Gene Therapy

Gene therapy: potential applications in Dentistry

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Abstract
Gene therapy refers to the treatment of genetic diseases using normal copies of the defective genes. It has the potential to cure any genetic disease with long-lasting therapeutic benefits. It remained an enigma for a long period of time, which was followed by a series of setbacks in the late 1990s. Gene therapy has re-emerged as a therapeutic option with reports of success from recent clinical studies. The United States and Europe has been pioneers in this field for over two decades. Gene therapy is a particular method by which defective gene is replaced or repaired by therapeutic gene. Vectors are vehicles which deliver the therapeutic gene into the host. Gene therapy can be used to treat wide range of diseases ranging from single gene disorder to multigene disorder. In dentistry the application of gene therapy includes bone repair, for treating auto immune disease, pain, DNA vaccination (for caries and periodontal disease) and cancer. Minor salivary glands and keratinocytes present in the oral mucosa are the 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.

Introduction
Gene therapy is an emerging field of biomedicine that has commanded considerable scientific and popular attention.1 Rapid progress in molecular biological technology has made researchers to
manipulate gene easier. A gene is a linear sequence of DNA that codes for a particular protein. Originally known as genes replacement therapy during the 1980s, ‘gene therapy’ and is applied to all manner of protocol that involve an element of gene transfer. Gene therapy is a technique in which defective genes that are responsible for disease development are corrected. The applications of gene therapy are based on the principle that a normal gene is inserted to compensate for a non functional gene and abnormal gene that can be repaired through selective reverse mutation. Scientists began gene therapy with bacteria in 1980 and first gene therapy in human (1990) was performed for treating severe combined immunodeficiency which worked for only few months. Several gene therapy clinical studies carried out in different parts of the world after 2008 have clearly demonstrated that gene therapy using viral vectors still holds promise to cure several genetic diseases. In October 2008, for the first time in the history of retinal genetic diseases, gene therapy had been shown to restore vision in three young adult patients with Leber’s congenital amaurosis, a common cause of blindness affecting infants and children. Each of the patients received a uniocular subretinal injection of recombinant adeno-associated virus serotype 2 (rAAV2) vectors containing a normal copy of the RPE65 gene. Initial follow-up after 3 months showed increased visual sensitivity compared to the control eye, demonstrating correction of the genetic defect. Gene therapy in recent days has grown by leaps and bounds and its application in dentistry includes bone repair, treatment of salivary gland diseases, autoimmune diseases, pain, DNA vaccination, dermatological disorder and cancer. This article will review few of the dental application of gene therapy.

Types of gene therapy and Gene transfer vectors
Gene therapy may be classified into two types; In somatic cell gene therapy (SCGT), the therapeutic genes are transferred into any of any cell other than a gamete, germ cell, gametocyte or undifferentiated stem cell. Any such modifications affect the individual patient only, and are not inherited by offspring. Somatic gene therapy represents mainstream basic and clinical research, in which therapeutic DNA (either integrated in the genome or as an external episome or plasmid) is used to treat disease. Over 600 clinical trials utilizing SCGT are underway in the US. Most focus on severe genetic disorders, including immunodeficiencies, haemophilia, thalassaemia and cystic fibrosis. Such single gene disorders are good candidates for somatic cell therapy. In germline gene therapy (GGT), germ cells (sperm or eggs) are modified by the introduction of functional genes into their genomes. Modifying a germ cell causes all the organism's cells to contain the modified gene. The change is therefore heritable and passed on to later generations. Australia, Canada, Germany, Israel, Switzerland and the Netherland prohibit GGT for application in human beings, for technical and ethical reasons, including insufficient knowledge about possible risks to future generations.

As described in 1995, there are two general ways to transfer genes: viral and non-viral. Viral are natural infectious agents for transferring genetic information. they are quite efficient, and at present they generally provide more preclinical and clinical utility than non-viral vectors. The principal viral vectors in clinical use today are based on modified adenoviruses, retroviruses and adenoviruses. An ideal gene transfer should be targeted to specific cells; express the transgene product at a
therapeutic level and under tight regulation for the required amount of time; show essentially no toxicity; and be administered with minimal invasiveness. There is no yet perfect gene transfer vector. However, some currently available vectors are quite useful for certain defined conditions such as adenovirus for gene therapy of head and neck cancers.1

Fig: Gene Therapy Using Adenovirus Vector

<table>
<thead>
<tr>
<th>Viral vectors</th>
<th>Nonviral vectors</th>
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<tr>
<td>Adenovirus</td>
<td>Lipid complex</td>
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<tr>
<td>Retrovirus</td>
<td>Liposomes</td>
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<tr>
<td>Adenovirus-associated virus (AAV)</td>
<td>Peptide/protein</td>
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<td>Lentivirus</td>
<td>Polymers</td>
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<td>Vaccinia virus</td>
<td>Mechanical</td>
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<td>Herpes simplex virus</td>
<td>Electroporation</td>
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<td>Gene gun</td>
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Areas of impact on dentistry

Bone repair
Bone loss caused by trauma, neoplasia, reconstructive surgery, congenital defect or periodontal diseases is major worldwide problem. Gene therapy represents an ideal approach towards augmenting bone regeneration. gene therapy can enhance osteoinduction via expression of growth factors, induce osteoblast differentiation and facilitate the production of osteoid matrix.3 Bone morphogenetic proteins (BMPs) are essential for the first requirement, osteoinduction, which is the activation of various cytokines or growth factors to attract osteoblasts and/or their progenitor cells to the repair site and induce them to produce bone.6 Bone defects in the oral and maxillofacial region can be repaired by transferring genes encoding BMP’s (Bone morphogenic Protein). It will be possible to directly deliver the BMP2 gene in vivo to tissues via an adeno viral vector to heal bone defects. Michigan research group has found non osteogenic fibroblasts (gingiva, dentalpulp), which can express BMP7 gene after being infected with an adeno viral vector.2
**Gene therapeutics for salivary glands**

Salivary glands are excellent target sites for gene transfer. They are capable of producing large amounts of proteins and are site where gene transfer can be readily accomplished in a minimally invasive manner by means of intraductal cannulation. Our original goal in developing gene transfer with salivary glands was to provide novel and effective therapies for patient who suffer from irreversible salivary gland dysfunction from either irradiation or sjogren’s syndrome. An adenovirus mediated water channel (aquaporin1 AQP1) gene transfer into irradiated submandibular gland showed increased saliva flow in rat model.

Gene transfer can also be utilized to augment salivary secretion by transferring genes that encode secretory proteins into salivary glands. A recombinant serotype 2 adenoassociated virus encoding the human VIP transgene was administered into submandibular gland of female mice to examine its ability to alter the progressive sjogren syndrome like dysfunction. The results showed that local delivery of recombinant serotype 2 adenoassociated virus can have a disease modifying and immunosuppress effects in submandibular gland of mice.

**Gene therapy for 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. Addition of a tumor suppressor Gene (gene addition therapy).
3. down regulation of the expression of genes that stimulate tumor growth.
4. Enhancement of immune surveillance (immunotherapy)
5. Activation of prodrugs that have a chemotherapeutic effect ("suicide" gene therapy).
6. Introduction of genes to inhibit tumor angiogenesis

The incidence of p53 in head and neck cancer is believed to be higher in recurrent disease. Replacing a mutated p53 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 80 to 90% of squamous cell carcinoma of head and neck cases show p16 inactivation. Heise and colleagues of ONYX pharmaceuticals in California, observed that an adenovirus with a mutant E1B-55KDa protein- termed ONYX-015 was able to replicate in cells with mutated p53 protein but spared cells with a normal p53 protein. This resulted in lysis of the mutant cell. Khuri and colleagues published clinical trial findings in which the ONYX-015 adenovirus was used with or without conventional chemotherapeutic drugs. They tested combinations of ONYX-015, cisplatinum and 5-fluorouracil in patients with squamous cell carcinoma of head and neck that had recurred after treatment by surgery, radiotherapy, or both. Patient were injected at the largest or more symptomatic tumor mass with 1010 plaque- forming units of ONYX-015 per day for five
consecutive days. Khuri and colleagues described 8 complete and 11 partial responses among 30 patients included in the study.1

**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.2 Several studies in animal models and University of Pittsburgh School of Medicine have shown that viral-mediated transfer of genes encoding opiate peptides to peripheral and central neurons can lead to antinociceptive effects. There also is a recent report from Okayama University dental school in Japan showing the feasibility of direct gene delivery to the articular surface of temporomandibular joint. While considering more research is needed before gene transfer can be tested clinically as a strategy for chronic pain management, the result of these recent studies suggest real promise.1

**DNA vaccination**
For many years, dental scientists have tried to use classical vaccination technology to eradicate dental caries or periodontal disease, thus far achieving mixed susceptibility. In the last decade, gene transfer research has led to a novel way to achieve vaccination: directly delivering DNA in plasmid vs the traditional administration of a purified protein or an attenuated microbe.1 The ability to induce an immune response to a protein antigen by administration of plasmid encoding the antigen has been successfully demonstrated in animal models.

Human periodontitis is thought to be initiated by a principal organism called *P. gingivalis*. Two separate *rgp*-encoding genes (*rgpA* and *rgpB*) are located on the chromosome of *P. gingivalis*. A study demonstrated that immunization of mice with *rgpA* DNA vaccine protects against challenge with invasive *P. gingivalis* strain W50B in the mouse lesion model.3 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.1

**Genes transfer to keratinocytes**
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. Expression of therapeutic genes can be achieved with use of topically applied agents. Gene therapy can be used to treat keratinocytes disorder like ichthyosis and epidermolysis bullosa. In future it can be used to treat most of dermatologic disorders.2 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. For successful keratinocytes gene therapy, stable and long term gene expression may be achieved through the use of endogenous, keratinocytes-specific promoters and by targeting stem cells. The longevity of genetically altered
keratinocytes in epidermal and oral epithelial grafts may be increased by identifying factors which will improve graft survival. Cell-marking studies through which grafted cells can be followed after being genetically marked with a reporter gene may shed light on the fate of grafted cells and on the persistence of expression in these cells in vivo. Ongoing work holds promise that it may soon be possible to characterize the phenotype of epithelial stem cells and to target gene delivery to them in vivo and in vitro. Such technical advances will open the door to clinical trials using keratinocytes to treat disease.3

**Gene therapy to grow new teeth**

Dental researchers hope to grow teeth in the laboratory that can be implanted into the mouths of patients who have lost their natural teeth. These would not be living teeth with 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. The Baylor College of Medicine was found PAX 9, a master gene critical for tooth development. The hope is we will able to bioengineer human teeth for replacement in future.3

**Is gene therapy safe?**

Gene therapy is under study to determine whether it could be used to treat disease. Current research is evaluating the safety of gene therapy; future studies will test whether it is an effective treatment option. Several studies have already shown that this approach can have very serious health risks, such as toxicity, inflammation, and cancer. Because the techniques are relatively new, some of the risks may be unpredictable; however, medical researchers, institutions, and regulatory agencies are working to ensure that gene therapy research is as safe as possible. Comprehensive federal laws, regulations, and guidelines help protect people who participate in research studies (called clinical trials). The U.S. Food and Drug Administration (FDA) regulate all gene therapy products in the United States and oversee research in this area. Researchers who wish to test an approach in a clinical trial must first obtain permission from the FDA. The FDA has the authority to reject or suspend clinical trials that are suspected of being unsafe for participants. The National Institutes of Health (NIH) also plays an important role in ensuring the safety of gene therapy research. NIH provides guidelines for investigators and institutions (such as universities and hospitals) to follow when conducting clinical trials with gene therapy. These guidelines state that clinical trials at institutions receiving NIH funding for this type of research must be registered with the NIH Office of Biotechnology Activities. The protocol, or plan, for each clinical trial is then reviewed by the NIH. Recombinant DNA Advisory Committee (RAC) to determine whether it raises medical, ethical, or safety issues that warrant further discussion at one of the RAC’s public meetings. An Institutional Review Board (IRB) and an Institutional Biosafety Committee (IBC) must approve each gene therapy clinical trial before it can be carried out. An IRB is a committee of scientific and medical advisors and consumers that reviews all research within an institution. An IBC is a group
that reviews and approves an institution's potentially hazardous research studies. Multiple levels of evaluation and oversight ensure that safety concerns are a top priority in the planning and carrying out of gene therapy.7

Dental surgeon as gene therapist
The role of dental surgeon in gene therapy is tenable. 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 produce 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. 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. Expression of therapeutic genes can be achieved with use of topically applied agents. Gene therapy can be used to treat disorders of keratinocytes like ichthyosis and epidermolysis bullosa. In future it can be used to treat most of dermatologic disorders. Dental surgeon can be the best fitting professional to administer gene therapy in the oral cavity which bears minor salivary glands and keratinocytes. Patient 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

Conclusion
Given the genetic basis for most diseases, instead of contemplating the future of gene therapy, it might be equally interesting to wonder about the future of gene therapy in the context of drug therapy. Although we still consider current gene transfer methods to fairly primitive and associated with significant problems, gene therapy's acceptance as part of the routine clinical armamentarium, at least for some applications (like head and neck cancer), seems very close.3

References