INTRODUCTION:

Genes, which are carried on chromosomes are the basic physical and functional units of heredity. The segment, composed of minute subunits called nucleotide bases, serves as the blueprint for manufacturing a single protein or enzyme needed for the structure or function of cells. In humans, genes are compressed and bundled into a set of 23 pairs of chromosomes, which stabilize and protect the DNA. Even a tiny error in the arrangement of a gene’s nucleotide bases can lead to the production of a protein or enzyme that works improperly or the needed compounds might not be produced at all.

The transfer of foreign genes into eukaryotic cells, production of coding proteins for therapeutic purposes and the development of strategies for gene therapy.

Gene therapy can be defined as the introduction of nucleic acids into cells for the purpose of altering the course of a medical condition or disease. Gene therapy is transferring recombinant genetic material (DNA or RNA) to the host cell in order to change the gene expression in the host cell to gain a therapeutic effect. Gene therapy involves the efficient introduction of functional gene into the appropriate cells of the patient in order to produce sufficient amount of protein encoded by transferred gene (transgene) so as to precisely and permanently correct the disorder.

There are three main strategies in gene therapy.
1. Gene addition.
2. Removal of a harmful gene by antisense nucleotide or ribozymes.
3. Control of gene expression.
Gene therapy has various potential advantages over drug therapy like:-
1. Functional gene can replace a dysfunctional gene or deficient gene.
2. Transgene can result into continuous production of a therapeutic protein that normally has a short half-life.
3. Gene therapy can be focused to a specific cell type to avoid potentially toxic systemic effects.
4. Gene therapy can improve patient’s compliance and decrease cost of therapy on long term bases.

Pre-requisites for human gene therapy are:
1. Identification of the gene responsible for disease.
3. Identification of gene mutations.
4. Linkage of gene mutation to the pathophysiology of the disease.
5. Identification or selection of the gene transfer target cell.
7. Gene transfer efficacy and safety testing system.

Gene therapy requires a certain number of elements:
1. Gene transfer efficacy and safety testing system.
2. Detection of gene expression and protein function.
3. Identification or selection of the gene transfer target cell.
4. Linkage of gene mutation to the pathophysiology of the disease.
5. Identification of gene mutations.
7. Gene transfer efficacy and safety testing system.

Methods of Gene Therapy

The gene delivery systems available for gene therapy now fall into two categories: viral vectors and non-viral vectors.

Non-viral Vectors:
1. Physical: Parenteral injections, micro-injections, aerosol, electroporation (high voltage current is passed to the target cell to produce pores on the cell surface through which transgene enters the cell) and gene guns
2. Chemical: Calcium phosphate, DEAE-dextran, liposomes and lipoplexes (for oral delivery of gene), surfactants and perfluorochemical liquids for aerosol delivery of gene

Viral vectors:
These are more promising system of gene delivery with various advantages over physical and chemical method-
1. Gene transferred is more efficient and specific than physical and chemical method.
2. Multiple and repeated doses are required in case of physical and chemical method, whereas in case of viral vector even a single dose is sufficient

A vector can be described as a system fulfilling several functions, including (a) enabling delivery of genes into the target cells and their nucleus, (b) providing protection from gene degradation, and (c) ensuring gene transcription in the cell. Vectors are frequently used to insert the genetic materials in the cells. Gene transfer via the viral vectors is called transduction while transfer via the non-viral vectors is called transfection. Viral vectors are retroviral, adenoviral, and adeno-associated viral (AAV). Non-viral vectors are liposomes, plasmid, and synthetic oligonucleotides.

Generally, the viral vectors can be divided into two types: integrating and nonintegrating viral vectors. The former, such as, retroviral, lentiviral, and adeno-associated viral vectors, can integrate into the human genome; whereas the non-integrating vector (e.g., adenoviral vector) is maintained in the nucleus without integrating into the chromosomal DNA.

Transfer of the genetic material to the cells
1. Percutaneous method
2. Surgical method
3. Cathether system

Applications of gene therapy in dentistry
1. Bone repair: An area of real clinical importance in dentistry which uses gene therapy to repair bony lesions. BMPs are the agents well established in introduction of both orthotopic and ectotopic bone formation. Genetically engineered
mesenchymal stem cells express BMP-2 induced increased production of blood vessels as well as new bone.

2. Gene transfer in salivary glands- Our original goal in developing gene transfer with salivary glands was to provide effective therapies for patients suffering from irreversible salivary gland dysfunction resulting from either irradiation for head and neck cancers or the autoimmune damage occurring with Sjögren's syndrome.

3. Pain-Managing or eliminating pain is a major part of dental practice. The use of gene transfer technology offers a potentially novel approach to manipulate specific, localized biochemical pathways involved in pain generation.

4. DNA vaccination- For many years, dental scientists have tried to use classical vaccination technology to eradicate dental caries or periodontal diseases, thus far achieving mixed success. In the last decade, gene transfer research has led to a novel way to achieve vaccination, directly delivering DNA into the buccal epithelium. As a result, the traditional administration of a purified protein or an attenuated microbe is obviated. Although applications of DNA vaccination are in the earliest stages of use with oropharyngeal tissues, it seems reasonable to suggest that these approaches will play a role in future strategies for preventing periodontal diseases and dental caries.

5. Gene transfer to keratinocytes- There are several features that make epidermal and mucosal keratinocytes attractive for treating local tissue disorders and as systemic gene therapeutics. First, most disorders involving the genetically modified tissue is accessible, second, preclinical assessment is accurate since culture models are established. Third, application of therapeutic genes can be achieved with use of topical agents applied agents. Fourth, procedures for stabilizing gene transfer sheets are already established because of their applications for burn patients. Finally, keratinocyte gene therapy is reversible because the genetically modified tissue can be excised readily. These efforts have relied on 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.

6. For head and neck cancer- Oral squamous cell carcinoma is an attractive tumour target due to its frequent genetic mutations and accessibility for intratumour administration.

a. Gene addition therapy- Aims at introducing normal or functional copy of gene into genome so as to restore the normal function of the cell. Several genetic alternations are seen in oral squamous cell carcinoma like mutation of p53, p21 and p16. Since the p53 plays a important role in regulating cell cycle and apoptosis, p53 gene transfer was initially tested in carcinoma patients by injecting primary tumour with an adenviral vector expressing wild type p53.

b. Antisense RNA therapy- Treatment of genetic disorders by introducing remedial gene that prevents the expression of a specific defective gene is called antisense therapy. Gene expression can usually be inhibited by RNA that is complementary to the strand of DNA expressing gene. Inhibition of expression of oncogene may alter the phenotype thus arresting the tumour growth.

c. Suicide gene therapy- Involves the introduction of gene into a cell that enables a prodrug to be activated into active cytotoxic drug. The most extensively studied approach utilizes Herpes Simplex Virus-Thymidine Kinase (HSV/TK) ganciclovir system.

d. Immunological gene therapy- The immunological gene therapy approach to oral cancer involves either increasing of immunologic potential of tumour cells or augmenting patients immune response to tumour.

7. Gene therapy in the field of implants- BMPs gene delivery for alveolar bone engineering at dental implant defects- Tooth loss as a consequence of trauma or disease leads to destruction of nearly half of alveolar bone. Treatment of Titanium implant fixture with Ad/BMP-7 resulted in enhancement of alveolar bone defect fill, coronal bone formation and new bone to implant contact.

Gene therapy is a novel approach to treat, cure, or ultimately prevent disease by changing the expression of a person's genes. In other words, it is a novel form of drug delivery that enlists the synthetic machinery of the patient's cell to produce a therapeutic agent.

Gene therapy may become an integral tool in dental practice early in the 21st century. It, and other biological therapies, are expected to be applied to oral diseases and disorders during the midpractice lifetime of today's dental professionals. If the applications of oral gene transfer are expanded to systemic diseases, oral health care providers in the future could routinely be "gene therapists" with therapeutic targets well outside the oral cavity.

References
1. North Calorina Association for Biomedical Research
13. Australian Genetics Resource Book