

Principles of gene therapy

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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. This article reviews the fundamentals in gene therapy and its various modes of administration with an insight into the role of gene therapy in Periodontics and future percepts and the technical and ethical issues of using gene therapy.

Key words: Ethics, gene therapy, vehicles

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INTRODUCTION

DNA, the constituent of genes, is made up of the four bases: adenine, cytosine, guanine and thymine. Just as the alphabets are put together to make words and sentences, so also these bases, represented by the initials A, C, G and T, are ordered to create the language of the genes [Figure 1]. Every individual inherits their genes from their parents and, in turn, passes them on to their children. Each person's genetic constitution is different and the changes in the genes determines the differences between individuals. Some changes, usually in a single gene, may cause serious diseases such as cystic fibrosis, muscular dystrophy or thalassaemia. More often, gene variants interact with the environment to predispose some individuals to cancer, heart disease or other such ailments.^[1]

Gene therapy uses purified preparations of a gene or a fraction of a gene, to treat diseases [Figure 2]. A common approach in gene therapy is to identify a malfunctioning gene and supply the patient with functioning copies of that gene. Other approaches include switching specific genes on or off or introducing genes to kill cancer cells, to suppress tumors by inhibiting the blood supply or to stimulate the immune system to attack certain types of cells.

Whichever approach is used, the aim of gene therapy is to introduce therapeutic material into the target cells, where it becomes active and exerts the intended therapeutic effect. In the mid 1980s, the focus of gene therapy was entirely on treating diseases caused by single-gene defects but by the

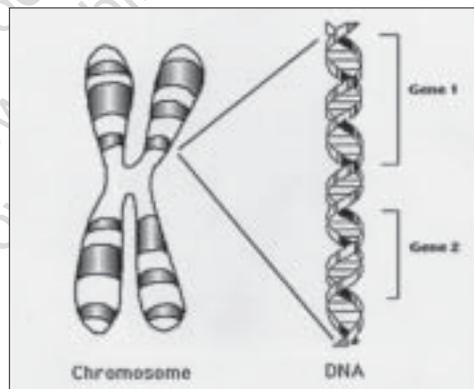


Figure 1: A chromosome

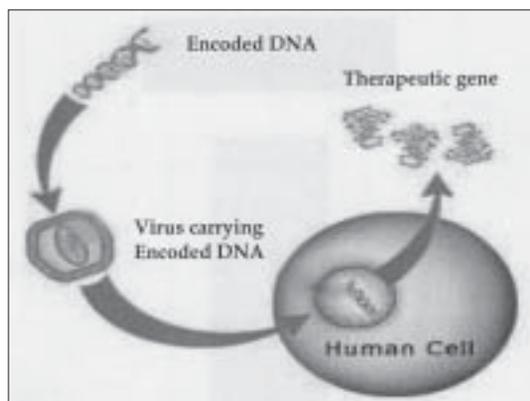


Figure 2: Gene therapy

late 1980s and early 1990s, the concept of gene therapy was being increasingly considered for the treatment of a number of acquired diseases.^[2]

This article reviews the fundamentals of gene therapy and its various modes of administration, with an insight into the

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role of gene therapy in periodontics. We also discuss the future percepts and the technical and ethical issues involved in the use of gene therapy.

FUNDAMENTALS OF GENE THERAPY

Genes that are present on the chromosomes form the basic unit of heredity. Genes are specific sequences of bases that encode instructions to make proteins. A genetic disorder results when an alteration in the genes present on the chromosomes occur. Gene therapy is designed to introduce genetic material into cells to compensate for abnormal genes or to enable the making of a beneficial protein.

For correcting faulty genes, one of several approaches may be employed:^[3]

- A normal gene may be inserted into a nonspecific location within the genome to replace a nonfunctional gene; this is the most common approach.
- An abnormal gene may 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 functional status.
- The regulation (the degree to which a gene is turned on or off) of a particular gene could be altered.

ADMINISTERING GENE THERAPY

A gene that is inserted directly into a cell usually does not function. Instead, a carrier called a vector is used to introduce the therapeutic gene into the patient's target cells^[4] [Figure 3]. The most common vector that is used is a virus that has been genetically altered to carry normal human DNA. Viruses cause diseases in humans by encapsulating and delivering the genes into cells. Some types of viruses, such as retroviruses, integrate their genetic material (which can be manipulated to include the therapeutic gene) into a chromosome in the human cell. Other viruses, such as

adenoviruses, introduce their DNA into the nucleus of the cell, but the DNA is not integrated into a chromosome.^[5-7]

The vector can be given intravenously or injected directly into a specific tissue in the body, where it is taken up by individual cells (the target cells). Alternatively, a sample of the patient's cells can be removed and exposed to the vector in a laboratory setting; the cells, containing the vector, are then reintroduced into the patient.

Some of the different types of viruses used as vectors in gene therapy are:^[8]

- Retroviruses (e.g. HIV): 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.
- Adenoviruses: A class of viruses with double-stranded DNA genomes; they cause respiratory, intestinal and eye infections in humans.
- Adeno-associated viruses: A class of small, single-stranded DNA viruses that can insert their genetic material at a specific site on chromosome 19.
- Herpes simplex viruses: A class of double-stranded viruses that can infect a particular cell type, i.e. neurons.

Besides viruses-mediated gene-delivery systems, there are several nonviral options for gene delivery. The simplest method is the direct introduction of therapeutic DNA into target cells. This technique has restricted use as it requires large amounts of DNA to bring out the desired effect.

Another nonviral approach involves the creation of an artificial lipid sphere (a liposome) with an aqueous core. This liposome, which carries the therapeutic DNA, is capable of transporting the DNA through the target cell's membrane. Therapeutic DNA can also be introduced into target cells by chemically linking the DNA to a molecule that will bind to special cell receptors. Once bound to these receptors, the therapeutic DNA constructs are engulfed by the cell membrane and passed into the interior of the target cell. This delivery system, however, tends to be less effective than the other options.

Experiments with the introduction of a 47th chromosome (an artificial, human techno-chromosome) into target cells are being carried out.^[9] This chromosome would exist autonomously alongside the standard 46, without affecting their functions or causing any mutations. It would be a large vector capable of carrying substantial amounts of genetic code and, because of its construction and autonomy, the body's immune system would not attack it. A disadvantage with this potential method is the difficulty in delivering such a large molecule into the nucleus of a target cell.

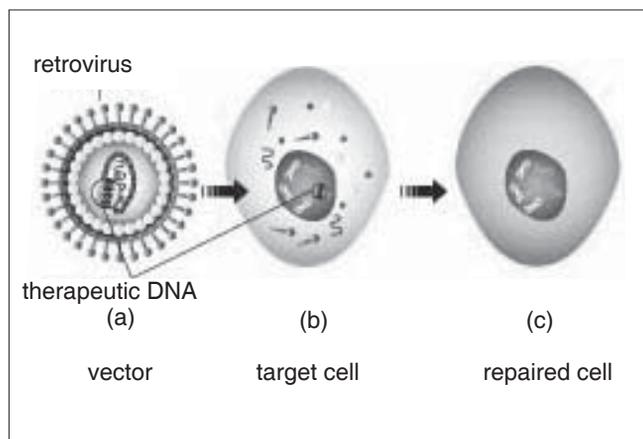


Figure 3: Gene therapy using virus

SOMATIC AND GERM LINE GENE THERAPY^[10]

Gene therapy can target somatic (body) or germ (egg and sperm) cells. In somatic gene therapy the recipient's genome is changed, but the change is not passed on to the next generation; whereas with germ line gene therapy the newly introduced gene is passed on to the offspring.^[11]

Germ line gene therapy is not being actively investigated, at least in larger animals and humans, although a lot of discussion is being conducted about its value and desirability.

TECHNICAL DIFFICULTIES IN GENE THERAPY

Gene delivery: Successful gene delivery is not easy or predictable, even in single-gene disorders. For example, although the genetic basis of cystic fibrosis is well known, the presence of mucus in the lungs makes it physically difficult to deliver genes to the target lung cells. Delivery of genes for cancer therapy may also be complicated by the disease being present at several sites. Gene-therapy trials for X-linked severe combined immunodeficiency (X-SCID), however, have been more successful.^[12] In this case, the genetic basis of the disease is well understood and the therapeutic material can be delivered using the established procedure of bone marrow transplant. A viral vector is used to introduce functioning copies of the gene (whose malfunction causes X-SCID) into blood-producing stem cells from the patient's bone marrow; these are then transplanted back into the patient.

Durability and integration: Some gene therapy approaches aim to achieve a long-term effect. Where such durability is required, the therapeutic material needs to remain functional for the intended duration. Two possible ways of achieving this are to either use multiple rounds of gene therapy or to integrate the therapeutic genes so that they remain active for some time. Integrating therapeutic DNA into the target cells to generate material may achieve long-lasting results, but there are concerns about possible undesirable side effects.

For instance, this approach has been used in trials to treat babies with the X-SCID syndrome. Three of the babies went on to develop leukemia-like symptoms.^[13] A possible explanation is that the therapeutic material might have integrated at a site where it affected another gene that may have produced the rapid growth of cancerous cells. Researchers are investigating ways of achieving long-term therapeutic effects without integration; for instance, by using stable, non-integrating vectors.^[14]

Other approaches seek more immediate effects, where integration of therapeutic DNA into the target cells is not

the aim. For instance, in gene therapy to treat cancer, the aim may be to use 'suicide' genes to kill cancerous cells as quickly as possible.

Immune response: When a viral vector is used to deliver gene therapy, the body may recognize it as 'foreign' and mobilize the immune system to attack it. In cancer, triggering such an immune response may be the aim of gene therapy. In other cases, immune responses may reduce the efficacy of gene therapy, causing the patient to stop responding after a few applications or by inducing serious side effects. Further, an enhanced response of the immune system to vectors encountered previously may make it difficult to give repeat applications of gene therapy.

Safety of vectors: Viruses, while the carrier of choice in most gene-therapy studies, present a variety of potential problems to the patient, e.g., 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.

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 multifunctional disorders such as these are especially difficult to treat effectively using gene therapy.

GENE THERAPY IN PERIODONTICS

Gene therapy has been used as a mode of tissue engineering in periodontics. The tissue engineering approach reconstructs the natural target tissue by combining three elements, namely, the scaffold, signaling molecules and cells.

The three basic approaches in tissue engineering are^[15] the following:

1. **Protein-based approach:** In this approach, growth and differentiation factors are used for regenerating periodontal tissues. Trials have been conducted using various growth factors, namely TGF- β , BMP-2, 6, 7, 12, bFGF, VEGF and PDGF.^[16]
2. **Cell-based approach:** Several preclinical studies using mesenchymal stem cells (MSC) have demonstrated efficient reconstruction of bone defects that are too large to heal spontaneously.^[17]
3. **Gene-delivery approach:**^[18] This approach involves two basic modalities:
 - a. ***In vivo* gene delivery:** In this approach, gene constructs, such as plasmid DNA or a viral particle, are physically entrapped within a scaffold or a matrix [Figure 4]. When the scaffold containing the

gene constructs is implanted into the tissue defect, the host cells migrate into the implant, take up the gene constructs and start producing the encoded protein.

- b. *Ex vivo* gene delivery: In this approach, cultured cells are transfected (in nonviral delivery systems) or transduced (in viral delivery systems) with gene constructs *in vitro* before they are transplanted into the tissue defect [Figure 5].

CLINICAL TRIALS USING GENE THERAPY

1. Platelet-derived growth factor (PDGF) gene delivery:
 - a. Jin *et al.*,^[19] demonstrated in their study that direct *in vivo* gene transfer of PDGF-B stimulated tissue regeneration in large periodontal defects.
 - b. Anusaksathien *et al.*,^[20] in an *ex vivo* investigation, showed that the expression of PDGF genes was prolonged for upto 10 days in gingival wounds.
 - c. Giannobile *et al.*,^[21] 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. Franceschi *et al.*,^[22] investigated *in vitro* and *in vivo* Ad gene transfer of BMP-7 for bone formation.
 - b. Dunn *et al.*,^[23] demonstrated that direct *in vivo* gene delivery of Ad/BMP-7 in a collagen gel carrier promoted successful regeneration of alveolar bone defects around dental implants.
3. Some of the ongoing clinical trials in the field of medicine:^[24,25]
 - a. Gene therapy and chemotherapy in treating patients with advanced solid tumors or non-Hodgkin's lymphoma.
Conditions: Adult Brain Tumor; adult non-Hodgkin's lymphoma; adult solid tumor.
 - b. Stem cell gene therapy to treat X-SCID.
Condition: Severe combined immunodeficiency.
 - c. Gene therapy to prevent cancer in patients with premalignant carcinoma of the oral cavity or pharynx.
Conditions: Lip and oral cavity cancer; oropharyngeal cancer.

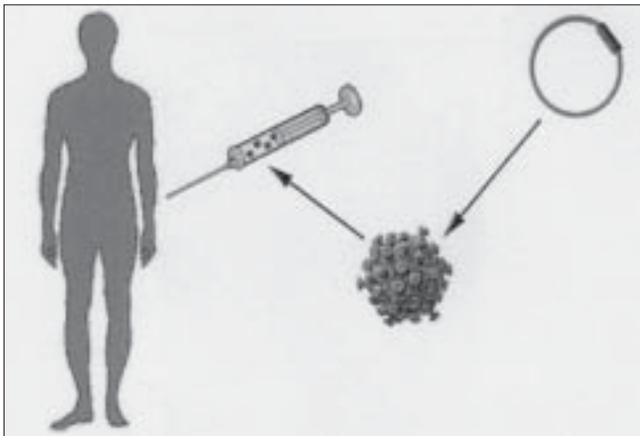


Figure 4: *In vivo* gene therapy

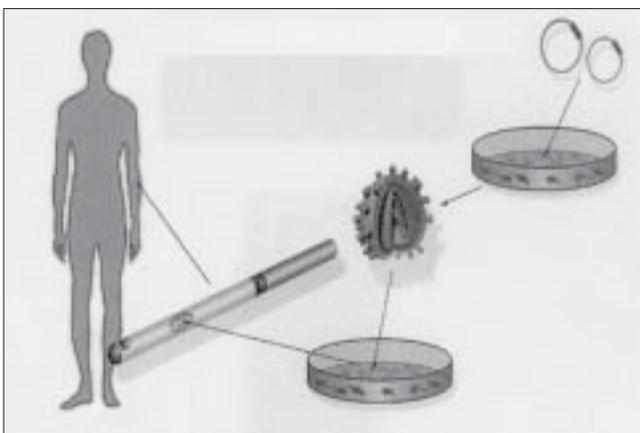


Figure 5: *Ex vivo* gene therapy

ETHICAL CONSIDERATIONS IN GENE THERAPY:^[26]

Some of the questions that need to be considered are the following:

- What is normal, what is a disability or disorder and who decides?
- Are disabilities diseases? Do they need to be cured or prevented?
- Does searching for a cure demean the lives of individuals presently affected by disabilities?
- Is somatic gene therapy (which targets the adult cells of persons known to have the disease) more or less, ethical than germ line gene therapy (which targets the egg and sperm cells and, thus, prevents the trait from being passed on to future generations)? In case of somatic gene therapy, the gene therapy may have to be repeated in future generations.
- At present, gene therapy is exorbitantly expensive. Who will have access to these therapies? Who will pay for their use?

CONCLUSION

New interventions, combining gene therapy with other approaches such as stem cell therapy, are emerging. Gene therapy has the potential to treat diseases such as cystic fibrosis, cancers, heart diseases and human immunodeficiency virus infection. However, to date, no clinical trial of gene therapy has resulted in the development of a commercially available treatment. Unsettled issues in gene therapy include effectiveness of delivery, longevity of the therapy and safety

of the procedures. While patient groups are largely satisfied with the current disease-led approach to gene therapy research, scientists have called for more studies on vector safety, delivery techniques, molecular causes of diseases and the uncertainty of outcomes.

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