Gene Therapy-Future in Regenerative Periodontics

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ABSTRACT:

Genes are specific sequences of bases that encode instructions to make proteins. Each person's genetic constitution is different and the changes in the genes determine the differences between individuals. A challenge faced by periodontal therapy is the predictable regeneration of periodontal tissues lost as a consequence of disease. Gene therapy is an emerging field of biomedicine. The procedure involves the transfer of genes to patients for clinical benefit. Gene therapy may achieve greater bioavailability of growth factors within periodontal wounds which may provide greater regenerative potential.

Key words: Genes, Regeneration, Gene therapy.

INTRODUCTION

Periodontitis is a multifactorial disease with a broad spectrum of inflammatory and destructive responses. While microbial and environmental factors are believed to initiate and modulate periodontal disease progression, genes also play a role in predisposition and progression of periodontal diseases. The ultimate goal of periodontal therapy is to regenerate the periodontium lost due to periodontal disease. Recombinant tissue growth factors provide encouraging results for periodontal regeneration (Figure 1) but there are limitations for topical protein delivery such as transient biological activity, protease inactivation and poor bio-availability from existing delivery vehicles. Therefore newer approaches employing methods to optimize growth factor targeting to achieve maximum therapeutic benefits have been recently developed.[1]

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 technology by which genes or small DNA or RNA molecules are delivered to human cells, tissues or organs to correct a genetic defect or to provide new therapeutic functions for the ultimate purpose of preventing or treating diseases.[2] This

article reviews the fundamentals of gene therapy and its implications in regenerative periodontics.

FUNDAMENTALS OF GENE THERAPY[3]

For correcting faulty genes one of the following approaches may be employed:

1. A normal gene may be inserted into a nonspecific location within the genome to replace a non-functional gene. This is the most common approach.
2. An abnormal gene could be swapped for a normal gene through homologous recombination.
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.
5. Spindle transfer is used to replace entire mitochondria that carry defective mitochondrial DNA.

REVIEW OF LITERATURE

In 1995, the potential impact of gene therapy on dentistry was described. In 2000, the first report of a fully successful gene therapy treatment-a French study involving a severe combined immuno-deficiency in young children was published. 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.[6]

TYPES OF GENE THERAPY

Gene therapy may be classified into the following types:

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.[7]

Somatic gene therapy: In the case of somatic gene therapy, the therapeutic gene is 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.[7]

GENE DELIVERY METHODS: Gene therapy involves the transfer of genetic information to target cells, which enables them to synthesize a protein of interest to treat disease. Various methods for gene delivery are:-

Viral vectors:[8]

1. Retrovirus: a class of viruses that can create double stranded DNA copies of their RNA genomes.
2. Adenoviruses: a class of viruses with double stranded DNA genomes.
3. Adeno-associated viruses (AAV): A class of small, single stranded DNA viruses that can insert their genetic material at a specific site on chromosome
4. Herpes simplex viruses: a class of double stranded viruses that can infect a particular cell type i.e. neurons.
5. Lenti viruses or hybrid viruses combine the traits of two or more viruses.

Non-viral vectors:[9]

1. Microseeding gene therapy: The therapeutic DNA is directly introduced into target cells using a gene gun. It is the simplest method of gene delivery. The disadvantage is that it requires large amounts of DNA to bring out the desired effect and hence this technique has restricted use.
2. Cationic liposomes: In this technique, an artificial lipid sphere (a liposome) with an aqueous core is created which carries the therapeutic DNA and is capable of transporting the DNA through the target cell's membrane. This delivery system tends to be less effective than others.

3. Gene activated matrices: The naked DNA is delivered to the target cells with the help of polymer matrix sponges.
4. Macromolecular conjugate: 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.

Difficulties in Using Gene Therapy[10]

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.

IMPLICATIONS OF GENE THERAPY IN PERIODONTICS

Gene therapy has been used as a mode of tissue engineering in periodontics. The tissue engineering approach reconstructs the natural target tissue by combining 3 elements namely scaffold, signalling molecules and cells.

Three basic approaches in tissue engineering are:

1. **Protein based approach:** In this approach growth and differentiation factors like TGF-β, BMP-2,6,7,12, VEGF and PDGF are used for regenerating periodontal tissues.[11]
2. **Cell based approach:** In this approach mesenchymal stem cell is used for reconstruction.[12]
3. **Gene delivery approach:**[13] It involves 2 basic modalities

a. In vivo gene delivery: In this approach, gene constructs such as plasmid or viral particles are entrapped physically in a scaffold which is implanted into the tissue defect. Host cells migrate into implant with the help of gene constructs and start producing proteins.

b. Ex vivo gene delivery: In this approach cultured cells are transfected using non-viral delivery systems or transduced using viral methods with gene constructs in vitro and then they are transplanted into the tissue defect.

TARGET GENES AND CLINICAL TRIALS USING GENE DELIVERY

Platelet Derived Growth Factor: PDGF is a member of a multifunctional polypeptide family which exerts its biological effects on cell proliferation, migration, extra-cellular matrix synthesis and anti-apoptosis.[1] Gene delivery of PDGF-β to human gingival fibroblasts revealed sustained signal transduction effects when compared to cells treated with recombinant human PDGF-BB protein alone.[6]

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.[14] Anusaksathien et al in 2003 reported that in an ex vivo investigation, the expression of PDGF genes was prolonged for up to 10 days in gingival wounds.[15] Giannobile 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.[16]

Bone Morphogenic Proteins: BMPs are multi-functional polypeptides belonging to the transforming growth factor-β super family of proteins. In dentistry, bone morphogenetic proteins (BMPs) have been shown to be potent growth factors stimulating alveolar bone formation.[17]

Franceschi et al in 2000 investigated in vitro and in vivo Ad gene transfer of BMP-7 for bone formation.[18] 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.[19]

Wingless (WNTS):

WNTS are a family of 19 secreted glycoproteins that are crucial for embryonic development and post-development physiology through regulation of cell proliferation, differentiation and apoptosis.[20]

Chang et al in 2007 found that retro viral WNT-4 transduced into human periodontal mesenchymal cells and transplanted into experimental periodontal defects promote healing of alveolar bone wounds in vivo.[21] Nemoto et al in 2009 showed that canonical wnt/β-catenin signaling inhibit murine cementoblasts differentiation and enhance cell

proliferation.[22]

Transcription Factors and Regulators:

Genes that are critical transcription factors and regulators of osteogenesis such as Runx2, osterix (Osx) and LIM domain mineralization protein (LMP) may hold promise in periodontal tissue engineering, especially in alveolar bone augmentation.[17]

Gene Delivery for Host Modulation

Gene therapy has also been investigated for the possibility of long term maintenance of therapeutic proteins by its application in host modulation. Tumor necrosis factor receptor immunoglobulin Fc (TNFR: Fc) fusion gene delivered to sites with experimental bone loss using AAV resulted in sustained therapeutic levels of serum TNFR protein for e"3 months and inhibition of bone loss.[23] Over expression of Tristetraprolin, a key cytokine-regulating RNA binding protein using an adenoviral vector significantly reduced the expression of interleukin-6, TNF-α and prostaglandin E2 in vitro and protected inflammation-induced bone loss and inflammatory infiltrate in an experimental periodontitis model.[24] Yu et al in 2011 demonstrated the potential of mitogen- activated protein kinase phosphatase 1 to prevent alveolar bone loss.[25]

FUTURE STRATEGIES OF GENE THERAPY IN PERIODONTICS

1. In prevention of periodontal disease-

- **Periodontal Vaccination:** Gene transfer research can lead to a novel way to achieve a vaccination like:

- Plasmid DNA encoding the Porphyromonas gingivalis fimbrial gene when injected in a salivary gland of a mouse produces fimbrial protein locally in the salivary gland tissue leading to the production of secreted IgA which could neutralize P. gingivalis and limit its ability to participate in plaque formation. Secreted fimbrillin in saliva could bind to pellicle components blocking the attachment of P. gingivalis.[26]
- P. gingivalis fimbrial antigen expressed by genetically engineered Streptococci gordoni vectors can be used as vaccine against P. gingivalis associated periodontitis in rats.[27]
- The recombinant hemagglutinin B (rHag B) when injected subcutaneously in Fischer rats gave protection against P. gingivalis induced bone loss.

- **Genetic Approach to Biofilm Antibiotic Resistance**

  Using a genetic approach ndvB mutant of Pseudomonas aeuroginosa have been isolated which is capable of forming biofilm but lacking the characteristic of periplasmic glucans, thereby, rendering microbial communities in biofilm more susceptible to conventional antibiotic therapy.[28]

- **An In vivo Gene Transfer by Electroporation for Alveolar Remodelling**

  Predictable alveolar bone remodelling has been found after, in vivo transfer of LacZ gene into the periodontium and using plasmid DNA as a vector along with electroporation (electric impulse) for driving the gene into cell.[26]

2. To control periodontal disease progression

- **Tight Adherence Gene for the Control of Periodontal Disease Progression**

  Development of mutant strains of "tight adherence gene" of Actinobacillus actinomycetemcomitans required for its adherence and virulence, limits colonization and pathogenesis of Actinobacillus actinomycetemcomitans.[29]

- **Antimicrobial Gene Therapy**

  When host cells were infected in vivo with defensin-2 (HBD-2) gene via retroviral vector, there was a potent antimicrobial activity which enhanced host antimicrobial defence.[30]

3. In the treatment of periodontal disease-

- **Gene enhanced tissue engineering:** Gene therapy provides long term exposure of the growth factor to the periodontal wound resulting in greater regeneration.

CONCLUSION

Results from preclinical and clinical trials, proves gene therapy to have a promising future in Regenerative Periodontics. Gene therapy is at the preclinical level at this time. Further details of mechanisms are to be understood and further researches need to be carried out to include these practically in day to day treatment modalities to achieve greater regeneration.

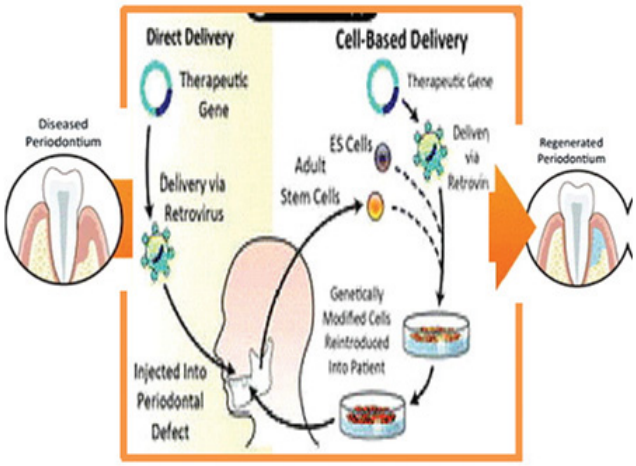

Figure 1: Gene Therapy in Periodontal Regeneration

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