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# GENE THERAPY IN DENTISTRY

**Aarthi A*, Sarumathi T, Hemalatha VT, and Amudhan A.**

Department of OMDR, Sree Balaji Dental College And Hospital, Chennai, Tamil Nadu, India.

**ABSTRACT**

Change and modification is a continuous process in advancement of technology. As in most of the diseases conventional method is not giving satisfactory results, thus focus is on gene therapy located to treat not only inherited disease but also acquired ones. It is a method by which defective gene is replaced or repaired by a therapeutic gene. Gene therapy can be used to treat wide range of diseases ranging from single gene disorder to multi-gene disorder[1]. It has variety of applications in the field of dentistry like in cancerous and precancerous condition, salivary gland disorders, autoimmune diseases, bone repair, DNA vaccination, bone repair etc. Minor salivary glands and keratinocytes present in the oral mucosa are excellent target sites for gene therapy since it can be readily accomplished with minimal invasive manner. Gene therapy is a promising field evolving in the current decade.

**Keywords:** Gene, Vector.

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***Corresponding author***

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## INTRODUCTION

Gene therapy is an emerging field of biomedicine that involves the transfer of genes to patients for clinical benefit. Gene therapy essentially consists of introducing specific genetic material into target cells without producing toxic effects on surrounding tissue. Joshua Lederberg and Edward Tatum laid out the fundamental tenets for gene therapy[2].

### Gene Therapy

It is the replacement of person's faulty genetic material with normal genetic material to treat or cure a disease or abnormal medical condition[3](US Food and Drug administration). Scientists started researching gene therapy with bacteria in 1980 and first gene therapy in human was performed in 1990 for treating severe combined immunodeficiency which worked for only few months [4]. Originally in 1980 gene therapy was known as gene replacement therapy.

There are two types of gene therapy: **Somatic cell And Germ line.**

**Somatic cell gene therapy[3,4]** is the only technique now in use. The purpose of the procedure is to eliminate the clinical consequences of a disease and the inserted gene is not passed on to the patient's offspring.

**Germ line gene therapy[4]** a healthy gene is inserted into the fertilized egg of an animal that has a genetic effect. Every cell that develops from this egg, including the reproductive cells, will have the new gene.

### Principles of Gene Therapy[5]:

- Regulation of particular gene (the degree to which the gene is turned on or off) can be changed.
- Faulty gene can be replaced for a normal gene through homologous recombination.
- Normal gene is inserted into nonspecific location within the genome to replace a nonfunctional gene.
- Abnormal gene is repaired through selective reverse mutation which returns the gene to its normal functional status[2].

### Requirements for Vector:

- It should not be identified by immune system (non-immunologic)
- Should be stable and easy to reproduce
- Should have longevity of expression
- Should have high efficiency (100% cells transfected)
- High specificity and low toxicity
- It should be able to protect and deliver DNA across the cell membrane into the nucleus.
- It should be able to target gene delivery to specific cells
- It should be easy to be produced in large amounts and be inexpensive
- Currently no single vector type will meet all needs for all tissues, that is different vectors will be needed for different c
- inical applications.

### VECTORS FOR GENE THERAPY

Vectors is defined as a vehicle to deliver the gene of interest.

1. Viral vectors
2. Nonviral vectors.

1. Viral Vectors[6]: adenovirus, adeno-associated virus, retrovirus and herpes simplex virus.
   a. Adenoviruses:
      They infect both dividing cells and nondividing cells. Adenoviruses do not integrate the foreign DNA into host cell's rather the foreign DNA exists independently in the nucleus (so called episome).

Adenoassociated virus, the smallest of three vectors listed, can accommodate only about half as much as foreign DNA as the others.

b. Retroviruses[6]:
Retroviruses infect only dividing cells. They integrate the foreign DNA into the host cell chromosome and thus lead to stable expression. However, the gene insertion is not controlled, and it occurs in such a way as to cause a mutation of the cell.

2. Nonviral Vectors can further classified into physical and chemical vectors.

- Physical vectors include electroporation, microinjection, use of Ballistic particles.
- Chemical vectors include calcium vectors, lipids, protein complexes.

a. **Electroporation:** In this method, electrical current creates transient holes in the cell membrane through which DNA can be transferred[6].

b. **Microinjection:** In this method, DNA is introduced in a single cell.

c. **Use of Ballistic Particles**: The plasmid DNA is coated onto tungsten or gold particles. Acclerated force is generated by high-voltage electronic spark, or helium discharge to propel the beads into the tissue.

d. **Calcium Vector[7]:** The ultra-low size, highly monodispersed DNA doped calcium phosphate nanoparticles protect from the external DNAse environment can be used safely to transfer the encapsulated DNA under in vitro and in vivo condition.

e. **Lipid Vectors:** They are produced by a combination of plasmid DNA and a solution that results in the formation of liposome. This fuses with the cell membrane of a variety of cell types, introducing plasmid DNA into the cytoplasm and where it is transiently expressed[8].

f. **Protein Complex:** Several groups developed cell-specific DNA delivery systems that utilize unique cell surface receptor on the target cell. By attaching the ligands recognized by such a receptor to the transfer DNA, the DNA ligands complex become selectively bound and internalized into the target cell[7].

**Types of delivery[6]**

**In vivo:** delivery of gene takes place in the body. During in vivo gene transfer, the foreign gene is injected into the patient by viral and non viral methods.

**Ex vivo:** delivery takes place outside the body and the cells are placed back in to the body. Ex vivo gene transfer involves a foreign gene transduced into tissue cells cultivated in laboratory outside the body, and then resulting genetically modified cells are transplanted back into the patient.

Successful gene therapy requires that[1]

- Genetic nature of the disease is completely understood
- Genes can be delivered to the target cells of affected tissue/organ
- Transfected gene should be active for intended duration
- Harmful side effects if seen should be manageable

Difficulties in gene therapy include[9]

- Difficulty to deliver genes in some sites like lung cells
- Genes might integrate at sites where it can affect the functioning of another gene
- Vectors may be recognized as —foreign‖ by immune system triggering immune response

- Viral vector may cause toxicity, inflammatory response and might recover their ability to cause disease
- Multigene disorders are difficult to treat by gene therapy
- Gene therapy is expensive

Principle:

- A "normal" gene is inserted into the genome to replace an "abnormal," disease-causing gene.
- 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.
- Target cells such as the patient's cells are infected with the viral vector.
- The vector then unloads its genetic material containing the therapeutic human gene into the target cell.
- The generation of a functional protein product from the therapeutic gene restores the target cell to a normal state[7].

**Applications of gene therapy in dentistry**

**In bone repair**

Bone loss is a major worldwide health problem. Regeneration of these bone structures would be enormously useful in the treatment of craniofacial and other bone anomalies, tooth loss, temporomandibular and other joint diseases, traumatic amputations and the consequences of tumor resection . The bone morphogenic proteins (BMPs)enable skeletal tissue formation during embryogenesis, growth, adulthood, and healing. Probably BMPs (BMPs 2, 4 and 7) are the only growth factors which can singly induce de novo bone formation both in vitro and at heterotopic sites. Bone defects in the oral and maxillofacial region can be repaired by transferring genes encoding BMP's (Bone Morphogenic Protein) [11]. It will be possible to directly deliver the BMP-2 gene in vivo to tissues via an adeno viral vector to heal bone defects . In one study genetically engineered mesenchymal stem cells expressing BMP-2 induced increased formation of new blood vessels as well as new bone .

Transferring platelet-derived growth factor gene to periodontal cells results in DNA synthesis and cellular proliferation. Delivery of PDGF by gene transfer has been shown to stimulate gingival fibroblast, PDL and tooth-lining cell (cementoblast) mitogenesis and proliferation above that of continuous PDGF administration in vitro. PDGF has also demonstrated positive effects in regenerating bone around teeth and dental implants[12,7] . This will facilitate localized regeneration of bone for periodontal and oral surgical procedures.

**Pain**

Gene transfer may be particularly useful for managing chronic and intractable pain viral-mediated transfer of genes encoding opiate peptides to peripheral and central neurons can lead to anti nociceptive effects[13]. Intrathecal injection of vectors derived from adenovirus, AAV or lipid encapsulated plasmids coding interleukin-10, transducing neurons of the Dorsal Root Ganglia by injection of herpes simplex virus based vectors into the skin and injecting vector virus carrying the gene for an endogenous opioid has been tried to control chronic pain[15].

**Systemic gene therapeutics as an application of gene transfer to salivary glands**

By means of an appropriate gene transfer vector—such as a recombinant adenovirus or an adeno-associated virus—a gene encoding a protein (a biopharmaceutical, or bio) is delivered to a healthy, functioning salivary gland via intraductal cannulation[17]. The gene product, bio, is secreted across the basolateral

membranes of the salivary epithelial cells into the bloodstream, rather than being secreted across the apical (lumenal) membrane into saliva.

**Autoimmune disease**

Salivary glands in patients with Sjögren's syndrome have a lymphocytic infiltrate with predominantly CD4+ cells, leading to the local production of cytokines that can lead to tissue damage (tumor necrosis factor, or TNF, -α; interleukin, or IL,-2; and interferon, or IFN, -γ), resulting in a derangement of the secretory acinar cells and their eventual loss. administration of a recombinant adeno-associated virus encoding human interleukin-10 (rAAVhIL10) to such tissue will restore an immunological balance in the salivary glands and result in a dissipation of the lymphocytic infiltrate[17].

**DNA vaccinations**

Dental scientists have tried to use classical vaccination technology to eradicate dental caries or periodontal Diseases by directly delivering DNA in a plasmid vs. the traditional administration of a purified protein or an attenuated microbe[18].

**Gene transfer to keratinocytes**

Ex vivo gene transfer to keratinocytes via the use of retroviruses and resulted in a normalization of tissue architecture and epidermal function for conditions such as ichthyosis and epidermolysis bullosa.

Keratinocytes are very favourable in these sites as[19]:

A. The area is easily accessible so monitoring is adequate.
B. Preclinical assessement is accurate since culture models are established.
C. Expression of therapeutic genes can be achieved with the use of topically applied agents.
D. Procedures for transplanting keratinocytes sheets already established because of their application for burn patients.
E. It is reversible because genetically modified tissue can be excised.

**Head and neck cancer**

Currently available vectors are quite useful for certain defined conditions, such as adenoviruses for gene therapy of head and neck cancers[20].

Gene therapy approaches to oral cancer

- Addiction gene therapy
- Suicidal gene therapy
- Oncolytic virus gene therapy
- Excision gene therapy
- Inhibition of tumorogenesis
- Immunotherapy

**Addiction gene therapy**

- Is to regulate tumor growth by introducing tumor suppressor genes, that inactivate the carcinogenic cells.
- Vector – Ad5CMV-p53, Ad5CMV-p27

**Protocol-**

- Day 1- IM injection followed 2hrs by a mouthwash
- From next day- mouthwash twice a day for 2-5 days
- This is repeated every 28 days
- Outcome: Inhibition of disease progression in the precancerous lesion without toxic effect
- The gene transfer application of immune modulation appears to have potential for treatment of autoimmune diseases such as Sjögren's syndrome.

**Suicidal gene therapy**

- Permits the expression of enzymes that transforms non toxic drugs into cytotoxic substances
- Vector- Adenovirus
  **Protocol-**
- Administration of Herpes simplex virus thymidine kinase gene in combination with ganciclovir. Ganciclovir change into ganciclovir phosphate, which is a cytotoxic substance that kills the neoplastic squamous cell

**Oncolytic virus gene therapy**

- Virus replicates only in the tumor cells that lacks p53 gene.
- Vector- ONYX-015 Adenovirus
  **Protocol –**
- IM inj. of ONYX-015 with 5-flurouracil
- Outcome: Appearance of immune response of tumor

**Excision gene therapy**

- Therapy involves removal of defective oncogenes, which inhibits growth of tumor cells.
- Vector- Adenovirus
  **Protocol** – IM injection with okadic acid
- Outcome – Reduction of expression of Egr1 that inhibits tumor activity

**Inhibition of tumorogenesis**

- Angiostatin, an angiogenic inhibitor is used in this technique.
- Vector – Adenovirus
  **Protocol-** Administration of encapsulated angiostatin
- Outcome - Angiostatin localized in the tumor and inhibit angiogenesis

**Immunotherapy**

- Immunotherapy increases patients immune response to tumor.
- Vector- Adenovirus
  **Protocol** – Intra tumoral injection with F/RGD
- Outcome – Increase in mononuclear cell infiltrations[17,20].

**Problems of genetherapy:**

Short Lived
- Hard to rapidly integrate therapeutic DNA into genome and rapidly dividing nature of cells prevent gene therapy from long time
- Would have to have multiple rounds of therapy

Immune Response
- new things introduced leads to immune response
- increased response when a repeat offender enters

Viral Vectors
- patient could have toxic, immune, inflammatory response
- also may cause disease once inside

Multigene Disorders
- Heart disease, high blood pressure, Alzheimer's, arthritis and diabetes are hard to treat because you need to introduce more than one gene may induce a tumor if integrated in a tumor suppressor gene because insertional mutagenesis

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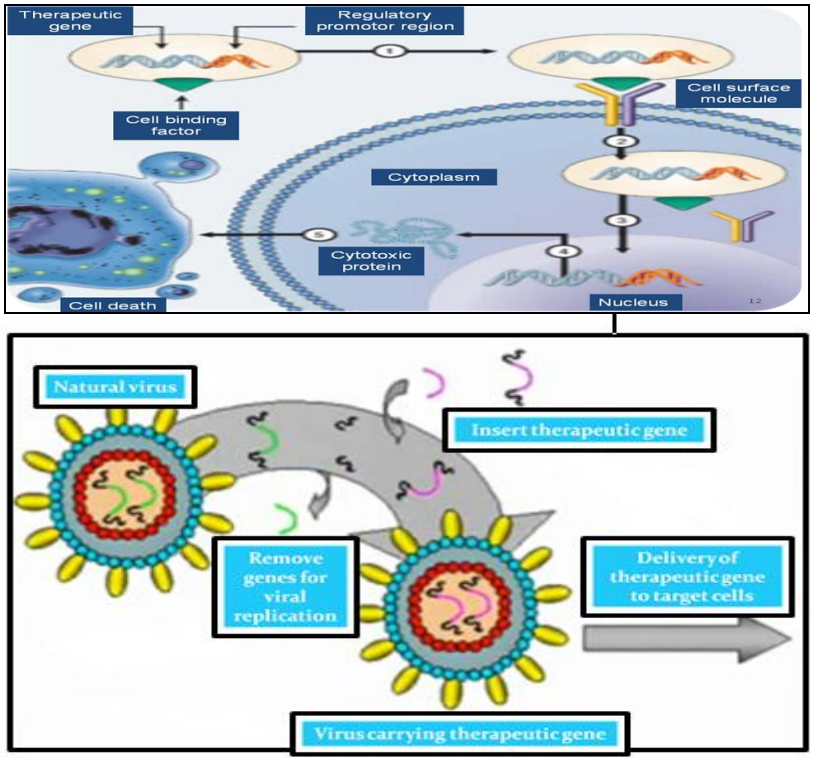
**Dental surgeon as Gene Therapist**

The role of dental surgeon in gene therapy is tenable. A main advantage for dentists in gene therapy studies is the ready accessibility of oral tissues. Salivary gland produces large amount of proteins and it is a site where gene transfer can be readily accomplished in minimal invasive manner[21].

1.PRINCIPLES OF GENE THERAPY:




**1.HSV VECTORS ENCODING THERAPEUTIC GENES IN PAIN TRT[14]**

| PAIN MODELS | GENE PRODUCT | INOCULATION |
|---|---|---|
| Acute pain | Preproenkephalin | Skin of dorsal hindpaw |
| Inflammatory pain | Preproenkephalin | Infected on scarified hind footpads |
| Neuropathic pain | Proenkephalin | Unilateral peripheral inoculation |
| Cutaneous Hyperalgesia | Preproenkephalin | Subcutaneous inoculation |
| Cancer Pain | Proenkephalin | Subcutaneous inoculation |

**2. AAV VECTORS ENCODING THERAPEUTIC GENES IN PAIN TRT[14,16]**

| PAIN MODELS | GENE PRODUCT | INOCULATION |
|---|---|---|
| Neuropathic pain | IL - 10 | Intrathecal administration |
| Neuropathic pain | Prepro-β-endorphin | Intrathecal administration |
| Inflammatory pain | μ-opioid receptor | Injected into DRG |

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## CONCLUSION AND FUTURE DIRECTION

No doubt future of gene therapy is very bright. Through researchers are facing various complications like uncertainity of the response of immune system to a viral vector, lack and the response of viral vector to other cells. Despite of these, gene therapy will definitely become a potential and promising treatment modality of number of diseases especially for head and neck cancers.

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