Gene Therapy for Periodontal Bioengineering

J. A. Cirelli[1], and W. V. Giannobile[1,2,3]*

[1] Department of Periodontics and Oral Medicine, University of Michigan, School of Dentistry, 1011 N. University Ave.Ann Arbor, MI 48109-1078MI, USA
[2] Center for Craniofacial Regeneration and Michigan Center for Oral Health Research, University of Michigan, Ann Arbor, MI, USA
[3] Department of Biomedical Engineering, College of Engineering, University of Michigan, Ann Arbor, MI, USA
*corresponding author, wgiannob@umich.edu

ABSTRACT

Although significant advancements have been achieved in periodontal therapy over the last decade, predictable regeneration of the tooth-supporting tissues is a challenge in periodontology and oral implantology. Recently, gene therapy, a new therapeutic approach for genetic and acquired diseases, has been applied for tissue bioengineering in multiple clinical situations, including the craniofacial complex, among them defects resulting from periodontal disease. The use of gene therapy vectors has enhanced the bioavailability and targeting of multiple growth and host immune factors to repair alveolar bone defects. Early pre-clinical studies utilizing both *ex vivo* and *in vivo* gene transfer strategies demonstrate the feasibility of using gene therapy for periodontal tissue engineering. This review highlights the current progress made in the field of periodontal regenerative medicine via gene targeting approaches.

Key words: gene therapy, growth factors, tissue engineering, periodontal regeneration, regenerative medicine

Tissue Engineering

Tissue engineering is an interdisciplinary field that applies the principles and methods of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function (Langer and Vacanti, 1993). It's a promising and revolutionary area that involves the most recent advances in molecular and cellular biology, polymer chemistry and physiology. An early concept of tissue engineering was first applied in the 1930's and considered the preparation *in vitro* of matrices containing viable cells which could be implanted in patients to substitute or facilitate the regeneration of damaged tissues (Bisceglie, 1933). Recently, it comprises a large variety of techniques and biomaterials stratified into substitutive, histioconductive and histioinductive approaches (Walgenbach *et al.*, 2001), which involve the use of polymeric matrices, cells and soluble regulators and allows engineering the tissues not only *in vitro* but also *in vivo*. Although significant progress has occurred in the development of organs and tissues in vitro, engineering tissues directly in vivo may be an advantage, considering that physiological environment *in vivo* facilitates the functional incorporation of the new tissue concomitantly to its formation, which is preferable for some tissues like craniofacial tissues, including alveolar bone and the periodontium (Spector, 1999; Taba *et al.*, 2005)

In Periodontics, the challenge for tissue engineering is to regenerate the tooth supporting tissues destroyed by periodontal infections. For decades, a number of different grafting biomaterials, comprising autografts, allografts, xenografts and alloplastic materials, have been developed for treatment of periodontal intrabony defects (Reynolds *et al.*, 2003). Although these biomaterials are proposed to act as scaffolds for bone regeneration, the resulting tissues are generally encased in a dense fibrous connective tissue and result in limited bone formation without improving cementum and periodontal ligament regeneration. Focusing in the regeneration of these two periodontal tissues, another principle of tissue engineering was applied in the 1980's - guided tissue regeneration (GTR) (Nyman *et al.*, 1982). GTR consists in the repopulation of the wounds by specific cells selected with the aid of a membrane used as physical barrier. GTR protects the defect and the root surface from gingival epithelial and connective tissue cells proliferation and allows the migration of periodontal ligament cells which is supposed to have greater regenerative capacity. Although attractive, this technique displays limited clinical predictability and begs for the need of a better understanding of cellular-molecular interactions in order to obtain periodontal regeneration (Murphy and Gunsolley, 2003).

Growth Factors in Periodontal Tissue Engineering

In the last decade, periodontal regeneration research has focused on soluble regulators involved in the modulation of the healing process. Mediators termed polypeptide growth factors (GFs) have been demonstrated to participate in several events required for tissue regeneration, including cellular chemotaxis, proliferation and differentiation (Anusaksathien and Giannobile, 2002). After tissue injury, wound healing is governed in part by a large number of GFs released by platelets, plasma exudates, macrophages and cells from the defect. GFs regulate the activity of cells by binding to specific cell surface receptors, that transduce signals to the cell nucleus via complex signal transduction pathways (Giannobile, 1996). In periodontia, GFs are present in the alveolar bone, cementum and periodontal ligament tissues. Several GFs have been associated with the promotion of periodontal healing, including platelet-derived-growth factor (PDGF), transforming growth factor-beta (TGF-β), basic fibroblast growth factor (FGF-2), insulin-like growth-factor-1 (IGF-

1), bone morphogenetic proteins (BMPs), vascular endothelial growth factor (VEGF), and parathyroid hormone (PTH) (Giannobile and Somerman, 2003).

Preclinical and initial clinical studies have demonstrated encouraging results after the therapeutic use of GFs alone or combined with other GF or regenerative techniques for periodontal engineering. PDGF has been one of the most investigated and has shown positive stimulatory effects on tissue regeneration in preclinical (Giannobile *et al.*, 1994; Giannobile *et al.*, 1996; Lynch *et al.*, 1989; Lynch *et al.*, 1991; Rutherford *et al.*, 1992) and clinical studies (Camelo *et al.*, 2003; Howell *et al.*, 1997; Nevins *et al.*, 2003). Most recently, PDGF-BB has received approval by the U.S. Food and Drug Administration (FDA) for the treatment of periodontal defects (Nevins *et al.*, 2005).

FGF-2 (or bFGF) is a multifunctional GF that has been shown to enhance human periodontal ligament and endothelial cell attachment and proliferation *in vitro* (Murakami *et al.*, 1999; Terranova *et al.*, 1989). These results suggest that FGF-2 plays a role in PDL-mediated mitogenesis and angiogenesis during the early wound healing process. Preclinical studies in higher animals reveals strong potential of FGF-2 to promote the closure of furcations (Murakami *et al.*, 2003; Rossa *et al.*, 2000; Takayama *et al.*, 2001) and repair of intrabony defects (Nakahara *et al.*, 2003).

Another promising group of GFs for periodontal regeneration are the BMPs. The human genome encodes at least twenty of these multifunctional polypeptides (Reddi, 1998). Among several functions, BMPs participate in the osteoblasts production by stimulating the cellular events of mesenchymal progenitor cells. Pre-clinical investigations have shown potential regeneration of tooth-supporting alveolar bone using BMP-2 (Wikesjo *et al.*, 2003; Kinoshita *et al.*, 1997), BMP-7 (Giannobile *et al.*, 1998) and BMP-12 (Wikesjo *et al.*, 2004).

In general, the effects of topical application of different GFs in periodontal therapy have shown significant improvement in tissue regeneration, but with insufficient predictability. Although *in vitro* studies have elucidated the role of GFs in the cellular events of the different type of cells, several factors may influence the results *in vivo*. Limitations include restrict understanding of the orchestrated molecular-cellular interactions during periodontal healing, which difficult the choice of the GFs to be employed, and the short half-live of GFs after delivered in vivo. This phenomenon is presumably due to proteolytic degradation, rapid diffusion, and the solubility of the delivery vehicle in the hostile wound healing environment (Giannobile, 1996).

The development of new delivery devices has improved the efficacy of GFs in vivo. Bioabsorbable controlled-release scaffolds have been fabricated to carry GFs and release them at optimal doses in a timely manner depending on the biological demand of the target tissue (Anusaksathien *et al.*, 2006). Several studies have shown release of GFs for up to 15 days when associated with poly (lactate-co-glycolide) (PLGA) scaffolds with microspheres (Elisseeff *et al.*, 2001; Murphy *et al.*, 2000). However, even with optimal scaffolds, the local application of GFs often requires a large amount of protein to stimulate significant effects in vivo, which increases the risk of unwanted side effects (Chang *et al.*, 2003). An alternative approach using gene transfer may therefore have the advantage of transferring into specific cells with specific promoter and appropriate vectors to attain a sustained gene expression and more efficient way of GF delivery *in vivo* (Nakashima *et al.*, 2003).

Gene Therapy

The improvement in the knowledge of the genetic and cellular mechanisms of human diseases allowed the development of a new therapeutic approach for genetic and acquired diseases called gene therapy. This new clinical strategy can be defined as an introduction of specific genetic changes by homologous vectors sequences (Hendrie and Russell, 2005). Although initially designed to permanently correct a single gene in monogenetic disorders, gene therapy purposes have included modification or elimination of malignant cells, modulation of host defenses and reengineering of diseased organs or tissues. Within this approach is the potential to genetically modify the cells to express the required GFs to bone regeneration and, more specifically, periodontal regeneration (Ramseier *et al.*, 2006).

A vector is a carrier that helps to circumvent the natural barriers to DNA internalization to the cell nucleus, where it can use the cellular machinery to express the exogenous gene (Worgall, 2005). In general, they can be divided into viral and non-viral vectors. Viral vectors work as gene-delivery vehicles by replacing part of their genome with a therapeutic gene. The most commonly employed vectors are retrovirus, lentivirus, adenovirus (Ad) or adeno-associated virus (AAV).

Each of these viral vectors has characteristics that make it more or less appropriated for specific applications. Advantages and disadvantages for clinical application of each gene transfer system are presented in Table 1. In general, safety is the primary concern and it includes the risk of reversion of a non-replicative vector to a wild-type virion, tumorigenesis and immunogenic reactions.

Non-viral vectors include plasmid DNA and synthetic vectors that consist of complexes of plasmid DNA with cationic lipids and polymers, known as lipoplexes and polyplexes respectively. Although they present improved safety and are easer manufactured than viral vectors, they have low gene-transfer efficiency, and in some cases toxicity and *in vivo* instability (Table 1).

Gene Therapy Applications in Periodontology

The application of gene therapy for tissue engineering has proved to be effective and has extended to multiple areas of medicine. Tissue repair requires a transient expression of genes, initiating a cascade of events directing a self-maintained process. Adenovirus has been largely employed for this purpose as it is non-integrating and a relatively safe virus, while inducing high level of transient gene expression and transduction of several cell types. In the craniofacial area, gene therapy has been evaluated in the regenerative treatment of bony anomalies, salivary gland injuries, dental pulp healing and periodontal diseases (Ramseier *et al.*, 2006). Table 2 dis-

Table 1. Characteristics of Delivery Vectors for Gene Therapy

Vector Characteristics	Retrovirus	Adenovirus (Ad)	Adeno-Associated virus (AAV)	Lentivirus	Non-viral (plasmids/ DNA complexes)
Transduction efficiency	Low	High	High	High	Low
Genomic integration	Yes	Rare ($<10^{-3}$)	Yes	Yes	No
Gene packaging capacity	High (8kb)	High (7.5kb)	Small (4.5kb)	High (8kb)	High
Time of expression	Long-term	Transient	Long-term	Long-term	Transient
Immune response	No	Yes	Low	No	Yes (plasmid)
Insertional mutagenesis	Possible	No	Rare	Possible	No
Vector production	Easy	Easy	Difficult	Difficult	Easy
Cellular infection	Only dividing cells	Dividing/ non-dividing cells	Dividing/ non-dividing cells	Dividing/ non-dividing cells	Dividing/ non-dividing cells

Table 2: Examples Gene Therapy Approaches For Craniofacial Tissue Engineering

Tissue	Vector	Purpose	References
Craniofacial skeleton	Ad- BMP-2	Bone regeneration (craniofacial defects)	(Lindsey, 2001) (Chang *et al.*, 2003)
	Ad-BMP-2 and 9	Bone regeneration (mandible)	(Alden *et al.*, 2000)
	Ad-VGF	Angiogenesis – tissue ischemia	(Mack *et al.*, 1998)
	Ad-BMP-7	Repair of skull defects	(Krebsbach *et al.*, 2000)
	Retro-BMP-4+VEGF	Repair of skull defects	(Peng *et al.*, 2002)
Salivary glands	Ad-aquaporin-1 (Ad-AQP1)	Stimulate salivary secretion	(Delporte *et al.*, 1997) (O'Connell *et al.*, 1999) (Zheng *et al.*, 2001)
	AAV-IL10	Autoimmune epitheliitis (Sjogren's Syndrome)	(Yamano *et al.*, 1999; Yamano *et al.*, 2001)
	Liposome/plasmid complex –"gene cocktail"	Reduce levels of superoxide radicals and hydrogen peroxide (protection against irradiation damage)	(Vitolo and Baum, 2002)
Tooth pulp	Gdf11-plasmid	Stimulate dentin production (pulp capping)	(Nakashima *et al.*, 2002; Nakashima *et al.*, 2003; Nakashima *et al.*, 2004)
	Ad-BMP7	Same purpose	(Rutherford, 2001)
Temporomandibular joint (TMJ)	Ad-LacZ	TMJ articular surface repair	(Kuboki *et al.*, 1999)
Periodontal tissues	Ad-PDGF-B	Periodontal regeneration	(Jin *et al.*, 2004)
	Ad-BMP-7	Periodontal regeneration	(Jin *et al.*, 2003)
Peri-implant bone	Ad-BMP-7	Extraction socket repair at dental implant defects	(Dunn *et al.*, 2005)

plays the recent applications of gene therapy in the craniofacial complex.

In periodontics, the notable role of PDGF in the periodontal regeneration has been previously discussed in the context of bone (Lattanzi *et al.*, 2005) and other tissues (Alsberg *et al.*, 2001) highlighting the potential of PDGF gene delivering for periodontal engineering. The initial studies evaluated the ability of Ad-PDGF-A to affect cells

derived from the periodontium (Giannobile *et al.*, 2001; Zhu *et al.*, 2001). Osteoblasts, periodontal ligament fibroblasts, gingival fibroblasts and cementoblasts displayed effective expression of the PDGF-A gene for up to 7 days following gene delivery, which resulted in enhanced mitogenic and proliferative responses in these cells (Giannobile *et al.*, 2001; Zhu *et al.*, 2001). Also, dermal fibroblasts presented prolonged signaling events and downregulation of PDGFαR up to 96h after Ad-PDGF-A delivery (Chen and Giannobile, 2002).

Simulating a clinical condition, a three-dimensional ex vivo wound-healing model was constructed using human gingival fibroblasts to evaluate the effects of gene transfer by Ad-PDGF-A and Ad-PDGF-B on cell repopulation and wound fill (Anusaksathien *et al.*, 2003). The expression of PDGF genes was prolonged for up to 10 days. Ad-PDGF-B resulted in 2-fold increased rate of defect fill and 4-fold greater cell densities inside the defect than Ad-PDGF-A or control groups. Upregulation of genes associated with PDGF signaling (PI3 kinase) and fibroblast migration (integrin α-5) suggested modulation of cellular and molecular events by Ad-PDGF-B therapy (Anusaksathien *et al.*, 2003).

The first evaluation of gene therapy for periodontal regeneration in vivo utilized ex vivo gene transfer in alveolar bone wounds in rats (Jin *et al.*, 2003). Syngeneic dermal fibroblasts (SDFs) were transduced *ex vivo* with Ad-BMP-7, seeded onto gelatin carriers and then transplanted to mandibular alveolar bone defects in a rat wound repair model. The treatment stimulated periodontal wound healing including bone, periodontal ligament and cementum. However, the *ex vivo* gene transfer has the limitations of cell procurement issues and the need for an additional surgical procedure for biopsy harvest. To overcome these disadvantages, an *in vivo* viral gene delivery approach was evaluated (Jin *et al.*, 2004). A collagen matrix containing Ad-PDGF-B were applied in a similar model of bone defect in rats. Localized transgene expression was observed up to 3 weeks resulting in proliferative and regenerative effects on periodontia. A four-fold increase in bridging bone and six-fold increase in cementum repair was observed in the Ad-PDGF-B treated sites in comparison to control sites (Jin *et al.*, 2004).

Another experiment using in vivo gene delivery evaluated the bone regeneration after the treatment of large defects surrounding dental implants in rats using Ad-BMP-7 administered in a collagen matrix (Dunn *et al.*, 2005). The treatment resulted in enhancement of alveolar bone defect fill, coronal new bone formation and new bone-to-implant contact.

SUMMARY

There remains strong potential for the role of gene delivery applications to improve the bioavailability, sustainability and targeting of growth factors to periodontal and alveolar bone defects. Much of the field is in its early stages for eventual human application to the field of periodontal regenerative medicine. Critical areas for study include safety with the use of viral vectors and control of the host immune response to chronic periodontal pathogen exposure. Improvements in vector optimization, combinatorial gene delivery approaches and carrier delivery systems may soon make gene therapy for periodontal repair a clinical reality.

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