

Gene Therapy in Periodontics: A Review and Future Implications

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Abstract

New advancements in gene therapy continue to have a significant impact on dentistry since 1995. At the same time, periodontal disease has attracted the attention of scholars and research scientists as a global concern. With a better understanding of disease progression and new advancement in biological science, gene therapy has emerged to enhance existing therapy and has radically recast approaches to the management of periodontal disease. Since the advent of gene therapy in dentistry, significant progress has been made to control periodontal disease and reconstruct the dentoalveolar apparatus. However, to date, gene therapy methods have not been developed to control periodontal disease due to its multifactorial origin, complex genetic predisposition, and risk associated with it. This review article provides a brief insight into the ever-expanding field of gene therapy and its possible future implication in the field of periodontics. Most of the modalities described in this article are more theoretical and still in infancy stage except for genetically fabricated materials used for regenerative purposes.

Keywords: Gene therapy, periodontics, alveolar regeneration, gene therapeutics, gene enhanced tissue engineering, gene transfer

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Introduction

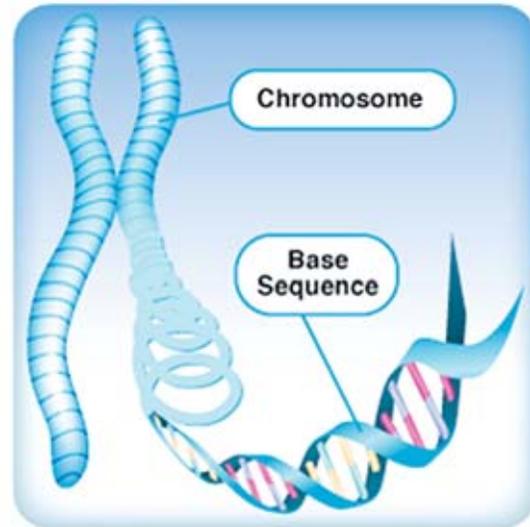
Gene therapy is a field of biomedicine. There have been tremendous advances in gene therapy relevant to dentistry since 1995.¹ However, in the field of periodontics gene therapy has not been applied with success primarily because the technology of gene therapy is still far from perfect and certainly has its own substantial problems.² Secondly, periodontal disease is multifactorial in origin comprised of microbial challenge and variable host immune responses modified by genetic and environmental factors.^{3,4,5} To complicate matters there are genetic variations in different populations like the involvement of multiple genes, interactions between genes and the environment, and no particular gene has been universally accepted as a causative factor for periodontal disease.⁶ Several initial attempts to apply gene therapy tools for periodontally relevant problems have been explored to date. However, they are still in the infancy stage and are more theoretical in nature even though some preliminary studies in animals have shown promising results.



In the near future a better understanding of complex periodontal diseases and approaches in gene therapy will enable us to apply gene therapy tools to control them allowing millions of people to retain their natural teeth for a lifetime. At present, gene therapy modalities for periodontal regeneration are at clinical trial levels in animal models. However, research is underway for using gene therapy approaches to alter host responses and specific periodontal pathogens. The understanding of basic principles and advances in gene therapy is essential to have an insight into the prospects and advances of this new field in periodontics. It is predicted that gene therapy may offer a wider range of treatment options in dentistry than in the past and may become an integral part in dental practice.

Basic Principles and Approaches in Gene Therapy²

Genes are specific sequences of bases that encode instructions on how to make proteins.



Genes are carried on chromosomes and are the basic physical and functional units of heredity. Gene therapy is a technique for correcting defective genes responsible for disease development.

Researchers may use one of several approaches for correcting faulty genes:

1. Inserting a normal gene into a nonspecific location within the genome to replace a nonfunctional gene.
2. An abnormal gene could be swapped for a normal gene through homologous recombination; this approach is the most common.
3. The abnormal gene could be repaired through selective reverse mutation, which returns the gene to its normal function.
4. The regulation (the degree to which a gene is turned on or off) of a particular gene could be altered.

Gene Transfer Techniques

Gene therapy works by delivering the therapeutic gene to the patients' target cells through the carrier molecule called a vector. Currently, the most common vector is a virus that has been genetically altered to carry normal human DNA. Once the vector enters target cells it unloads its genetic material containing the therapeutic human gene. The generation of a functional protein product from the therapeutic gene restores the target cell to a normal state.

The clinical application of gene transfer can be accomplished in either two ways: *in vivo* or *ex vivo*. During *in vivo* gene transfer the foreign gene is injected into the patient by viral and nonviral methods. The *in vivo* gene delivery systems are summarized in Table 1.

In contrast an *ex vivo* gene transfer involves a foreign gene transduced into the cells of a tissue biopsy, outside the body, and then resulting genetically modified cells are transplanted back into patient.

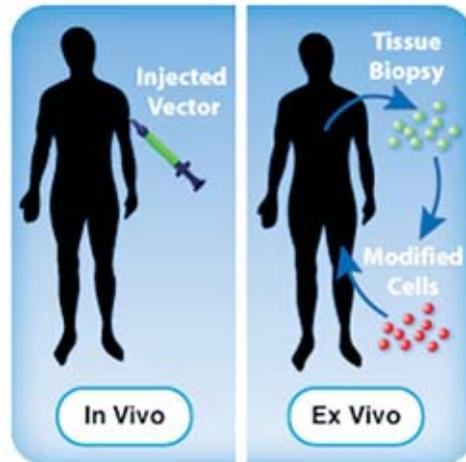
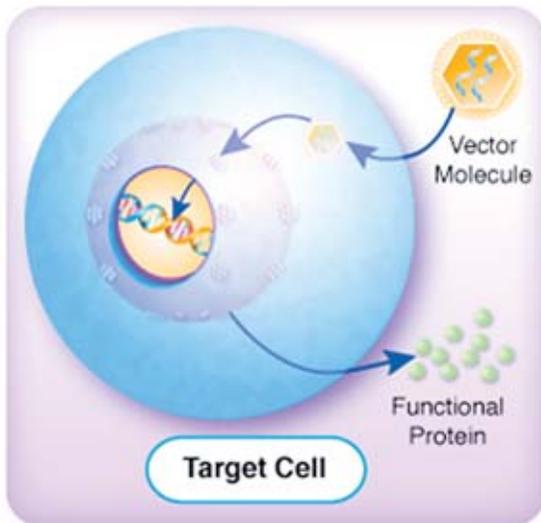


Table 1. Summary of *in vivo* gene delivery systems.

Viral Method	Non Viral Methods	
Viruses serving as viral vectors: Retroviruses Adenoviruses Adeno-associated viruses Herpes simplex viruses Lenti virus	Micro Seeding Gene Therapy:	Direct injection of therapeutic DNA into target cells using a gene gun. This approach is limited in its application because it can be used only with certain tissues and requires large amounts of DNA (simplest method).
	Cationic Liposomes:	Creation of artificial lipid spheres with an aqueous core. This liposome, which carries the therapeutic DNA, is capable of passing the DNA through the target cell's membrane.
	Macromolecular Conjugate:	Here therapeutic DNA gets inside target cells by chemically linking the DNA to a molecule that will bind to special cell receptors. Once bound to these receptors, the therapeutic DNA constructs are engulfed by the cell membrane and passed into the interior of the target cell. This delivery system tends to be less effective than other options.
	Gene Activated Matrices (GAMS):	These deliver naked DNA via polymer matrix sponges.

Limitations of Gene Therapy

The following are limiting factors on the use of gene therapy:

1. Short-lived nature of gene therapy.
2. Immune response of the patient.
3. Problems with viral vectors like patient-toxicity, immune and inflammatory responses, and gene control and targeting issues.
4. Limitation of sufficient quantity of the engineered gene that can be delivered.
5. Extreme cost.
6. Ethical restrictions.

Clinical Implications of Gene Therapy in Animal Models

Reconstructive surgery (autogenous or allograft placement) for patients with significant alveolar bone loss is preferred rather than sacrificing teeth, but the results are unpredictable.⁷ A gene therapy approach is an attempt to use the body's own bone growth mechanisms to produce new bone by supplementing the regenerative site with therapeutic protein. To date, considerable research progress has been made in gene therapy to repair bony lesions. While most of the studies were conducted in animal models, they have shown promising results. For ethical reasons and the risk associated with human gene therapy, more research has to be done before these approaches can be tested in humans.

Gene Enhanced Tissue Engineering

The general strategy of tissue engineering is to supplement the regenerative site with a therapeutic protein-like growth factor. However the problem with the delivery of growth factor is its short life (a few hours). This is due to proteolytic breakdown and receptor mediated exocytosis and solubility of the delivery vehicle. To overcome this problem, gene therapy has been developed which provides long-term exposure (at least two weeks) of the growth factor to the periodontal wound.

This can be done in the following ways:

- a. *Ex vivo* gene transfer is done in a tissue culture environment and the transduced cells carrying the foreign genes are placed back into the host. University of Michigan researchers have shown several different

cell types such as non-osteogenic fibroblast (from human gingiva and dental pulp), oral keratinocytes, myoblasts, as well as osteoblasts can express the BMP-7 (Luciferase gene) and BMP-9 gene (Bone Morphogenetic Proteins are multifunctional growth factor) after being infected with an adenoviral vector. These cells are then able to differentiate into bone forming cells when placed into the osseous defect *in vivo*.^{8,9,10}

- b. Mesenchymal stem cells are pluripotent stem cells. When they are genetically engineered and placed into an osseous defect *in vivo*, they induce new bone and blood vessels by expressing BMP-2. It has also been shown that genetically engineered stem cells were able to engraft, differentiate, and display regulatory behaviors.¹¹
- c. By directly delivering the BMP-2 gene¹ and BMP-7 gene¹² *in vivo* to tissue via an adenoviral vector, i.e., Ad-BMP (vs. using *ex vivo* cellular re-engineering), researchers have achieved repair of osseous defects in a short amount of time.
- d. The delivery of Platelet Derived Growth Factor (PDGF) for tissue engineering periodontal wound has become an active area of interest because of its potent effect on the regeneration of hard and soft tissues. A prolonged period of exposure of approximately >7 hrs is required for its potent effect on the cellular proliferation to occur. Since the "growth arrest specific (gas) gene" encodes the PDGF receptor, there is a down regulation of PDGF activity leading to transient biological activity and bioavailability of PDGF at the wound site. To overcome this limitation, recently researchers at the University of Michigan have developed an *in vivo* PDGF-A gene transfer through adenovirus vector (Ad-PDGF-A). The bioactive Ad-PDGF-AA protein released induces sustained tyrosine phosphorylation and corrective down regulation of PDGF receptor which is encoded by "growth arrest specific (gas) gene." This extends the effect of PDGF on cell signaling which is critical for cellular proliferation. The use of gene therapy as a mode of growth factor delivery offers a novel approach to periodontal tissue engineering.¹³

- e. Bone sialoprotein (BSP) is a major non-collagenous protein in bone and other mineralized tissues. *Cbfa*¹ is a “master gene” in osteogenesis and is involved in BSP gene expression which controls the cell differentiation during bone repair and regeneration. By the *in vivo* delivery of a BSP-gene into an osseous defect, it has been shown to regenerate periodontal alveolar bone.¹⁴
- f. NTF-hydrogel therapy is a novel, innovative method of regenerating bone. Pre-clinical trials have shown injecting a NTF gene (non-viral non-immunogenic gene) together with a synthetic, non-immunogenic hydrogel made from hyaluronic acid into the site of bone degeneration or loss, induces neighboring cells to produce new bone tissue. This therapy represents a significant improvement over conventional treatment.¹⁵
- g. Introduction of a Bcl2 gene (antiapoptosis gene) in conjunction with gene activated matrix technology (GAM) when introduced into a highly localized tissue injury site like those found in periodontal disease will improve the clinical outcome of the tissue injury by means of tissue repair and/or tissue regeneration.¹⁶
- h. A new class of product, known as “DNA devices,” has been introduced for the first time using selective genetics for the fulfillment of mechanical targeting (one of the forms of gene transfer). This gene delivery technology employs proprietary formulations incorporating intact DNA into polymers capable of being used as coatings on implantable devices such as periodontal implants creating a new class of site-specific gene therapy products. The use of DNA devices will improve the biocompatibility between the implanted device and human tissue.¹⁷

Future Strategies of Gene Therapy in Preventing Periodontal Diseases

Gene Therapeutics-Periodontal Vaccination

For many years researchers have been exploring vaccination techniques in animal models to eradicate periodontal disease with mixed success. In the last decade gene transfer research has led to a novel way to achieve a vaccination like

1. A salivary gland of a mouse when immunized using plasmid DNA encoding the *Porphyromonas gingivalis* (*P. gingivalis*) fimbrial gene produces fimbrial protein locally in the salivary gland tissue resulting in the subsequent production of specific salivary immunoglobulins A, or IgA and immunoglobulin G, or IgG, antibodies and serum IgG antibodies. This secreted IgA could neutralize *P. gingivalis* and limit its ability to participate in plaque formation. Similarly, secreted fimbrillin in saliva could bind to pellicle components blocking the attachment of *P. gingivalis*.¹⁸
2. Scientists have also demonstrated the efficacy of immunization with genetically engineered *Streptococci gordonii* vectors expressing *P. gingivalis* fimbrial antigen as vaccine against *P. gingivalis* associated periodontitis in rats.¹⁹
3. The gene hemagglutinin, which is an important virulence factor of *P. gingivalis*, has been identified, cloned, and expressed in *Escherichia coli*. The recombinant hemagglutinin B (rHag B) when injected subcutaneously in Fischer rats infected with *P. gingivalis* showed serum IgG antibody and interleukin-2 (IL-2), IL-10, and the IL-4 production which gave protection against *P. gingivalis* induced bone loss.²⁰



Genetic Approach to Biofilm Antibiotic Resistance

Some microorganisms have the ability to form a microbial community attaching to surfaces and are generally referred to as biofilm. Researchers have found bacteria growing in biofilms become up to 1,000 fold more resistant to antibiotics as compared to a planktonic counter part making them hard to control. The mechanism behind microbial biofilm resistance is not clear. Recently Mah et al.²¹ identified gene *ndvB* encoding for glycosyltransferase required for the synthesis of periplasmic glucans in wild form of *Pseudomonas aeruginosa* RA14 strain. This remarkably protected them from the effects of antibiotics,

biocides, and disinfectant. Using a genetic approach researchers have isolated ndvB mutant of *Pseudomonas aeruginosa*, still capable of forming biofilm but lacking the characteristic of periplasmic glucans, thereby, rendering microbial communities in biofilm more susceptible to conventional antibiotic therapy.

An *In vivo* Gene Transfer by Electroporation for Alveolar Remodeling

Periodontal tissue reacts to stimuli such as mechanical stress and inflammation by active remodeling with the expression of various molecules. Using an *in vivo* transfer of LacZ gene (gene encoding for various remodeling molecules) into the periodontium and using plasmid DNA as a vector along with electroporation (electric impulse) for driving the gene into cell, has shown predictable alveolar bone remodeling.²²

Tight Adherence Gene for the Control of Periodontal Disease Progression

Colonization of target tissue by a periodontal pathogen like *Actinobacillus actinomycetemcomitans* is an essential first step involved in the pathogenesis of localized aggressive periodontitis. It has been shown a "tight adherence gene" of *Actinobacillus actinomycetemcomitans* is required for its adherence and virulence. Researches have developed mutant strains lacking the "tight adherence gene" which could predictably control periodontal disease progression by limiting colonization and pathogenesis of *Actinobacillus actinomycetemcomitans*.²³

Antimicrobial Gene Therapy to Control Disease Progression

One way to enhance host defense mechanism against infection is by transfecting host cells with an antimicrobial peptide/protein-encoding gene. Researchers have shown when host cells were infected *in vivo* with β defensin-2 (HBD-2) gene via retroviral vector, there was a potent antimicrobial activity which enhanced host antimicrobial defense.²⁴

Gene Therapy to Grow New Teeth

Dental researchers hope to grow teeth in the laboratory that can be implanted into the mouths of patients who have lost their natural teeth. These would not be living teeth with nerves

and blood vessels, but they would be made of the same substances as human teeth. In order to accomplish this researchers must find the genes responsible for building the 25 major proteins making up tooth structures. In addition there may be dozens of other genes involved in instructing the body when, how, and where to form a particular tooth. There may be as many as 10% of the total number of genes somehow involved in the formation of teeth. The Baylor College of Medicine has found PAX 9, a master gene critical for tooth development. The hope is we will be able to bioengineer human teeth for replacement in the future.²⁵



Designer Drug Therapy in Treating Periodontal Disease

If genes necessary for normal development are known, then "*designer drug therapies*" aimed at one area of the gene or the other can be developed. These designer drugs will be safer than today's medicines because they would only affect the defect in a gene clearly identified through genetic research.²⁵

Conclusion

Today's improvements in technology coupled with the changing pattern of diseases have stimulated research on genetics. Since gene transfer tools are in their infancy, it is not likely gene transfer approaches will be used for routine periodontal care on humans. However, it can be predicted in the future there will be a growing recognition of genetic approaches in dental practice. In the near future, gene therapy can be attempted to prevent periodontal disease in a genetically susceptible individual at an early stage.

It is important for dentists to recognize human science is evolving rapidly. Our profession must pay attention to the advances taking place in the field of biotechnology, especially in genetic engineering, if we are to continue as an essential part of mainstream healthcare. This field will change the future of dental practice within the next two decades by providing an advanced standard of care for the dental patients.

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