

Gene Therapy: Principles and Applications in Dentistry.

Vagish Kumar L S*.

Department of Oral Medicine and Radiology, Yenepoya Dental College and Hospital, Yenepoya University, Mangalore-575018, Karnataka, India.

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*For Correspondence

Department of Oral Medicine and Radiology, Yenepoya Dental College and Hospital, Yenepoya University, Mangalore-575018, Karnataka, India.

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ABSTRACT

Change and modification is a continuous process in advancement of technology. Research is being done to understand the cellular and molecular basis of every disease. Most of the conventional methods to treat diseases have not been giving satisfactory results, so currently there is increasing focus on gene therapy to treat wide variety of inherited and acquired diseases. Gene therapy is the use of genes as medicine. 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. 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. This makes dentists as suitable candidate for gene therapy. Aim of this article is to focus in brief on the basic principles of gene therapy, its applications in the field of dentistry and its limitations and disadvantages.

INTRODUCTION

Scientists are trying to eradicate diseases by changing the genes that are responsible for it. The process by which this is done is called gene therapy. Joshua Lederberg and Edward Tatum laid out the fundamental tenets for gene therapy [1]. Advances in molecular biological technology such as recombinant DNA technology have made researchers to manipulate genes easier. Genes are the smallest functional units of the genetic system, which control the development and function of all organisms. A gene is a linear sequence of DNA that codes for a particular protein [2]. Proteins are large molecules that perform various essential functions in the body. DNA the constituent of genes is made up of 4 bases: adenine, guanine, cytosine and thymine. These bases are ordered to create the language of genes [3]. When genes are altered, the encoded proteins are unable to carry out their normal function resulting in diseases.

Genes are mainly concerned with two types of function—determining the structure of the thousands of different proteins that are present in the human body and controlling where, when and in what quantity each protein is made [4]. Gene therapy is based on principle that a normal gene is inserted to compensate for a nonfunctional gene and abnormal gene can be repaired through selective reverse mutation. It uses purified preparations of a gene or a fraction of gene to treat diseases. 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 [2]. Originally in 1980 gene therapy was known as gene replacement therapy. Genetic Diseases like cystic fibrosis, blood disorders, muscular dystrophy and diabetes etc can be attempted to be cured by gene therapy [5]. Gene therapy in recent days has grown by leaps and bounds and its application in dentistry includes bone repair, treatment of salivary gland diseases, auto immune diseases, cancerous and precancerous lesions, pain, DNA vaccination (periodontal diseases, caries) and dermatological disorders [2, 4, 6]. It is used to replace a faulty gene or to introduce a new gene whose function is to cure or to favorably modify the clinical course of the condition. Transferred genes can be used for either reparative or pharmacological purposes.

Principles of Gene Therapy

Gene therapy is the replacement of person's faulty genetic material with normal genetic material to treat or cure a disease or abnormal medical condition (US Food and Drug Administration) [7]. The procedure involved in gene therapy includes

- Pinpoint gene of interest,
- Acquiring a normal copy of gene (therapeutic gene) by restrictive endonuclease enzyme (cutting and splicing) and
- Finally cloning of therapeutic gene into a vector, which is a vehicle to deliver the gene of interest [2].

Gene transfer efficacy and safety testing system should be done before therapy [5].

Faulty genes can be corrected by several methods [3]

- 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.

GENERAL PRINCIPLES OF GENE TRANSFER

The concept of gene therapy involves the introduction of exogenous genes into somatic cells that form the organs of the body to produce a desired therapeutic effect. The selected DNA fragment is first cleaved using restriction endonucleases. Then vector or vehicle is prepared to transfer the genetic material. The vector is isolated, purified and cleaved to allow insertion of the DNA fragment. The DNA fragments then must be joined to the cleaved ends of the vector, effectively closing the molecule. This successful insertion of an exogenous DNA molecule into a vector results in a DNA chimera. These vector constructs are the basis of recombinant DNA techniques. Next step involves introduction of the construct into a cell, allowing the production of a line of genetically identical cells containing the DNA sequence introduced by the vector. This allows mass production of cells with a specifically designed genetic make-up [1, 3, 4].

The vector can be administered intravenously or injected directly into a specific tissue in the body, where it is taken by individual target cells. Alternatively patient's representative cells that are removed from the body can be exposed to the vector in laboratory. The cells which now contain the vectors are reintroduced into the patient for therapeutic action. Vector delivers the therapeutic gene into patient's target. The target cells become infective with therapeutic gene through vector. Functional proteins are created from the therapeutic gene causing the cell to return to a normal stage [3, 4].

Requirements for Vector

The ideal requirements for vectors are [1, 2, 4]

- 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 clinical applications.

Types of Vector for gene therapy

Vectors can be either Viral or Non-Viral.

Viral vectors-Commonly used viral vectors are *Adenovirus*, *Adeno associated virus (AAV)*, *Retro virus* and *Herpes simplex virus*. Among these, *adenovirus* is commonly used, as it can be cultured easily and is of lower pathogenicity [2]. These viruses are attenuated to transfect genes, but they cannot replicate or cause infection.

Eliminating their ability to replicate through genetic manipulation of the wild type virus eliminates the pathogenicity of virus [4]. A variety of virus vectors that have been employed to deliver genes have their own advantages and disadvantages. Gene transfer mediated by viral vectors is referred to as transduction [8].

Adenoviruses

They infect both dividing cells and non dividing cells. *Adenoviruses* do not integrate the foreign DNA into host cells; rather the foreign DNA exists independently in the nucleus. *Adeno associated virus*, the smallest of three vectors listed, can accommodate only about half as much as foreign DNA as the others. This vector can insert their genetic material at specific site of chromosome 19 [9]. Disadvantage of these vectors lies in the activation of both the innate and adaptive parts of the recipient's immune system when applied in vivo [10].

Retroviruses

Retroviruses infect only dividing cells. They permanently integrate the foreign DNA into the host cell chromosomes 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. Retroviral vectors require mitotic cell division for transduction [11].

Herpes simplex viruses

Double stranded viruses that infect particular cell type i.e. neurons [9, 12].

Non Viral Vectors- can further be classified into physical and chemical vectors. Gene transfer mediated by non viral vectors is referred to as transfection [8].

Physical vectors

Electrophoration, microinjection and use of Ballistic particles [6].

Chemical vectors

Include calcium vectors, lipids and protein complexes.

Electrophoration

In this method, electrical current creates transient holes in the cell membrane through which DNA can be transferred.

Microinjection

In this method, DNA is introduced in a single cell.

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

Calcium Vector: The ultra-low size, highly monodispersed DNA doped calcium phosphate nano particles protect from the external DNase environment can be used safely to transfer the encapsulated DNA under *in vitro* and *in vivo* condition.

Lipid Vectors: Creation of artificial lipid sphere with aqueous core, liposome - carries therapeutic DNA through membrane. 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 [1, 2].

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 gets internalized into the target cell [4].

Other Non-Viral options include,

Direct introduction of therapeutic DNA - Disadvantage being it can be used only with certain tissues and requires a lot of DNA [3].

Trying to introduce a 47th chromosome ^[2] - This chromosome would be alongside chromosome 46 and act autonomously without affecting the function of it or causing any mutations. Advantages are that it can carry large amount of genetic code and also because of its autonomy, immune system would not attack it. Disadvantage is difficulty in introducing such a large molecule into the nucleus of the target cell ^[3].

Non viral methods present certain advantages over viral methods, with simple large scale production and low host immunogenicity being just two. Previously, low levels of transfection and expression of the gene held nonviral methods at a disadvantage; however, recent advances in vector technology have yielded molecules and techniques with transfection efficiencies similar to those of viruses. Other than viral vectors, stem cells are also used in gene therapy. Stem cells are manipulated in laboratory in order to make them receive new genes that can change their functions ^[4, 5].

Types of Gene Therapy ^[2, 3]

Germ line gene therapy: Repair or replace defective gene in germ line cell. Modified gene would be inherited.

Somatic gene therapy: Repair or replace defective gene in some or all body cells of an individual. But the change is not passed to next generation.

Types of delivery

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 ^[4]

- 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

The most frequently observed adverse reactions to gene therapy are severe inflammatory processes and coagulopathies, generally in relation to the viral vectors employed ^[10, 13, 14].

Applications in Dentistry

Bone repair

Bone loss caused by trauma, neoplasia, reconstructive surgery, congenital defects or periodontal disease 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 ^[15, 16]. 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) ^[17]. It will be possible to directly deliver the BMP-2 gene *in vivo* to tissues via an *adeno viral* vector to heal bone defects ^[2, 5]. In one study genetically engineered mesenchymal stem cells expressing BMP-2 induced increased formation of new blood vessels as well as new bone ^[6]. Michigan research group has found non osteogenic fibroblasts (gingiva, dental pulp) expresses BMP-7 gene after being infected with an adeno viral vector. BMPs are agents well established in induction of both orthotopic and ectopic bone formation. In *ex vivo* studies,

researchers accomplish the actual gene transfer in a tissue culture environment and then place the transduced cells carrying the foreign genes back into the host [6]. 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 [18]. This will facilitate localized regeneration of bone for periodontal and oral surgical procedures. Bone sialoprotein (BSP) is a major non collagenous protein in bone and other mineralized tissues. By the *in vivo* delivery of a BSP gene into an osseous defect, it has been shown to regenerate periodontal alveolar bone [4]. Studies have demonstrated the potential use of gene delivery to regenerate alveolar bone and cementum around teeth and alveolar bone associated with dental implant fixtures [18].

Ex vivo Gene Transfer for Bone Repair

The advantage of an *ex vivo* gene transfer approach is that specific cells like bone marrow cells or stem cells can be selected as the cellular delivery vehicle for specific clinical problems. In addition, *ex vivo* strategies have a high efficiency of cell transduction. It is possible to harvest cells from the patient, have a very short period of infection and reimplant the transduced cells at the appropriate anatomic site. The cells that have received the most interest as a cellular delivery vehicle are mesenchymal stem cells, muscle-derived stem cells, adipose-derived stem cells, buffy coat cells from bone marrow or blood and skin fibroblasts [4, 6]. Delivery of multiple genes may enhance bone repair.

Tissue engineering

Gene constructs, such as plasmid DNA or a viral particle are physically entrapped within a matrix. When this matrix/ scaffold is implanted into the tissue defects, host cells migrate into the implant, take up the gene construct and start producing the encoded protein [16].

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. Gene transfer may be particularly useful for managing chronic and intractable pain. Several studies in animal models have shown that viral mediated transfer of genes encoding opiate peptides to peripheral and central neurons can lead to antinociceptive effects. More research is needed before gene transfer can be tested clinically as a strategy for chronic pain management. The use of gene transfer in place of drug delivery to achieve the continuous release of short-lived bioactive peptides in or near the spinal dorsal horn underlies the most common strategies for gene therapy of pain. 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. Also direct gene delivery to the articular surface of the temporomandibular joint has been found to be feasible. Research in this may result in novel treatment strategies for chronic temporomandibular pain [4, 6].

DNA Vaccination

Dental scientists have tried to use classical vaccination technology to eradicate dental caries or periodontal diseases, thus far achieving mixed success. Modern focus is on directly delivering DNA in a plasmid rather than the traditional administration of a purified protein or an attenuated microbe. The ability to induce an immune response to a protein antigen by administration of plasmid DNA encoding the antigen has been successfully demonstrated in animal models. Applications of DNA vaccination are in the earliest stages of its use with oropharyngeal tissues. DNA vaccination will play a role in future strategies for preventing periodontal diseases and dental caries. Immunization of salivary gland using plasmid DNA encoding the *Porphyromonas gingivalis* fimbrial gene leads to the production of fimbrial protein locally in the salivary gland tissue with consequent production of specific salivary immunoglobulin A, or IgA, and immunoglobulin G, or IgG, antibodies and serum IgG antibodies. Also generation of antigen specific cytotoxic T lymphocytes can be achieved resulting in protection from *P. gingivalis*. Also any secreted fimbrial protein in saliva could bind to pellicle components and also inhibit the attachment of *P. gingivalis* to the developing plaque. It has been reported that the plasmid pCIA-P encoding *pac* gene of *S. mutans* could induce protective anticaries immune responses in rats by targeted salivary gland immunization [4, 6]. Future research strategies should be directed in this direction for preventing periodontal diseases and dental caries.

Keratinocyte

Keratinocyte are the cells which are present in oral mucosa. Several features make epidermal and mucosal keratinocytes, attractive for treating local tissue disorders and as systemic gene therapeutics. Presence of stem cells in keratinocytes is also an advantage. They are easily accessible and so monitoring can be accurate. Preclinical assessment can be done accurately since culture models are established. Procedures for transplanting keratinocytes sheets are established because of their application for burn patients. It is reversible because genetically modified tissue can be excised. Cultured oral keratinocytes have been grafted to oral surgical defects. They persist at these sites and exhibit normal epithelial morphology. Expression of therapeutic genes can be achieved with use of topically applied agents. The ability of transduced human keratinocytes to synthesize and secrete biologically active recombinant proteins has been demonstrated. Human growth hormone, apolipoprotein E and the coagulation cascade factor IX are successfully delivered by genetically modified keratinocytes. Gene therapy can be used to treat keratinocytes disorders and dermatologic disorders like ichthyosis and epidermolysis bullosa [2, 4, 6]. Advances in future gene therapy research may make use keratinocytes to treat diseases.

Salivary Glands

Salivary Glands produce large amount of proteins and are sites easily accessible for gene transfer with minimum invasiveness through intraductal cannulation. The opening of the main duct in the oral cavity is cannulated and gene delivery vectors, viral or nonviral, are infused by a retrograde injection. Salivary glands are encapsulated which minimizes the undesirable access of administered vectors and transgenes to other tissues. Aim is to provide gene therapy to 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 by augmenting salivary secretions by transferring genes that encode secretory proteins into salivary glands. The proteins are subsequently secreted in an exocrine manner. Salivary glands are secretory tissues and local (oral, pharyngeal and esophageal) applications of gene therapeutics can be accomplished through exocrine secretion of transgene products in saliva. Salivary glands can also be used for gene therapy for systemic single-protein deficiency disorders [2, 4, 6]. A study showed that gene therapy using IL-27 ameliorates Sjögren's syndrome-like autoimmune exocrinopathy [19].

Oral Cancer

The general strategy in cancer treatment is to express a gene product that will result in cancer cell death. It can be achieved by [1, 2, 20]

- Addition of a tumor-suppressor gene (gene addition therapy);
- Deletion of a defective tumor gene (gene excision therapy);
- Down-regulation of the expression of genes that stimulate tumor growth;
- Enhancement of immune surveillance (immunotherapy);
- Activation of pro-drugs that have a chemotherapeutic effect and cause toxicity only to tumor cells ("suicide" gene therapy);
- Introduction of genes to inhibit tumor angiogenesis;
- "Cancer vaccination" with genes for tumor antigens [21].

The goal of gene therapy in cancer is to introduce new genetic material into cancer cells that will selectively kill the cancerous cells, causing no toxicity to surrounding normal cells. Major concern about using gene transfer vectors with patients, who have autoimmune disease, is the possibility of an immunological reaction to the vector. Vectors such as *adenoviruses* are useful for gene therapy of head and neck cancers. *Adeno associated Virus* vectors are much less immunogenic than adenoviral vectors. p53 tumor suppressor gene is a gene that is deleted or mutated in over 50% of human cancers. The incidence of p53 mutations in head and neck cancers believed to be higher in recurrent disease. Replacing a mutated p53 gene with a wild-type (normal) p53 gene is a potential approach to head and neck cancer treatment. Another tumor suppressor gene that could be replaced in head and neck cancer therapy is p16, since in Squamous Cell Carcinoma of Head and Neck cancer 80% to 90% of cases show p16 inactivation. Inactivation of p16 is believed to be one of the first steps in head and neck cancer carcinogenesis, and may therefore be an ideal target for gene replacement therapy. Gene transfer of gene p27 was found to inhibit the cell cycle of tumour cells, inducing apoptosis and triggering the suppression of tumour growth [20]. It has been demonstrated that gene p27 mutations are highly related to the appearance of tongue cancer. The tumor suppressor genes p16, p21, p27, and Rb are frequently mutated in head and neck cancer, and therefore are potential gene therapy targets [4, 6]. A novel method is gene-directed enzyme-prodrug therapy. In this a recombinant virus is generated which encodes a prodrug-activating enzyme such as nitroreductase, thymidine kinase or cytosine deaminase. Once delivered to the tumour cell, the enzyme is able to convert a harmless prodrug (administered locally or systemically) into a highly toxic cytotoxic drug. One example is thymidine kinase gene of *Herpes Simplex Virus* (HSV) transforms ganciclovir into ganciclovir phosphate. The activated drug is able to leech out of the virus-

infected cell to kill surrounding non-infected cells, creating a 'bystander' effect in cancer. Some viruses like vesicular stomatitis virus have natural ability to infect and replicate selectively in tumor cells. Gene therapy with these types of attenuated virus vectors with tumor specific promoters integrated in them can cause oncolysis. Genetic mutation already present in the tumor cells results in virus replication, cell lysis, virus spread and tumor destruction [13]. Increasing the sensitivity of the tumour to normal therapeutic processes by suppressing NF-kB activity with the use of gene therapy is also a good approach. NF-kB appears to contribute towards the progression and metastasis of various cancers, including OSCC, therefore its inhibition may be a useful adjuvant treatment in oral cancer therapy [20]. Future studies can utilize multigene strategies to target multiple pathways in cancer cells [21].

Orthodontic Tooth Movement

Tooth movement depends on the remodeling of alveolar bone, which is controlled by osteoclasts and osteoblasts. Gene therapy with osteoprotegerin (OPG) and RANKL has been used to inhibit and accelerate orthodontic tooth movement in a rat model. Local RANKL gene transfer to the periodontal tissue accelerated orthodontic tooth movement by approximately 150% after 21 days, without eliciting any systemic effects. Thus it can be helpful for shortening orthodontic treatment and also for moving ankylosed teeth. Local OPG gene transfer inhibited tooth movement by about 50% after 21 days of forced application [6]. In future similar procedures may be used by orthodontist to reduce treatment time and improve results.

Gene therapy to grow new teeth

This approach is generally presented in terms of adding molecules to induce *de novo* tooth initiation in the mouth. It might be combined with gene-manipulated tooth regeneration; that is, endogenous dental cells *in situ* can be activated or repressed by a gene-delivery technique to produce a tooth. More than 200 genes are known to be expressed during tooth development. The Baylor College of Medicine has found PAX 9, a master gene critical for tooth development. *de novo* repression or activation of genes such as RUNX2 or USAG-1 might be used to stimulate the third dentition in order to induce new tooth formation in the mouse [22]. Dental researchers can also hope to grow teeth in the laboratory that can be implanted into the mouths of patients who have lost their natural teeth in another way. These teeth would be without nerves and blood vessels, but they would be made of the same substances as human teeth. In order to accomplish this, researchers must find the genes responsible for building the 25 major proteins making up tooth structures. In addition, there may be dozens of other genes involved in instructing the body when, how and where to form a particular tooth. There may be as many as 10% of the total number of genes somehow involved in the formation of teeth [4]. The hope is researchers will be able to bioengineer human teeth for replacement in the future.

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. The oral cavity serves as a convenient window to the body. 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. Dental surgeon has got easy approach to areas like salivary glands and keratinocytes (oral epithelium). Salivary glands are excellent target sites for gene transfer, readily accomplished in minimal invasive manner. There are about 500-1000 minor salivary gland in oral cavity. Salivary gland produces large amount of proteins and it is a site where gene transfer can be readily accomplished in minimal invasive manner. Salivary glands could be used for gene therapeutic applications with single protein deficiencies. Irreversible salivary gland dysfunction due to autoimmune diseases and irradiation can also be corrected using gene therapy. Keratinocytes are the cells which are present in oral mucosa. Several features make epidermal and mucosal keratinocytes, attractive for treating local tissue disorders and as systemic gene therapeutics. Expression of therapeutic genes can be achieved with use of topically applied agents. Dental surgeon can be the best fitting professional to administer gene therapy in the oral cavity which bears minor salivary glands and keratinocytes. Also with future research and advances in gene therapy patients with intractable pain in any part of the body can walk in to dental clinic to get his/her pain relieved through gene therapy. In future dentist will have inseparable role in the field of gene therapy [2, 4, 6, 23].

CONCLUSIONS AND FUTURE DIRECTION

Gene therapy essentially consists of introducing specific genetic material into target cells without producing toxic effects on surrounding tissue. Major impediment to the successful application of gene therapy for the treatment of a range of diseases is not a paucity of therapeutic genes, but the lack of an efficient nontoxic gene delivery system. Research should be focused on generation of less immunogenic vectors with reduced immunogenic properties and to prevent inflammatory responses mediated by vectors. Future of gene therapy is excellent and encouraging. Gene therapy has potential and is promising treatment modality of number of diseases especially for head and neck cancers. There are many non-life-threatening conditions that adversely affect a

patient's quality of life, for which there are no effective treatments. The ability of *herpes simplex* virus to reside in neurons in a latent state without affecting normal cellular physiology can be used in the treatment of neurological disorders in future. Third dentition that might be locally induced by gene therapy to replace missing teeth presents an attractive concept and challenges to researchers. Understanding the mechanisms of supernumerary tooth formation which literally represent third dentition and applying it in gene therapy will help eliminate the problem of missing tooth. The lack of suitable treatment has permitted morbidity to become a rational basis for extending the scope of gene therapy.

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