Abstract
Genes are specific sequences of bases that encode instructions to make proteins. When genes are altered so that encoded proteins are unable to carry out their normal functions, genetic disorders can result. Gene therapy is designed to introduce genetic material into cells to compensate for abnormal genes or to make a beneficial protein. Gene therapy has a promising era in the field of dentistry. Gene therapy has been used as a mode of tissue engineering in dentistry. The tissue engineering approach reconstructs the natural target tissue by combining four elements namely: scaffold, signaling molecules, cells and blood supply and thus can help in the reconstruction of damaged tissue.

Keywords: Gene therapy, Gene delivery, Scaffold, Signaling molecule, Designer drug therapy

A broad definition of gene therapy is the genetic modification of cells for therapeutic purposes[1]. This approach is becoming possible owing to the increased understanding of the molecular basis for many diseases and the advances in the technology of gene transfer. 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 protein (termed recombinant protein) expressed.

Currently genetic principle are being applied along with tissue engineering for rehabilitation. This article reviews the fundamentals of gene therapy, and its implication indendistry.

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.

Fundamental Of Gene Therapy [2]
There are a variety of different methods to replace or repair the genes targeted in 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[3]
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.

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.

Gene Delivery:-
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[4-6]. The technology can be used to treat disorders that result from single point mutations[7] or to increase the protein. The preferred strategy for gene transfer depends on the required duration of protein release and the morphology of the target site. production[8].
There are various methods for gene delivery:-
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.

Some of the different types of viruses used as gene therapy vectors [9]:

1. Retroviruses - 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.
2. Adenoviruses - 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.
3. Herpes simplex viruses - 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.

2) Non viral

- The simplest method is the direct introduction of therapeutic DNA into target cells. This approach is limited in its application because it can be used only with certain tissues and requires large amounts of DNA.
- Another nonviral approach involves the use of an artificial lipid sphere with an aqueous core. This liposome, which carries the therapeutic DNA, is capable of passing the DNA through the target cell’s membrane.

MAJOR DEVELOPMENTS IN GENE THERAPY

In 1999, a trial treatments of SCID have been gene therapy’s only success; since 1999, gene therapy has restored the immune systems of at least 17 children with two forms (ADA-SCID and X-SCID) of the disorder. In 2002 a question was raised when two of the ten children treated developed a leukemia-like condition [10]. In 2006 scientists have successfully treated metastatic melanoma in two patients using killer T cells genetically retargeted to attack the cancer cells [11]. As well as in March again, scientists announced the successful use of gene therapy to treat two adult patients for a disease affecting myeloid cells [12].

Italy reported a breakthrough for gene therapy in which they developed a way to prevent the immune system from rejecting a newly delivered gene. Similar to organ transplantation.[13]

In 2007, the world’s first gene therapy trial for inherited retinal disease [14]. The safety of the subretinal delivery of recombinant adeno associated virus (AAV) carrying RPE65 gene, and found it yielded positive results.[15]

In 2009, gave trichromatic vision to using gene therapy, a hopeful precursor to a treatment for color blindness in humans. [16]

Limitation Of Gene Therapy [2]

1. Viral vector may be recognized as antigen and leads to activation of immune response. This may lead the efficacy of gene therapy and can induce serious side effect.

2. Chance of inducing a tumor (insertional mutagenesis) - If the DNA is integrated in the wrong place in the genome, for example in a tumor suppressor gene, it could induce a tumor. This has occurred in clinical trials for X-linked severe combined immunodeficiency (X-SCID) patients, in which hematopoietic stem cells were transduced with a corrective transgene using a retrovirus, and this led to the development of T cell leukemia in 3 of 20 patients.

3. Safety of vector - Viruses, the carrier of choice in most gene therapy studies, present a variety of potential problems to the patient toxicity, immune and inflammatory responses, and gene control and targeting issues. In addition, there is always the fear that the viral vector, once inside the patient, may recover its ability to cause disease.

4. Difficulty to treatment of Multigene disorders - Conditions or disorders that arise from mutations in a single gene are the best candidates for gene therapy. Unfortunately, some of the most commonly occurring disorders, such as heart disease, high blood pressure, Alzheimer’s disease, arthritis, and diabetes, are caused by the combined effects of variations in many genes. Multigene or multifactorial disorders such as these would be especially difficult to treat effectively using gene therapy.

5. Short-lived nature of gene therapy - Before gene therapy can become a permanent cure for any condition, the therapeutic DNA introduced into target cells must remain functional and the cells containing the therapeutic DNA must be long-lived and stable. Problems with integrating therapeutic DNA into the genome and the rapidly dividing nature of many cells prevent gene therapy from achieving any long-term benefits. Patients will have to undergo multiple rounds of gene therapy.

6. Expensive: gene therapy is costly and very expensive procedure.

7. Ethical restriction.

IMPLICATION IN DENTISTRY:--

There have been tremendous advances in gene therapy relevant to dentistry since 1995. Currently genetic principle are being applied along with tissue engineering for rehabilitation of tissue.

Tissue engineering Can be categorized into three major classes:

1. conductive,
2. Inductive, and
3. Cell transplantation approaches

Conductive approaches utilize biomaterials in a passive manner to facilitate the growth or regenerative capacity of existing tissue. An example of this that is very familiar to dentists, and particularly periodontists, is the use of barrier membranes in guided tissue regeneration. Nyman et al. were the first to successfully use osteoconductive mechanisms in providing a means for selective wound healing by supporting the in growth of the periodontal supporting cells, while excluding gingival epithelial and connective tissue cells from reconstruction sites.[17]

Inductive Activating cells in close proximity to the defect site with specific biological signals. The origins of this mechanism are rooted in the discovery of bone morphogenetic proteins (BMPs). Urist first showed that new bone could be formed at nonmineralizing, or ectopic, sites after implantation of powdered bone (bone demineralized and ground into fine particles).[18] Limitation of inductive approaches is that the inductive factors for a particular tissue may not known cell transplantation, becomes very attractive. This approach involves direct transplantation of cells grown in the laboratory.[19] The cell transplantation strategy truly reflects the multidisciplinary nature of tissue engineering, as it requires the clinician or surgeon, the bioengineer, and the cell biologist

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.

A. Platelet-derived growth factor (PDGF) gene delivery:
1. Early studies in dental applications using recombinant adenoviral vectors that encode PDGF demonstrated the ability of these vector constructs to transduce potently the cells isolated from the periodontium (eg, osteoblasts, cementoblasts, PDL cells, and gingival fibroblasts)[23]. Continuous exogenous delivery of PDGF-α may delay mineral formation induced by cementoblasts, whereas PDGF clearly is required for mineral neogenesis[24].
   b. Anusaksathien et al.[26] in an ex vivo investigation, showed that the expression of PDGF genes was prolonged for up to 10 days in gingival wounds.
   c. Giannobile et al.,[27] reviewed different mechanisms of drug delivery and novel approaches to reconstruct and engineer oral- and tooth-supporting structures, namely the periodontium and alveolar bone.

2. Bone morphogenetic protein delivery:
   a. In vitro and in vivo Ad gene transfer of BMP-7 for bone formation.[28]
   b. Direct in vivo gene delivery of Ad/BMP-7 in a collagen gel carrier promoted successful regeneration of alveolar bone defects around dental implants.[29]

GENE THERAPY CAN BE APPLIED IN NUMEROUS WAYS. They are as Following:-

8. An In vivo Gene Transfer by Electroporation for Alveolar Remodeling
9. Antimicrobial Gene Therapy to Control Disease Progression
10. Designer Drug Therapy in Treating Periodontal Disease

1. Gene Therapeutics-Periodontal Vaccination
   a. 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.
   b. Scientists have also demonstrated the efficacy of immunization with genetically engineered Streptococci gordoni vectors expressing P. Gingivalis is fimbrial vaccine as vaccine against P. gingivalis associated periodontitis in rats.[30]
   c. 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.[31]

2. Bone
   "Replacing bony defects include the utilization of autografts, allografts, and synthetic biomaterials. "Both conductive and inductive approaches can be used to regenerate small bony defects." Guided tissue regeneration (GTR) after periodontal surgery represents a conductive approach to regeneration of bone."BMPs, related proteins, and the genes encoding these proteins allow one to engineer bone using inductive approaches in situations" where GTR is not sufficient. In contrast, cell transplantation approaches offer the possibility of pre-forming large bone structures (e.g., complete mandible) that may not be achievable using the other two strategies. These structures may even be completely developed in the lab prior to use in large-scale reconstructive procedures.

3. Cartilage
   "Transplantation of cells without a carrier is now used clinically to repair small articular cartilaginous defects[32]

   "Investigators have also demonstrated in animal models that new cartilaginous tissue with precisely defined sizes and shapes relevant to maxillofacial reconstruction (e.g., nasal septum, temporomandibular joint) can be engineered using appropriate biodegradable scaffolds for transplanting the cells[33-34]

4. Skin and oral mucosa
   "Engineering and transplantation of oral mucosa and gingiva could be potentially important as a new technique in periodontal graft surgery and in the treatment of gingival recession

5. Dentine and dental pulp
   "There is now evidence suggesting that even if the odontoblasts (cells that produce dentin) are lost due to caries, it may be possible to induce formation of new cells from pulp tissue using certain BMPs.[35-37]

   "These new odontoblasts can synthesize new dentin. Tissue engineering of dental pulp itself may also be possible using cultured fibroblasts and synthetic polymers.[38]

6. Salivary gland
   "The loss of salivary gland tissue and/or function, whether it be a sequela to radiation therapy to treat cancer or part of a disease such as Sjogren's syndrome, is a problem that can significantly affect quality of life, particularly for medically compromised individuals.

   "One method in treating salivary gland functional deficiencies makes use of an inductive gene therapy approach. " The aim in this approach is to make existing non-secretory ductal epithelial cells (following irradiation therapy) into secretory cells capable of fluid movement. Success in animal models has been demonstrated.[39,40]" Baum et al. have recently initiated the development of an artificial salivary gland substitute composed of polymer tube lined by epithelial cells.[41]

7. 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[42] 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.

8. An In-vivo Gene Transfer by Electroporation for Alveolar Remodeling[43]

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.

9. Antimicrobial Gene Therapy to Control Disease Progression[34]

"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 ? defensin-2 (HBD-2) gene via retroviral vector; there was a potent antimicrobial activity which enhanced host antimicrobial defenses.

10. Designer Drug Therapy in Treating Periodontal Disease[45]

"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.

CONCLUSION Gene therapy has a promising role in the field of dentistry but it do encompasses serious ethical issue to be dealt with. It is evident that gene therapy have emerged from its stage of infancy of mere theoretical and hypothetical quotations to factual scientific researches which reveals potential hopes. There is still lot of research and details of mechanisms to be understood to include these practically in day to day treatment modalities.

REFERENCES


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