Research Article

Implication of Growth Factors in Periodontal Tissue Engineering- A Literature Review

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Abstract

Periodontitis, a recognized disease worldwide, is bacterial infection-induced inflammation of the periodontal tissues that results in loss of alveolar bone. Once it occurs, damaged tissue cannot be restored to its original form, even if decontaminating treatments are performed. For more than half a century, studies have been conducted to investigate true periodontal regeneration. Periodontal tissue engineering deals with the repair of alveolar bone, tooth-associated cementum, and periodontal ligament. Polypeptide growth factors are a class of naturally occurring biological mediators that regulate the proliferation, migration, and extracellular matrix synthesis of a variety of cell types including those derived from the periodontium.

In this review, we focus on the implications of growth factors in tissue engineering studies and discuss future perspectives on periodontal regeneration.

Keywords: *Periodontitis, Periodontal regeneration, tissue engineering, growth factors, gene therapy.*

Tissue engineering is an emerging multidisciplinary field involving biology, medicine, and engineering that is likely to revolutionize and improve the health and quality of life for millions of people worldwide by restoring, maintaining, or enhancing tissue and organ function. Periodontal tissue engineering deals with the repair of alveolar bone, tooth-associated cementum, and periodontal ligament (PDL).**(**1**)**

For tissue to be engineered a triad of factors must be intricately incorporated and woven together delicately for an apt outcome, the factors being, the scaffold, which acts as a housing for the cells to grow, a continuous supply of cells, and the incorporation of signaling molecules at an appropriate time. This totality of the factor needed is often called **Tissue engineering triad (**1**)**

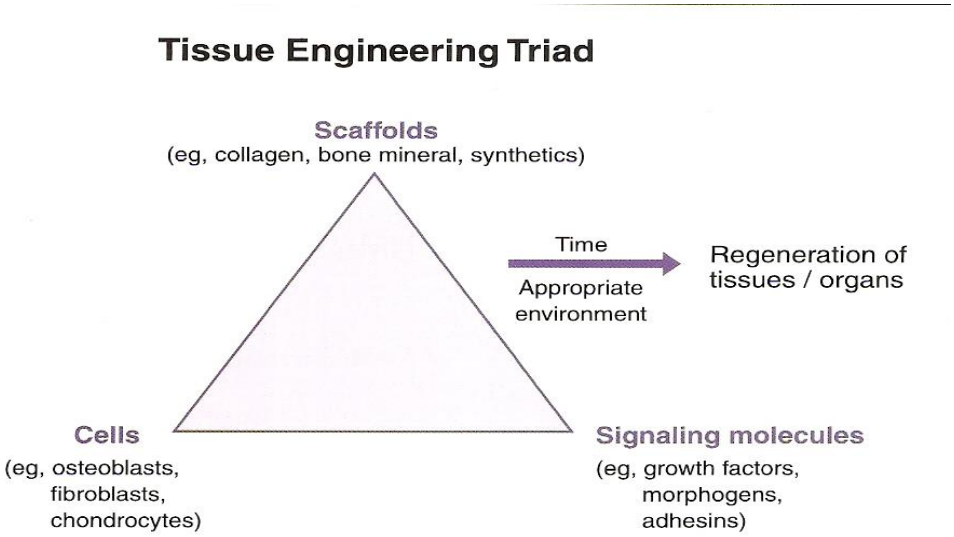

Figure 1

Mechanism of action of gene delivery system

The gene-delivery therapy for periodontal regeneration identifies a malfunctioning gene and supplies the patient with functioning copies of that particular gene through an in-vivo and ex- Vivo gene delivery system. The transfer of the therapeutic gene into the patient's target cell requires a carrier called a vector, which are either viruses or D.N.A.

plasmids. When the scaffold containing the gene constructs is implanted into the tissue defect, the host cells migrate into the implant, take up the gene constructs and start producing the encoded protein. **(**2**)**

Introduction to Growth Factors

Polypeptide growth factors are a class of naturally occurring biological mediators that regulate the proliferation, migration, and extra- cellular matrix synthesis of a variety of cell types including those derived from the periodontium.**(3)** They were first described in 1960 in blood fluid of fetal calf serum. Recent advances in molecular cloning have proven the application of growth factors in tissue engineering used as an alternative treatment approach for periodontal regeneration. In general growth factors are synthesized as pro-peptide forms which are biologically active and stored in the cytoplasm. Growth factors and their cognates interaction mediate several intracellular signaling pathways and modulate target cell response by altered gene activity, local acting growth factors, regulate the development and function of cells, offer the potential for regenerating tissue types. Growth factors are proteins that may act locally or systemically to affect the growth and function of cells in several ways. The application of growth factors to restore damaged tissues aims at regeneration through biomimetic processes or mimicking the processes that occur during embryonic and post-natal development. **(**4**)**

Types of signaling molecules/Biochemical mediators/growth factors include:

Bone morphogenic Proteins, fibroblast growth factor, platelet-derived growth factor, transforming growth factor, epidermal growth factor, cementum derived growth factor, parathyroid derived growth factor and insulin-like growth factor.

Figure 2

Mechanism of Action

The growth factors release may affect target cells either as autocrine, juxtracrine, or intracrine fashion. In certain concentrations growth factors substantially increase the rate of cell turnover through secondary intra- cellular messengers of phosphor di esterase diacylglycerol and C. kinase that can regulate D.N.A synthesis, resulting in mitosis. Few growth factors stimulate cell differentiation and regulate the synthesis of the extracellular matrix. Platelet-derived growth factor acts as a chemo-attractant for a variety of cells. Growth factors operate through highly complex intracellular pathways in order to regulate intra-cellular PH. thereby influencing genetic activity. The activation of receptor by growth factor initiates a cascade of intracellular biochemical changes.**(**5**)**

Implication of Growth Factors in Periodontal Tissue Engineering

Platelet-derived growth factor was first discovered by Lynch and his co-workers in late 1805 to promote regeneration of bone, cementum, periodontal ligament. It is the first growth factor to be evaluated in preclinical periodontal and peri-implant regenerative studies. As it contains biological mediators that regulate the proliferation and migration of gingival and periodontal ligament fibroblasts, cementoblasts, pre- osteoblasts, and osteoblastic cells at a wound site. **(**6**)** There are four isoforms of Platelet-derived growth factors namely platelet-derived growth factor -A, -B, -C, -D. The mature parts of A and B chains are 100 amino acids that share 60% of the amino acids. The C & D chains are activated by proteolysis.

PDGF stimulates DNA synthesis and cell replication in osteoblasts, as well as increases bone collagen synthesis and the rate of bone matrix apposition. **(**7**)** PDGF-BB is most effective on PDL cell mitogenesis and matrix biosynthesis **(**8**)**. Recombinant human PDGF BB homodimer (rhPDGF-BB) is a potent recruiter and a strong mitogenic factor for cells crucial to musculoskeletal tissue repair, including mesenchymal stem cells (MSCs), osteogenic cells, and tenocytes. rhPDGF-BB also upregulates angiogenesis. These properties allow rhPDGF-BB to trigger the cascade of bone and adjoining soft tissue repair and regeneration.**(**9**)**

Howell et .al **(10)** conducted a study to evaluate the regenerative potential of a combination of recombinant human (rh) platelet-derived growth factor-BB (PDGF-BB) and (rh) insulin-like growth factor-I (IGF-I)in the treatment of periodontal infra-bony defects in humans. They concluded that the local application of rhPDGF-BB and rhIGF-I to periodontal lesions was safe and efficacious in regenerating lost bone. Several studies were conducted by Park et.al11, Cho.et.al, **(**12,13**)**

Camelo et.al **(**14**)** Nevin et.al, **(**15**)** Jayakumar et .al **(**16**)** to evaluate the regenerative potential of PDGF-BB either alone or in combination to allografts including demineralized frozen dried bone graft and beta-tricalcium phosphate and have concluded that PDGF –BB has proven to be a good adjuvant to bone grafts in the regeneration of bone.

Platelet-derived growth factor gene delivery Jin et al.(17) demonstrated that direct in vivo gene transfer of PDGF-B stimulated periodontal regeneration in infra-bony periodontal defects in rats. Descriptive histology and histomorphometry revealed that human PDGF-B gene delivery promotes the regeneration of cementum and alveolar bone, whereas PDGF-1308, a dominant-negative mutant of PDGF-A, has minimal effects on periodontal tissue regeneration

Platelet-derived growth factor (PDGF) is an active growth factor, which is a product of two distinct genes PDGF-A and PDGF-B. PDGF stimulates DNA synthesis and cell replication in osteoblasts, (18) as well as increases bone collagen synthesis and the rate of bone matrix apposition.(19)

Fibroblast Growth Factor -2

Takayama et al.(20), investigated the role of fibroblast growth factor -2 in periodontal regeneration as a chemo- attractant for periodontal ligament cells during periodontal wound healing. Terranova et al. (21) Kitamura et al. (22,23) investigated the efficacy of the local application of recombinant human fibroblast growth factor-2 (FGF-2) in periodontal regeneration They concluded that FGF-2 can be efficacious in the regeneration of human periodontal tissue that has been lost by periodontitis. Basic fibroblast growth factor (bFGF or FGF-2) has been demonstrated to have potent angiogenic activity and the potential to induce the growth of immature PDL cells. The mRNA level of laminin in PDL cells, which plays an important role in angiogenesis, is up-regulated by FGF-2 stimulation. Thus it may in turn accelerate periodontal regeneration.

Transforming Growth Factor-β is the name given to a group of homodimeric proteins involved in the formation and development of many tissues. Once secreted, the ligand binds to transmembranous heterodimeric receptors, activating a group of intracellular proteins. The phosphorylated intracellular proteins start an intracellular signaling pathway which activates a set of genes. (24)

In vitro, transforming growth factor β has been observed to promote extracellular matrix production in many cell types, such as periodontal ligament and fibroblast Transforming growth factor.- β, when used alone or in combination with platelet, derived growth factor-BB stimulates the proliferation of periodontal ligament fibroblast.**(**25**)** It enhances collagen gel construction in vitro when combined with platelet-derived growth factor and insulin growth factors. Besides Transforming growth factor β, stimulates the biosynthesis of type I collagen fibronectin and induces depositions of bone matrix.**(**26**)** In vivo transforming growth factor β increases bone regeneration when applied with gelatin scaffold. Transforming growth factor. cementum-derived growth factor β when applied with a biodegradable osteogenic material has proven to cause bone regeneration in rabbits. In a recent study the application of rh transforming growth factor.- β in conjunction with nonreasonable barrier membrane greatly enhanced bone regeneration in oral osseous defects. Transforming growth factor.- β activates fibroblasts to form procollagen, which results in the deposition of collagen within the wound.**(**27**)**

Transforming growth factor –β (TGF- β) is a multifunctional growth factor structurally related to bone morphogenetic protein. Markopoulou et al. **(**28**)** evaluated the in vitro effect of a combination of recombinant human transforming growth factor-beta 1 (rhTGF-β1) and calcified freeze-dried bone allograft (FDBA) and a porous biphasic calcium phosphate (BC) bone graft on human PDL (hPDL) cell differentiation. They concluded that TGF-β1 was an effective adjuvant to calcified freeze-dried bone allograft (FDBA) and a porous biphasic calcium phosphate (BC) bone graft and had good regenerative potential. TGF-β1 seems to play an important role in inducing fibroblastic differentiation of PDL stem/progenitor cells and in maintaining the PDL apparatus

Bone Morphogenic Proteins

Another important therapeutic application of BMPs is for maxillary bone regeneration to allow the replacement of lost teeth by osteo -integrated dental implants. This approach involves the regeneration of peri-implant bone after implant fixation or bone height improvement in areas below the maxillary sinus. Preclinical and clinical studies have shown improved bone formation after treatment with BMP-2. However, the use of

different carriers and the association of barrier membrane (GTR technique) or other biomaterials seem to be critical factors in influencing the therapeutic outcome.(29,30,31,32)

Bone Morphogenetic Protein Gene Delivery Bmp-2

Lieberman et al.(33) Baltzer et al. (34) demonstrated gene therapy for bone regeneration, they transduced the bone marrow stromal cells with rhBMP-2 to form bone into a bony segmental defect in rabbits. Franceschi et al. (35) investigated in vitro and in vivo Ad gene transfer of BMP-7 for bone formation. Ad transduced nonosteogenic cells also were found to differentiate into bone-forming cells and produce BMP-7 or BMP-2 in vitro and in vivo. A study by Huang et al. using plasmid DNA encoding for BMP-4 with a scaffold delivery system was found to enhance bone formation when compared with blank scaffolds.(36)

BMP-7 or osteogenic protein-1 is a potent modulator of osteogenesis and bone cell differentiation. Its effect in periodontal-regenerative treatment was evaluated in bony defects around tooth roots in preclinical studies. Significant improvement in bone and cementum regeneration was observed in dogs (37). An extensive cementogenesis was considered the most significant effect of BMP-7 in bony defects in baboons 38. Improvement of bone formation around titanium implants was also demonstrated by studies in animals (39).

The potential of BMP-12 to repair the tendon and PDL tissues has been shown in vitro and in vivo studies (40). A preliminary study in dogs compared rhBMP-12 with rhBMP-2 for the treatment of periodontal defects. The results showed less bone and more functionally oriented PDL between the new bone and new cementum after BMP-12 treatment, contrasting with a more parallel fiber arrangement of BMP-2-treated defects (41).

Type of B. M. P	Studies Conducted	Results
Bone Morphogenetic protein gene delivery BMP-2	Lieberman et al.[33] Baltzer et al. [34]	Demonstrated gene therapy for bone regeneration, they transduced the bone marrow stromal cells with rhBMP-2 to form bone into a bony segmental defect in rabbits.
BMP-7	Franceschi et al (2000)	Investigated in vitro and in vivo Ad gene transfer of BMP-7 for bone formation. Ad transduced nonosteogenic cells also were found to differentiate into bone-forming cells and produce BMP-7 or BMP-2 in vitro and in vivo
BMP-4	Huang et al. (2005)	Study was conducted using plasmid DNA encoding for BMP-4 with a scaffold delivery system was found to enhance bone formation when compared with blank scaffolds. [36]
BMP-7 or osteogenic protein-1	Giannobile WV et.al (1998)	Concluded a Significant improvement of bone and cementum in dogs[37]
		An extensive cementogenesis was considered the most significant effect of BMP-7 in bony defects in a study conducted on baboons [38]
		Improvement of bone formation around titanium implants was also demonstrated by studies in animals[39]
BMP- 12	Wolfman NM (1997)	Studied the potential of BMP-12 to repair tendon and periodontal tissues through vitro and in vivo studies

Bone Morphogenetic protein gene delivery

In an early approach to regenerate alveolar bone in an animal model, the ex vivo delivery of Ad-encoding murine BMP-7 was found to promote periodontal tissue regeneration in large mandibular periodontal bone defects. BMP-7 gene transfers not only enhanced alveolar bone repair but also stimulated cementogenesis and PDL fiber formation. Of interest, the alveolar bone formation was found to occur via a cartilage intermediate When genes that encoded the BMP antagonist were delivered, inhibition of periodontal tissue formation resulted. **(**42,43**)** A recent study by Dunn et al. **(**44**)** showed that direct in vivo gene delivery of Ad/BMP-7 in a collagen gel carrier promoted successful regeneration of alveolar bone defects around dental implants. These experiments provide promising evidence that shows the feasibility of in vivo and ex vivo gene therapy for periodontal tissue regeneration and peri-implant osseointegration.

Gene therapy for periodontal tissue engineering causes the transfer of genetic information into cells leading to the synthesis of a protein of interest which would aid in the regeneration of lost periodontium. Hence gene therapy could be considered as an advancement towards achieving periodontal regeneration.

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