-trisphosphate; XIAP, X-linked inhibitor of apoptosis protein.

**Conclusion:**

These growth factor superfamilies have recognized with specific transmembrane receptors. Many specific examples were presented for illustration of the growth factors for promoting development. For, the complexity of feedback mechanisms through which growth factors coordinate embryogenesis. And, several distinct types of mechanisms are accomplished with which growth factor signaling is conducted.

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