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RESEARCH ARTICLE

GENE THERAPY IN PERIODONTICS: A REVIEW

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ABSTRACT

The term gene therapy originally referred to the treatment of a disease by means of genetic manipulation. It involves the transfer of a therapeutic or working gene copy into specific cells of an individual in order to repair a faulty gene copy. Thus, it may be used to replace a faulty gene, or to introduce a new gene whose function is to cure or to favourably modify the clinical course of a condition. With the better understanding of the 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 diseases. Since the advent of gene therapy in dentistry, significant progress has been made to control periodontal disease and reconstruct the dentoalveolar apparatus. Gene therapy is one of the recent advancements and its applications in the field of periodontics are reviewed in general here.

INTRODUCTION

Periodontal diseases have a broad spectrum of inflammatory and destructive responses, and are thought to be multifactorial in origin. Genetic variance has been considered as a major risk factor for periodontitis. With the advent of gene therapy in dentistry, significant progress has been made to control periodontal disease and reconstruct the dentoalveolar apparatus (Chatterjee *et al*., 2013). Gene therapy is a field of Biomedicine. A broad definition of gene therapy is the genetic modification of cells for therapeutic purposes (Kinane *et al*., 2005). Genes are specific sequences of bases present in the chromosome that form the basic unit of heredity. Each person's genetic constitution is different and the changes in the genes determine the differences between individuals. Some changes usually in a single gene, may cause serious diseases. More often, gene variants interact with the environment to predispose some individuals to various ailments. The goal of gene therapy is to transfer the DNA of interest, for example, growth factor and thrombolytic genes into cells, thereby allowing the DNA to be synthesized in these cells and its proteins (termed recombinant protein) expressed. According to Strayer(1994), gene therapy may involve (1) supplying or increasing the expression of a mutant gene that is insufficiently expressed (e.g., to treat enzymatic deficiencies); (2) blocking a gene that is detrimental (e.g., using antisense constructs to inhibit tumor proliferation); or (3) adding a foreign gene to treat a situation beyond the capability of the normal genome (e.g., introduce an enzyme into a cell or tissue that allows the tissue to become more sensitive to the effects of a pharmacologic agent).

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History

The first gene therapy trials on humans began in 1990 on patients with Severe Combined Immunodeficiency (SCID). In 2000, the first gene therapy "success" resulted in SCID patients with a functional immune system. These trials were stopped when it was discovered that two of ten patients in one trial had developed leukemia resulting from the insertion of the gene-carrying retrovirus near an oncogene (Chatterjee *et al*., 2013).

Major Developments in Gene Therapy

1. In 1995, the potential impact of gene therapy on dentistry was described.
2. In 2000, the first report of a fully successful gene therapy treatment-a French study involving a severe combined immunodeficiency in young children was published.
3. *Wikesjo et al.* in 2004, showed the effect of rhBMP-12 on regeneration of alveolar bone and periodontal attachment.
4. *Goncalves et al.* in 2008, demonstrated that root cementum may modulate the expression of growth and mineral-associated factors during periodontal regeneration.
5. *Lin et al.* in 2008, demonstrated that gene delivery of PDGF-B displays sustained signal transduction effects in human gingival fibroblasts that are higher than those conveyed by treatment with recombinant human platelet-derived growth factor-BB protein.

Approaches for Gene Therapy

- A normal gene may be inserted into a nonspecific location within the genome to replace a nonfunctional gene. This approach is most common.
- An abnormal gene could be swapped for a normal gene through homologous recombination.
- The abnormal gene could be repaired through selective reverse mutation, which returns the gene to its normal function.
- The regulation (the degree to which a gene is turned on or off) of a particular gene could be altered.
- Spindle transfer is used to replace entire mitochondria that carry defective mitochondrial DNA.

Types of Gene Therapy

1. Germ line gene therapy - In the case of germ line gene therapy, germ cells, i.e., sperm or eggs are modified by the introduction of functional genes, which are ordinarily integrated into their genomes. Therefore, the change due to therapy would be heritable and would be passed on to later generations (Friedmann, 1996).
2. Somatic gene therapy - In the case of somatic gene therapy, the therapeutic genes are transferred into the somatic cells of a patient. Any modifications and effects will be restricted to the individual patient only, and will not be inherited by the patient's offspring (Friedmann,1996)

Gene Delivery Methods

In general, gene therapy involves the transfer of genetic information to target cells, which enables them to synthesize a protein of interest to treat disease (Baum *et al*., 2002). The technology can be used to treat disorders that result from single point mutations. Various methods for gene delivery are :

1. VIRAL - A carrier molecule called a 'vector' must be used to deliver the therapeutic gene to the patient's target cells. Currently, the most common vector is a virus that has been genetically altered to carry normal human DNA. Viruses have evolved a way of encapsulating and delivering their genes to human cells in a pathogenic manner. Scientists have tried to take advantage of this capability and manipulate the virus genome to remove disease-causing genes and insert therapeutic genes (Chatterjee *et al*., 2013)

NON-VIRAL – The various non-viral approaches are

a. Micro-seeding gene therapy – Simplest method of gene therapy which involves direct introduction of therapeutic DNA into target cells using a gene gun. The disadvantage is that it requires large amounts of DNA to bring out the desired effect and hence this technique has restricted use.
b. Cationic liposomes – This technique involves the creation of an artificial lipid sphere (a liposome) with an aqueous core. This liposome which carries the therapeutic DNA is capable of transporting the DNA through the target cell's membrane. The disadvantage is that this delivery system tends to be less effective than others.
c. Gene activated matrices – It involves polymer matrix sponges to deliver naked DNA to the target cells.
d. Macromolecular conjugates – In this technique, DNA is linked to a molecule that binds to special cell receptors. Once bound, the therapeutic DNA is engulfed by the cell membrane and passed into the interior of the target cell.
e. Human Techno-chromosome – Experiments with the introduction of 47^{th} chromosome (an artificial, human techno-chromosome) into target cells are being carried out. This chromosome would exist autonomously alongside the stranded 46^{th} chromosome, without affecting their functions or causing any mutations. It would be a large vector capable of carrying substantial amounts of genetic code and because of its construction and autonomy, the body's immune system would not attack it.

Technical Difficulties in Using Gene Therapy

1. Difficulty in delivery of gene.
2. Short-lived nature of gene therapy.
3. Activation of immune response.
4. Chance of inducing a tumor – Insertion mutagenesis.
5. Safety of vectors.
6. Difficulty to treat multigene disorders.
7. Durability and integration.
8. Expensive.

Table 1. Viral gene therapy vectors used in gene therapy

VECTOR	FEATURES	ADVANTAGES	DISADVANTAGES
Retrovirus	A class of viruses that can create double-stranded DNA copies of their RNA genomes. These copies of its genome can be integrated into the chromosomes of host cells. Human immunodeficiency virus (HIV) is a retrovirus.	Nonimmunogenic	-Infects only dividing cells. -Insertional mutagenesis.
Adenovirus	A class of viruses with double-stranded DNA genomes that cause respiratory, intestinal, and eye infections in humans. The virus that causes the common cold is an adenovirus.	-Infects dividing and non dividing cells. - Does not integrate into target cell genome.	-Potentially immunogenic.
Adeno-associated virus	A class of small, single-stranded DNA viruses that can insert their genetic material at a specific site on chromosome 19.	-Infects dividing and non dividing cells. -Low immunogenicity. -Nonpathogenic in human.	-Difficult to produce at high titres. -Small transgenes.
Herpes Simplex virus	A class of double-stranded DNA viruses that infect a particular cell type, neurons. Herpes simplex virus type 1 is a common human pathogen that causes cold sores.	-	-

Implications of Gene Therapy in Periodontics

There have been tremendous advances in gene therapy relevant to dentistry since 1995. Even in the field of periodontics, it has been studied extensively. Currently genetic principles are being applied along with tissue engineering for periodontal rehabilitation (Chatterjee *et al*., 2013). The tissue engineering approach reconstructs the natural target tissue by combining four elements, namely, the scaffold, signaling molecules, blood supply and cells.

Approaches of Tissue Engineering in Periodontics

1. Protein based approach - Growth and differentiation factors are used for regeneration of periodontal tissues likes TGF-β, BMP-2,6,7,12, bFGF, VEGF and PDGF.
2. Cell based approach - Several studies using mesenchymal stem cell have demonstrated efficient reconstruction of bone defect that are too large to heal spontaneously.
3. Gene delivery approach - To overcome the short half-lives of growth factor peptides **in vivo**, gene therapy that uses a vector that encodes the growth factor is utilized to stimulate tissue regeneration. So far, two main strategies of gene vector delivery have been applied to periodontal tissue engineering.

a. IN VIVO - Gene therapy is done by targeting the gene delivery system to the desired cell type in the patient using either physical means such as tissue injection (brain tumor) or biolistics (dermal DNA vaccination), or potentially in the future, using systemic infusion of cell-specific receptor-mediated DNA carriers (reconstructed liposome's or viruses). Importantly, neither of these gene therapy strategies involve reproductive germline cells nor therefore the genetic alteration will not be transmitted to the next generation.

b. EX VIVO - **Ex-vivo** gene therapy is performed by transfecting or infecting patient-derived cells in culture with vector DNA and then reimplanting the transfected cells into the patient. Two types of **ex-vivo** gene therapies under development are those directed at fibroblasts and hematopoietic stem cells.

Clinical Trials Using Gene Therapy

Platelet-derived growth factor gene delivery

The application of PDGF-gene transfer strategies to tissue engineering originally was generated to improve healing in soft tissue wounds, such as skin lesions. But, recently various trials have been done with PDGF using Plasmid and Ad/PDGF gene delivery,for regeneration of periodontal tissue.

a. Jin *et al*. in 2004, demonstrated in their study that direct *in vivo* gene transfer of PDGF-B stimulated tissue regeneration in large periodontal defects.

b. Anusaksathien *et al*. in 2003, in an *ex vivo* investigation, showed that the expression of PDGF genes was prolonged for up to 10 days in gingival wounds.

c. Kaiger *et al*. in 2006, reviewed different mechanisms of drug delivery and novel approaches to reconstruct and engineer oral- and tooth-supporting structures, namely the periodontium and alveolar bone.

1. Bone morphogenic protein delivery

a. Franceschi *et al*. in 2000, investigated *in vitro* and *in vivo* Ad gene transfer of BMP-7 for bone formation.

b. Dunn *et al*. in 2005, demonstrated that in case of direct *in vivo* gene delivery of Ad/BMP-7 in a collagen gel carrier promoted successful regeneration of alveolar bone defects around dental implants.

Gene Enhanced Tissue Engineering

The general strategy of tissue engineering is to supplement the regenerative site with a therapeutic protein like growth factors. However the problem with the delivery of growth factor is its short life. This is due to protelytic breakdown and receptor mediated exocytosis and solubility of delivery vehicle. To overcome these problems, gene therapy has been developed which provides long term exposure of growth factor to periodontal wound (Chatterjee *et al*., 2013).

Clinical Applications in Periodontics

1. Antimicrobial Gene Therapy to Control Disease Progression
2. Gene Therapeutics-Periodontal Vaccination
3. Designer Drug Therapy in Treating Periodontal Disease
4. Genetic Approach to Biofilm Antibiotic Resistance
5. An *In vivo* Gene Transfer by Electroporation for Alveolar Remodeling

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 that 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 defenses.

Gene Therapeutics-Periodontal Vaccination

- The 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.
- Scientists have also demonstrated the efficacy of immunization with genetically engineered Streptococci gordoni vectors expressing *P. Gingivalis* is fimbrial antigen as vaccine against *P. gingivalis* associated periodontitis in rats (Katz *et al*., 1999).
- 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 (Chatterjee *et al*., 2013).

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.

Genetic Approach to Biofilm Antibiotic Resistance

Researchers have found bacteria growing in biofilms become up to 1,000 fold more resistant to antibiotics as compared to a planktonic counterpart making them hard to control. Recently Mah *et al*., identified gene ndvB encoding for glycosyltransferase required for the synthesis of periplasmic glucans in wild form of Pseudomonas aeuroginosa RA14 strain (Mah *et al*., 2003). This remarkably protected them from the effects of antibiotics biocides, and disinfectant. Using a genetic approach. Researchers have isolated ndvB mutant of Pseudomonas aeuroginosa still capable of forming biofilm but lacking the characteristic of periplasmic glucans there by rendering microbial communities in biofilm more susceptible to conventional antibiotic therapy.

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

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.

- Step A- Cells obtained from outpatient skin biopsy.
- Step B- Gene of therapeutic interest is introduced into cells by electroporation.
- Step C- Genetically engineered cells are propagated and characterized.
- Step D- Genetically engineered cells are returned back to clinician.

Conclusion

Today's improvements in technology coupled with the changing pattern of diseases have stimulated research on genetics. Gene therapy has a promising role in the field of periodontics but it does encompass serious ethical issue to be dealt with. It is evident that gene therapy has emerged from its stage of infancy of mere theoretical and hypothetical quotations to factual scientific researches, which reveals potential hopes. There are still lots of research and details of mechanisms to be understood to include these practically in day to day treatment modalities.

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