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. Gene therapy is one of the recent advancements and its applications in the field of periodontics are reviewed in general here.

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 of the recent advancements and its applications in the field of periodontics are reviewed in general here.

Copyright © 2014 Dr. Prabhati Gupta et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
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 47th chromosome (an artificial, human techno-chromosome) into target cells are being carried out. This chromosome would exist autonomously alongside the stranded 46th 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.
5. Safety of vectors.
6. Difficulty to treat multigene disorders.
7. Durability and integration.
8. Expensive.

<table>
<thead>
<tr>
<th>VECTOR</th>
<th>FEATURES</th>
<th>ADVANTAGES</th>
<th>DISADVANTAGES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retrovirus</td>
<td>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.</td>
<td>Nonimmunogenic</td>
<td>-Infected only dividing cells. -Insertional mutagenesis.</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>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.</td>
<td>-Infected dividing and non dividing cells. -Does not integrate into target cell genome.</td>
<td>-Potentially immunogenic.</td>
</tr>
<tr>
<td>Adeno-associated virus</td>
<td>A class of small, single-stranded DNA viruses that can insert their genetic material at a specific site on chromosome 19.</td>
<td>-Infected dividing and non dividing cells. -Low immunogenicity. -Nonpathogenic in human.</td>
<td>-Difficult to produce at high titres. -Small transgenes.</td>
</tr>
<tr>
<td>Herpes Simplex virus</td>
<td>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.</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 1. Viral gene therapy vectors used in gene therapy

5974 Dr. Prabhati Gupta et al. Gene therapy in periodontics: a review
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 like 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 improved 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.

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 proteolytic 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
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 (Kat 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 aeruginosa 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 aeruginosa 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.

**REFERENCES**


*******