GROWTH FACTORS IN PERIODONTAL REGENERATION

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ABSTRACT

The term growth factor refers to a naturally occurring protein capable of stimulating cellular growth, proliferation and cellular differentiation. Polypeptide growth factors represent a class of biological mediators that regulate critical cellular activities, including migration, proliferation, differentiation, and matrix synthesis. Since the late 1980s, there has been a concerted effort to increase the knowledge of how polypeptide growth factors influence the repair and regeneration of tissues. The various types of growth factors were classified and studied to regenerate the lost periodontal tissues. The aim of this review is to study the growth factors (GFs) used in the periodontal regeneration and also to compare the various studies to understand the probable mode of action of growth factors for regenerating the lost periodontal tissues.

Key Words: Growth Factors, Periodontitis, Periodontal Regeneration, Signalling Molecules.

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INTRODUCTION:

Periodontitis, evoked by the bacterial biofilm (dental plaque) that forms around teeth, progressively destroys the periodontal tissue supporting the teeth, including the periodontal ligament, cementum, alveolar bone and gingiva.[1] Ultimately, this chronic inflammatory disease can lead to loss of the affected teeth. All over the world this disease remains highly prevalent and is considered to threaten quality of life (QOL) of middle-aged and older populations as far as ''oral'' functions are concerned. Some success has been achieved in suppressing progression of periodontitis by mechanically removing bacterial biofilm.[2]

However, removal of the cause, bacterial plaque, with conventional periodontal and/or surgical treatments can, at best, reduce pocket depth and diminish inflammation in the affected region.[3] No such treatment can ever regenerate lost periodontal tissue or normal structure and functionality. Considering that the ''mouth'' and ''teeth'' have various aesthetic and functional roles to play, establishing a brand-new treatment that enables the regeneration and rebuilding of periodontal tissue once destroyed by periodontal disease represents a task of tremendous importance.[4]

Growth Factors:

The term growth factor refers to a naturally occurring protein capable of stimulating cellular growth, proliferation and cellular differentiation. Growth factors are important for regulating a variety of cellular processes. Growth factors typically act as signalling molecules between cells. Examples are cytokines and hormones that bind to specific receptors on the surface of their target cells. Polypeptide growth factors represent a class of biological mediators that regulate critical cellular activities, including migration, proliferation, differentiation, and matrix synthesis.[5]

Since the late 1980s, there has been a concerted effort to increase the knowledge of how polypeptide growth factors influence the repair and regeneration of tissues. These naturally occurring ligands have been shown to have pleiotropic effects; they support regeneration in several settings and accelerate healing processes. They exert their effects by binding to specific cell membrane receptors to initiate complex cascades that eventually reach a nuclear target gene to generate signals for specific phenotype expression.[6]

Examples of polypeptide growth factors in bone, cementum, and healing tissues include platelet derived growth factor, vascular endothelial growth factor etc. They often promote cell differentiation and maturation, which varies between growth factors.[7] For example, bone morphogenetic proteins stimulate bone cell differentiation, while fibroblast growth factors and vascular endothelial growth factors stimulate blood vessel differentiation (angiogenesis).[8]

Growth factors vs cytokines:

The term growth factor is sometimes used interchangeably among scientists with the term cytokine. Historically, cytokines were associated with hematopoietic (blood forming) cells and immune system cells (e.g., lymphocytes and tissue cells from spleen, thymus, and lymph nodes). For the circulatory system and bone marrow in which cells can occur in a liquid suspension and not bound up in solid tissue, it makes sense for them to communicate by soluble, circulating protein molecules.[9]

While growth factor implies a positive effect on cell division, cytokine is a neutral term with respect to whether a molecule affects proliferation. While some cytokines can be growth factors, such as G-CSF and GM-CSF, others have an inhibitory effect on cell growth or proliferation. Some cytokines, such as Fas ligand are used as "death" signals; they cause target cells to undergo programmed cell death or apoptosis.

History of Growth Factors:

In 1917, Neuhof was the first to describe heterotopic osteogenesis, followed by Huggins in 1930. Growth factors were first described when substances such as blood fluids (for example, foetal calf serum) and tissue extracts were added to cells in tissue culture. Their effect was to alter, usually stimulate, cell proliferation, differentiation or migration.[8]

The study of growth factors is still largely a tissue culture-based (in vitro) science; however, currently there is a great deal of interest in the identification of growth factors in living animal and human systems (in vivo) and in their clinical applications. In the early days of growth factor/cytokine discovery they were being described with great rapidity and, in many cases, the same molecules were given different names by different investigators.[11] Urist et al. (1965)[12] reported that in rats, demineralized bone tissue intramuscular implantation led to formation of new bone.

Functions of Growth Factors

Growth factors regulate cell activity by a number of mechanisms. Importantly, all these may occur simultaneously, and in different tissues where the effects may be different, depending upon the conditions.[13]

1. Mitogenic activity

This was one of the earliest discovered mechanisms of growth factor action because in certain concentrations growth factors substantially increase the rate of cell turnover. It has been proposed that mitosis is stimulated by many growth factors through secondary intracellular messengers. Platelet derived growth factor (PDGF) and epithelial growth factor (EGF) are typical growth factors which stimulate mitosis through the regulation of intracellular calcium and pH.

2. Cell differentiation

The growth factors stimulate cell differentiation and inhibit mitosis. A classic example of this is TGF-ß. It is considered that at least one of the TGF-ß families of growth factors can initiate cellular differentiation.

3. Cell migration

Cell migration is an extremely complex cellular activity. The precise interaction between extracellular substance, growth factors and intracellular events is important in the overall process of migration. Many growth factors influence cell migration.

4. Gene regulation

Growth factors that regulate mitosis and differentiation obviously regulate gene activity. It is now well understood that growth factors operate through highly complex intracellular pathways to regulate intracellular pH and calcium, thereby influencing genetic activity. Insulin-like growth factor 2 (IGF-2), EGF and PDGF are examples of factors which regulate genes.

Mechanism of Action:[14]

Growth factors are among the most potent of biological substances being biologically active at concentrations of picograms per millimetre. The specificity and magnitude of the response is believed to be mainly due to the type and abundance of membrane bound receptors rather than availability of the growth factors. Growth factors are not typical cell nutrients which act within cells, but rather are secreted by cells and interact with specific, membrane-bound glycoprotein receptors, which are crucial in the conversion of the first message into second message or intracellular message which can act within the cell and initiate a series of biochemical reactions that eventually result in cell division.

Biological mediators for growth factors activation:[15]

1)Growth factor receptor 2)Second messengers

3)Transcription factors

Growth factor receptor (biochemistry):

In biochemistry, a receptor is a protein molecule, embedded in either the plasma membrane or cytoplasm of a cell, to which a mobile signalling (or "signal") molecule may attach.

Types of receptors :[16]

Receptors can be roughly divided into two major classes:

1. Cell-surface receptors and

2. Intracellular receptors

Ligand-gated ion channel receptors are a class of receptor that may occur both at the cell-surface and intracellularly. There are many different classes of transmembrane receptor that recognize different extracellular signalling molecules. The examples of receptors are:

1. G-protein coupled receptors, e.g., Chemokine receptors.

2. Receptor tyrosine kinases, e.g., Growth factor receptors.

3. Integrins

4. Toll-like receptors

Individual growth factor proteins tend to occur as members of larger families of structurally and evolutionarily related proteins. There are many families which are listed below:

All this growth factors Stimulates cell cycle from G0 phase to G1 phase.

E P MU RF D? ARMP	MP CE CL Q	CDDCA RQ
Cngbcpk _j epmu rf d_armp	èActivated macrophages •Salivary glands •Keratinocytes	èI cp_rgl mawrc _l b dg`pm`j_qr mitogen •Keratinocyte migration •Granulation tissue formation
Rp_l qdmpk gl e epmu rf d_armp-α	è? arg t _rcb macrophages •T-lymphocytes •Keratinocytes	èF cn_rma wrc _l b cngrhelial cell proliferation •Expression of antimicrobial peptides
F cn_rma wrc epmu rf d_armp	èK cqc l af wk _j cells	èCngrf cjg j _l b cl bmrf cjg_j cell proliferation •Hepatocyte motility
T _qa sj_p cl bmrf cjg_j epmu rf d_armp	èK cqc l af wk _j cells	èT _qa sj_p permeability •Endothelial cell proliferation
Nj_rcjcr bcpg t cb epmu rf d_armp	èNj_rcjcrq •Macrophages •Endothelial cells •Smooth muscle cells •Keratinocytes	èE p_l sjma wrc*k _apmmf_ec* fibroblast and smooth muscle cell chemotaxis •Granulocyte, macrophage and fibroblast activation •Fibroblast, endothelial cell and smooth muscle cell proliferation •Matrix metalloproteinase, fibronectin and hyaluronan production •Angiogenesis •Wound remodelling •Integrin expression regulation
Dg`pm`j_qr epmu rf d_armp/ _l b 0	èK _arophages •Mast cells •T-lymphocytes •Endothelial cells •Fibroblasts	èDg`pm`j_qr af ck mr_vgq •Fibroblast and keratinocyte proliferation •Keratinocyte migration •Angiogenesis •Wound contraction •matrix deposition
Rp_l qdorming growth factor-β	èNj_rcjcrq •T-lymphocytes •Macrophages •Endothelial cells •Keratinocytes •Smooth muscle cells •Fibroblasts	èE p_l sjma wrc*k _apmmf_ec* lymphocyte,fibroblast and smooth muscle cell chemotaxis •TIMP synthesis •Angiogenesis •Fibroplasia •Matrix metalloproteinase production inhibition •Keratinocyte proliferation
I cp_rgl mawrc epmu rf d_armp	èDg`pm`j_qrq	èI cp_rgl mawrc k gep_rgml* proliferation and differentiation

Application of Growth Factors in Periodontal Regeneration

Bone Morphogenetic Proteins (BMPs):

The studies indicated that the primary actions of BMPs are to differentiate mesenchymal precursor cells into cartilage- and bone-forming cells. TGF-β, IGFs, and FGFs all affect the already differentiated or committed bone-forming cells present in the bone, causing them to divide and/or increase secretion of extracellular matrix molecules. By affecting the cells of the bone itself, they have somewhat limited capacity for regeneration.[17] On the other hand, rhBMP-2 will affect the precursor cells, presumably cells from the marrow environment and the soft tissue surrounding the defect site, and stimulate them to infiltrate the defect area and differentiate into cartilage and bone cells.[18]

Wikesjo¨ et al. evaluated hBMP-12 for periodontal tissue regeneration, particularly periodontal ligament formation. hBMP-12 and hBMP-2 were implanted on absorbable collagen sponges in periodontal defects and the results were compared after 60 days of healing. Greater bone regeneration was observed in implants treated with hBMP-2, but ankylosis was noted. Defects treated with hBMP-12 showed less bone regeneration, but exhibited a functionally oriented periodontal ligament system inserting into newly formed cementum.[19]

Platelet Derived Growth Factor:

Its primary effect is as a potent mitogen, initiating cell division, and as a chemotactic factor for cells of mesenchyme origin, including osteoblasts. Several subtypes of PDGFs exist; they consist of homodimers or heterodimers of the PDGF-A and PDGF-B gene products. The most intense area of PDGF research involves the use of platelet-rich-plasma (PRP).

Only recently it has been found that a platelet-derived growth factor receptor is present in osteoblast.[20] Although little is known about the regulation of platelet-derived growth factor AA synthesis, some evidence suggested that platelet derived growth factor AA activity is regulated at the level of receptor binding. Platelet-derived growth factor BB might be critical in wound healing or fracture repair, since it is released after platelet aggregation. It may also play an entirely different role in bone cell physiology than that of platelet derived growth factor AA.[21]

The use of recombinant human PDGF for Periodontal Regeneration

Histological evidence of periodontal regeneration was first reported in defects in beagle dogs. During the development of platelet-derived growth factor for clinical use, recombinant human platelet derived growth factor was used in conjunction with allogenic bone to correct class II furcations and interproximal intrabony defects on teeth with poor prognosis which are to be extracted.[22] Histological evidence of periodontal regeneration was present with excellent furcation fill in this study as well as other studies also.[23]

Subsequently, the effectiveness of 0.3 mg / ml of recombinant human platelet-derived growth factor and tricalcium phosphate (GEM-21) in the improvement in clinical attachment level and bone level were studied. The tricalcium phosphate demonstrated a significant amount of regeneration after 6 months. Another case series suggested that recombinant human platelet derived growth factor with freeze-dried bone allograft can be combined to achieve excellent results in severe periodontal intrabony defects.

Transforming Growth Factor-β

Transforming growth factor-β belongs to a large superfamily of related proteins that also includes BMPs, growth and differentiation factors, activins, inhibins and anti-Mullerian hormone. All members play important roles in regulating cell proliferation and differentiation and the production of extracellular matrix. There are five isoforms of transforming growth factor-β (transforming growth factor-β1 to transforming growth factor-β5).[24] Most cells synthesize and respond to transforming growth factor-β, but high levels were found in bone, platelets and cartilage.

Transforming growth factor β is synthesized by osteoblasts in inactive (latent) form. Transforming growth factor β has been shown to stimulate pre-osteoblast cell replication, osteoblastic collagen synthesis, bone matrix apposition and alkaline phosphatase activity. However, transforming growth factor β also appears to retard terminal differentiation of osteoblasts.[25]

Fibroblast Growth Factor

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. To mediate their range of effects, fibroblast growth factor proteins signal via membrane-spanning tyrosine kinases and there are a wide variety of mechanisms for receptor regulation and availability. In normal adult tissues, the most abundant proteins are fibroblast growth factor-1 and fibroblast growth factor-2.[26] Fibroblast growth factor-2 is expressed by osteoblasts and is generally more potent than fibroblast growth factor-1, although the expressions of other fibroblast growth factors are not nearly as ubiquitous.[27]

The use of recombinant human FGF-2 for Periodontal Regeneration:

Several growth factors have recently received attention because of their ability to actively regulate various cellular functions of periodontal ligament (PDL) cells and the effects of topical application of such factor(s) on periodontal tissue regeneration. A recent randomized controlled double-masked Phase II clinical trial at 13 Japanese dental facilities compared the therapeutic response to varying doses of fibroblast growth factor-2 vs. the control.[28] The finding of the study suggested that topical application of fibroblast growth factor-2 can be efficacious in regenerating periodontal tissue of patients with two-walled or three-walled intrabony defects.

Keratinocyte Growth Factor

Regional and temporal changes in KGF expression played important roles in the development and maintenance of epithelial structures and in epithelial wound healing. A lack of KGF expression by periodontal fibroblasts in vivo was expected to hinder apical epithelial migration and thus stabilize the epithelial attachment. The effects of retinoic acid (RA) on KGF expression in vitro provided an indirect mechanism by which KGF may regulate the growth and differentiation of gingival epithelia.[29]

Insulin-Like Growth Factors

There are two types of insulin like growth factors-IGF-I and IGF-II- that function similarly but are independently regulated. As their name indicates, IGFs are biochemically and functionally similar to insulin. They are primarily produced by the liver and circulate in the vascular system.[30]

IGF-I has been shown to be chemotactic for cells derived from the PDL.53 IGF-I also has strong effects on periodontal ligament fibroblasts (PLF) mitogenesis and protein synthesis in vitro. IGF-I receptors have also been localized on the surface of PLFs.38 The role of IGF-II on parameters of PLF and gingival fibroblast metabolism have not been reported to date.[30]

Vascular Endothelial Growth Factor

It has been demonstrated that human cultured epithelial cell sheets prepared by tissue engineering techniques provides useful graft material for wound healing and tissue regeneration.[31] This study indicated that meaningful amounts of VEGF and TGF-α and -βl were released from human cultured gingival epithelial sheets (HCGES), which suggested potential for promoting wound healing and tissue regeneration. However, limited information was available with regard to biological effects such as release of growth factors from human cultured gingival epithelial sheets (HCGES).

Epidermal Growth Factor

High levels of bound EGF were noted on periodontal ligament fibroblasts, preosteoblasts, and prechondrocytes. The EGF receptors were expressed in high amounts by the cells of the epithelial rests, and these cells were responsive to the various actions of EGF. It can be speculated that activation of the epithelial rest cells in various pathologic conditions is associated with a local rise in the tissue level of EGF. The mitogenic, chemotactic, and synthetic responses of rat periodontal ligament (PDL) fibroblastic cells to epidermal growth factor (EGF), TGF-β, IGF-I,II were examined in vitro using PDL cells obtained from the coagulum of healing tooth sockets.[32] They concluded that all this growth factors may be useful for clinical application in periodontal regenerative procedures.

CONCLUSION

The explosion of knowledge and the understanding of the role of growth factors, their mechanisms of actions and molecular signalling pathways, which have been reviewed in this topic, suggest the potential for many novel therapeutic targets, not only for applying growth factors but also for potential use of growth factors inhibitors or agents that target specific part of the intracellular signalling pathways.

There remains an enormous challenge to convert some of the knowledge from basic studies of the bone cell physiology to therapeutically useful techniques for the future. Such novel approaches may result in real qualitative improvements in clinical outcomes over currently available therapeutic modalities.

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