ISSN (O) : 2582-3271
PubMed-National Library of Medicine - ID: 101766917

International Journal of Dental Science and Clinical Research (IJDSCR)

Dr. Angel Vaidic Publication

Available Online at: http://www.ijdscr.org

Volume 3, Issue 3, May - 2021, Page No. : 01 - 12

Gene Therapeutics: A New Path of Future Prospective in Periodontics

[1]Dr. Aparna Chandel, PG Student, Department of Periodontology and Implantology, National Dental College and Hospital, Derabassi, Mohali (Punjab) India

[2]Dr. Gurpreet Kaur, Professor and HOD, Department of Periodontology and Oral Implantology, National Dental College and Hospital, Derabassi, Mohali (Punjab) India

Citation of this Article: Dr. Aparna Chandel, Dr. Gurpreet Kaur, "Gene Therapeutics: A New Path of Future Prospective in Periodontics", IJDSCR – May - 2021, Vol. – 3, Issue - 3, P. No. 01-12.

Corresponding Author: Dr. Aparna Chandel, PG Student, Department of Periodontology and Implantology, National Dental College and Hospital, Derabassi, Mohali (Punjab) India

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Change and modification is a continuous process in the advancement of technology. Research is being done to understand the cellular and molecular basis of every disease. In research facilities around the world, scientists are attempting to prevent diseases at their very roots. Most of the conventional methods to treat disease are not giving satisfactory results. Instead of curing illness and diseases with drugs, they are trying to modify the genes that cause the diseases. Genes are specific sequences of bases that encode instruction to make proteins. Genetic disorder results when these genes are altered, and their encoded proteins are unable to carry their normal functions. Gene therapy involves the transfer of new genetic material or transformation of the existing material for treating human diseases. Gene therapy is designed to introduce genetic materials into the cell to compensate for abnormal genes or to make a beneficial protein. Gene therapy has a promising era in the field of 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 four elements namely: Scaffold, signalling molecules, cells and blood supply and thus can help in the reconstruction of damaged periodontium including cementum, gingival, periodontal ligament and bone.

Keywords

Gene therapy, gene delivery, gene therapeutics, gene enhanced tissue engineering, gene transfer

Introduction

Periodontal diseases, have a broad spectrum of inflammatory and destructive responses, and are thought to be multi-factorial in origin. Genetic variance has been considered as a major risk factor for periodontitis. With the advent of gene therapy in dentistry, significant progress has been made in controlling the periodontal disease and reconstruction of damaged periodontal tissue[1]. Research is done by scientists to control diseases at their very root in various parts of the globe. They are trying to alter the gene that causes disease instead of finding drugs to cure illness or diseases. The process by which this is done is called as gene therapy[2].

Gene therapy is a field of biomedicine. There have been tremendous advances in gene therapy relevant to dentistry since 1995. However, in the field of periodontics gene therapy has not been applied with success primarily because the technology of gene therapy is still far from perfect and certainly has its own substantial problems. Secondarily, periodontal disease is multifactorial in origin comprised of microbial challenge and variable host immune responses modified by genetic and environmental factors[5].

The goal of gene therapy is to transfer the DNA of interest (for example, growth factor and thrombolytic genes) into cells, thereby allowing the DNA to be synthesized in these cells and its protein (termed recombinant protein) expressed[1].

The understanding of basic principles and advances in gene therapy is essential to have an insight into the prospects and advances of this new field in periodontics. It is predicted that gene therapy may offer a wider range of treatment options in dentistry than in the past and may become an integral part in dental practice[4].

Definition of Gene Therapy

A gene is a distinct sequence of nucleotides forming the chromosome portion of a cell's DNA or chromosomal RNA. Genes are the smallest functional units of the genetic system with two main 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.

The **US Food and Drug Administration (FDA) defines** gene therapy as products "that mediate their effects by transcription and/or translation of transferred genetic material and/or by integrating into the host genome and that are administered as nucleic acids, viruses, or genetically engineered microorganisms. The products may be used to modify cells in vivo or transferred to cells ex vivo prior to administration to the recipient[3].

Major Development in Gene Therapy

The first gene therapy trials on humans began in 1990 on patients with Severe Combined Immunodeficiency (SCID)

Brief history of gene therapy

1909 - Wilhelm Johannsen introduced the term "gene".

1928 - Griffith performed the first experiment suggesting that bacteria are capable of transferring genetic information through a process known as "transformation".

1952 - Zinder and Lederberg introduced the term "transduction" as a mechanism of genetic transfer. 1968- Rogers and Pfuderer demonstrated a proof of concept for virus-mediated gene transfer.

1972 - Friedman and Roblin suggested gene therapy for genetic diseases.

1988 - The first officially approved clinical protocol to introduce a foreign gene into humans was approved by the Recombinant DNA Advisory Committee (RAC). 1990 - FDA approved the first time a gene therapy trial with a therapeutic attempt in humans.

1999 - FDA restricted all clinical trials using gene therapy for nearly a decade because of the outcomes of the first gene-therapy-based clinical trial on ADASCID (adenosine deaminase: severe combined immune deficiency) as patients eventually developed leukemia (4/10). 2002- In 2002 a question was raised when two of the ten children treated developed a leukemia-like condition.

2006 - Scientists have successfully treated metastatic melanoma in two patients using killer T cells genetically retargeted to attack the cancer cells. As well as in March again, scientists announced the successful use of gene therapy to treat two adult patients for a disease affecting myeloid cells. Italy reported a breakthrough for gene therapy in which they developed a way to prevent the immune system from rejecting a newly delivered gene. Similar to organ transplantation.

2007- The world's first gene therapy trial was done for inherited retinal disease. The sub retinal delivery of recombinant adeno associated virus (AAV) carrying RPE65 gene was found to be safe and yielded positive results.

2009 - The journal Nature reported that researchers at the University of Washington and University of Florida were able to give trichromatic vision to squirrel monkeys using gene therapy, a hopeful precursor to a treatment for color blindness in humans.

2003- China became the first country to approve a gene therapy based product for clinical use.

2012 - EMA (The European Medicines Agency) recommended for the first time a gene therapy product (Glybera) for approval in the European Union. **December 2017** - First FDA approval of an in vivo gene-therapy product, for Luxturna from Spark Therapeutics.

August 2018 - US National Institutes of Health advisory committee declared "gene therapies on recombinant. DNA"no longer need to be reviewed before clinic studies[5].

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. The 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 end of the vectors, effectively closing the molecule. The second stage involves the 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.

Requirements for an ideal gene transfer vector are[7]

- It should be targeted to specific cells
- It should express the transgene product (protein) at a therapeutic level
- It should have longevity of expression under tight regulation
- It should have high efficiency
- It should show essentially no toxicity
- It should be administered with minimal invasiveness.

Various vectors are used in gene therapy. Vectors that have been targeted and used include viral and non-viral vectors. Viral vectors are natural infectious agents for transferring genetic information and are quite efficient. These viruses are attenuated to transfect genes, but they cannot replicate or cause infection. Various viral vectors used in experiment today are retrovirus, adenovirus, adeno-associated virus (AAV), lentivirus, vaccinia virus, and herpes simplex virus. Among all viral vectors currently being studied, adenoviruses and retroviruses are very commonly used. Examples of non-viral vectors include electroporation, polymers, microinjection, calcium vectors, lipid vectors, and protein complexes.

Fundamentals of Gene Therapy

There are a variety of different methods to replace or repair the genes targeted in gene therapy.

- A normal gene may be inserted into a nonspecific location within the genome to replace a non-functional gene. This approach is most common.
- An abnormal gene could 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 function.
- The regulation (the degree to which a gene is turned on or off) of a particular gene could be altered.
- Spindle transfer is used to replace entire mitochondria that carry defective mitochondrial DNA[6].

Types of Gene Therapy

Gene therapy may be classified into the following types:

1. Germ line gene therapy

In the case of germ line gene therapy, germ cells, i.e., sperm or eggs are modified by the introduction of functional genes, which are ordinarily integrated into their genomes. Therefore, the change due to therapy would be heritable and would be passed on to later generations.

2. Somatic gene therapy

In the case of somatic gene therapy, the therapeutic genes are transferred into the somatic cells of a patient. Any modifications and effects will be restricted to the individual patient only, and will not be inherited by the patient's offspring.

Implications of Gene Therapy in Periodontics

There have been tremendous advances in gene therapy relevant to dentistry since 1995. Even in the field of periodontics, it has been studied extensively. Currently genetic principles are being applied along with tissue engineering for periodontal rehabilitation.

Approaches for regenerating tooth-supporting structures

1. Guided tissue regeneration uses a cell occlusive barrier membrane to restore periodontal tissues.
2. Alternatively, an example of gene therapy uses vector-encoding growth factors aimed stimulating the regeneration of host cells derived from the periodontium.

Gene Enhanced Tissue Engineering

The general strategy of tissue engineering is to supplement the regenerative site with a therapeutic protein like growth factors. However the problem with the delivery of growth factor is its short life. This is due to proteolytic breakdown and receptor mediated exocytosis and solubility of delivery vehicle. To overcome these problems, gene therapy has been developed which provides long term exposure of growth factor to periodontal wound.

There are three approach of tissue engineering in periodontics

1. **Protein based approach**[8]

   Growth and differentiation factors are used for regeneration of periodontal tissues likes TGF-β, BMP-2,6,7,12, bFGF, VEGF and PDGF.

2. **Cell based approach**[9]

   Several studies using mesengymal stem cell have demonstrated efficient reconstruction of bone defect that are too large to heal spontaneously.

3. **Gene delivery approach**[10]

   To overcome the short half-lives of growth factor peptides in vivo, gene therapy that uses a vector that encodes the growth factor is utilized to stimulate tissue regeneration. So far, two main strategies of gene vector delivery have been applied to periodontal tissue engineering. Gene vectors can be introduced directly to the target site (in vivo technique) or selected cells can be harvested, expanded, genetically transduced and then replanted (ex vivo technique).

Route of Gene Delivery

1. **In Vivo**
2. **Ex Vivo**

In Vivo

In vivo gene delivery is a one-step process that may be necessary for disorders requiring immediate treatment. One of main challenges of this approach is low transduction efficiency and the possibility of causing inflammatory/immune response. Another limitation is the difficulty in targeting the cell population of interest[11].

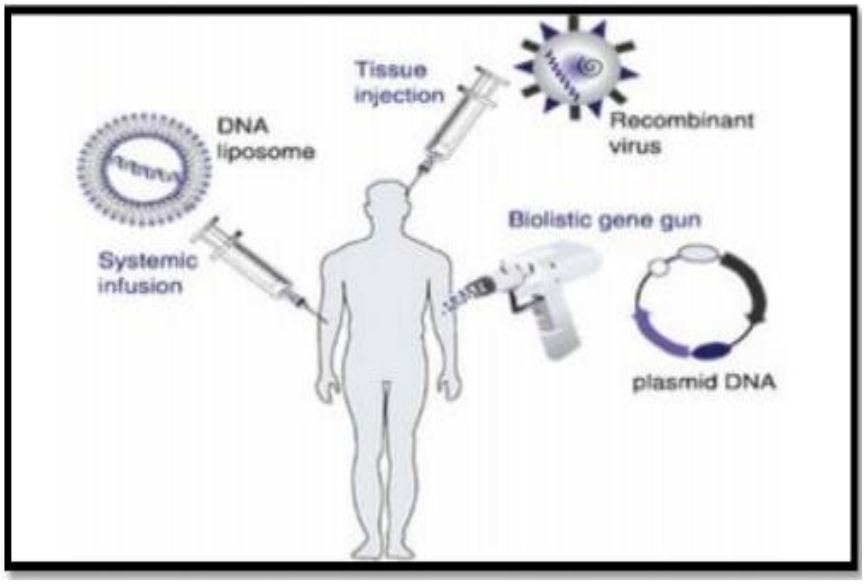

Figure 1: In Vivo Method Of Gene Delivery

Ex Vivo

While in ex vivo gene transfer, the foreign gene is transduced into the cells of a tissue biopsy, followed by genetic modification of these cells under in vitro conditions. After this preparation phase outside the body, these cells are subsequently implanted into the site of injury. Delivery by ex vivo gene vehicle can be considered as a safer method, because cells can be screened for tumorigenicity before implantation into the host. However, these initial steps are often labor intensive, complex and involve significant cost[11].

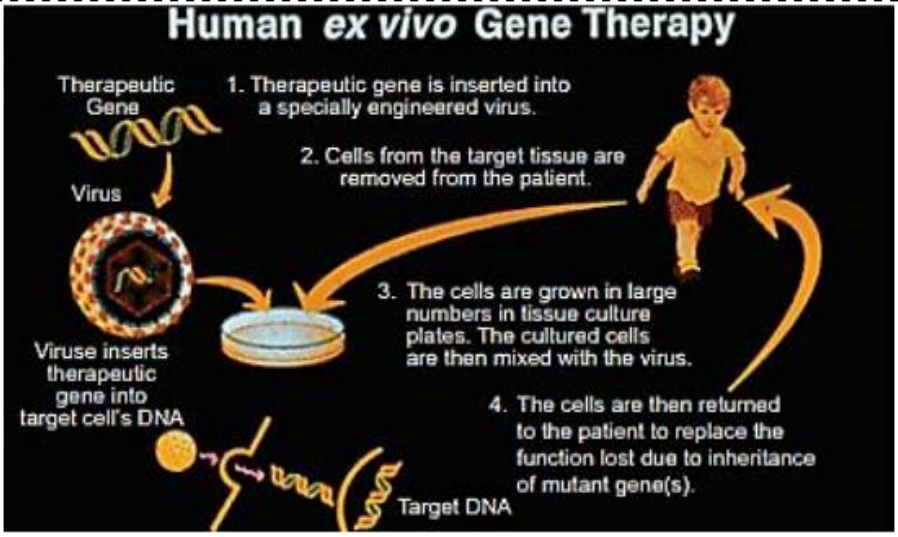

Figure 2: Ex Vivo Method Of Gene Delivery

Clinical Trials Using Gene Therapy

The application of PDGF-gene transfer strategies to tissue engineering originally was generated to improve healing in soft tissue wounds, such as skin lesions. But, recently various trial have been done PDGF using Plasmid and Ad/PDGF gene deliver, for regeneration of periodontal tissue.

Platelet-derived growth factor gene delivery

Early studies in dental applications using recombinant adenoviral vectors that encode PDGF demonstrated the ability of these vectors constructs to transduce potently the cells isolated from the periodontium (e.g. osteoblasts, cementoblasts, PDL cells, and gingival fibroblasts). Continuous exogenous delivery of PDGF-α may delay mineral formation induced by cementoblasts, whereas PDGF clearly is required for mineral neogenesis.

- Jin et al. demonstrated in their study that direct in vivo gene transfer of PDGFB stimulated tissue regeneration in large periodontal defects[12].
- Anusaksathien et al. reported that in an ex vivo investigation showed that the expression of PDGF genes was prolonged for up to 10 days in gingival wounds[13].
- Giannobile et al. reviewed different mechanisms of drug delivery and novel approaches to reconstruct and engineer oral- and tooth-supporting structures, namely the periodontium and alveolar bone[14].

Bone morphogenetic protein delivery

BMPs are multifunctional polypeptides that belong to the transforming growth factorB superfamily of proteins. The human genome encoded at least 20 BMPs. BMPs bind to type I and II receptors that function as serine- threonine kinases. The type I receptor protein kinase phosphorylates intracellular signalling substrates called Smades (Sma gene in C elegans and Mad gene in Drosophila). The most remarkable feature of BMPs is the ability to induce ectopic bone formation. BMPs not only are powerful regulators of cartilage and bone formation during embryonic development and regeneration in postnatal life but also participate in development and regeneration in postnatal life but also participate in the development and repair of other organs, such as the brain, kidney, and nerves. Studies have demonstrated the expression of BMPs during tooth

development and periodontal repair, including alveolar bone.

- Franceschi et al. investigated in vitro and in vivo Ad gene transfer of BMP-7 for bone formation[15].
- Dunn et al. demonstrated that in case of direct in vivo gene delivery of Ad/BMP-7 in a collagen gel carrier promoted successful regeneration of alveolar bone defects around dental implants[16].

Limitations of Gene Therapy[17]

The following are limiting factors on the use of gene therapy:

- Short-lived nature of gene therapy.
- Immune response of the patient.
- Problems with viral vectors like patient toxicity, immune and inflammatory responses, and gene control and targeting issues. Limitation of sufficient quantity of the engineered gene that can be delivered.
- Extreme cost. Ethical restrictions

Technical Difficulties in Using Gene Therapy

1. **Difficulty in delivering of gene**

   Delivery of successful gene in gene therapy is not easy or predictable, even for single gene disorders. For example genetic basis of cystic fibrosis is well known but delivery of gene therapy is still difficult because of presence of mucus in lungs[6].

2. **Short-lived nature of gene therapy**

   Before gene therapy can become a permanent cure for any condition, the therapeutic DNA introduced into target cells must remain functional and the cells containing the therapeutic DNA must be long-lived and stable. Problems with integrating therapeutic DNA into the genome and the rapidly dividing nature of many cells prevent gene therapy from achieving any long-term benefits. Patients will have to undergo multiple rounds of gene therapy.

3. **Activation of immune response**

   Viral vector may be recognized as antigen and leads to activation of immune response. This may lead the efficacy of gene therapy and can induce serious side effect.

4. **Chance of inducing a tumor (insertion mutagenesis)**

   If the DNA is integrated in the wrong place in the genome, for example in a tumor suppressor gene, it could induce a tumor. This has occurred in clinical trials for X-linked severe combined immunodeficiency (X-SCID) patients, in which hematopoietic stem cells were transduced with a corrective transgene using a retrovirus, and this led to the development of T cell leukemia in 3 of 20 patients.

5. **Safety of vector**

   Viruses, the carrier of choice in most gene therapy studies, present a variety of potential problems to the patient 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.

6. **Difficulty to treat 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 multifactorial disorders such as these would be

especially difficult to treat effectively using gene therapy.

7. **Expensive**

Gene therapy is costly and very expensive procedure.

Future Strategies Of Gene Therapy In Preventing Periodontal Diseases

The complexity of gene-enhanced periodontal regenerative therapeutics lies in determining which gene or gene combinations are necessary and sufficient to enhance the multiple tissue types regeneration in the periodontium. In the future, it might be possible to mimic the natural healing process by developing novel biomimetic scaffolds that react to environmental stimuli or release their cargo (proteins or genes) according to individual cellular demand. Cooperation between tissue engineers and periodontal clinicians may eventually help to overcome the challenges. Scaffolds form an important component of the periodontal regenerative armamentarium and it is expected in the near future that the use of gene activated scaffolds, from pre-clinical studies to the clinics, will be easily accepted not only by the patients but also by the dental community, owing to its long history of acceptance. It is also expected that the cost of manufacturing such non-viral gene delivery systems will decrease, allowing treatment for a vast majority of patients.

Although gene therapy to enhance periodontal regeneration is still at a primitive stage, the constant growth in the development of non-viral vectors with better transfection efficiency suggests that DNA-based therapeutics may play a significant role in periodontal regeneration in the coming years.

They are as following

- Gene Therapeutics-Periodontal Vaccination
- Genetic Approach to Biofilm Antibiotic Resistance
- An In vivo Gene Transfer by Electroporation for Alveolar Remodelling
- Antimicrobial Gene Therapy to Control Disease Progression
- Designer Drug Therapy in Treating Periodontal Disease
- Tight Adherence Gene for the Control of Periodontal Disease Progression
- Gene Therapy to Regenerate New Teeth.

Gene Therapeutics - Periodontal Vaccination

For many years researchers have been exploring vaccination techniques in animal models to eradicate periodontal disease with mixed success. In the last decade gene transfer research has led to a novel way to achieve a vaccination like

1. A salivary gland of a mouse when immunized using plasmid DNA encoding the Porphyromonas gingivalis (P. gingivalis) fimbrial gene produces fimbrial protein locally in the salivary gland tissue resulting in the subsequent production of specific salivary immunoglobulins A, or IgA and immunoglobulin G, or IgG, antibodies and serum IgG antibodies. This secreted IgA could neutralize P. gingivalis and limit its ability to participate in plaque formation. Similarly, secreted fimbrillin in saliva could bind to pellicle components blocking the attachment of P. gingivalis.
2. Scientists have also demonstrated the efficacy of immunization with genetically engineered Streptococci gordoni vectors expressing P. gingivalis fimbrial antigen as vaccine against P. gingivalis associated periodontitis in rats[18].
3. The gene hemagglutinin, which is an important virulence factor of P. gingivitis, has been identified,

cloned, and expressed in Escherichia coli. The recombinant hemagglutinin B (rHag B) when injected subcutaneously in Fischer rats infected with P. gingivalis showed serum IgG antibody and interleukin-2 (IL-2), IL-10, and the IL-4 production which gave protection against P. gingivalis induced bone loss[19].

Genetic Approach to Biofilm Antibiotic Resistance

Some microorganisms have the ability to form a microbial community attaching to surfaces and are generally referred to as biofilm. Researchers have found bacteria growing in biofilms become up to 1,000 fold more resistant to antibiotics as compared to a planktonic counter part making them hard to control. The mechanism behind microbial biofilm resistance is not clear. Recently Mah et al. identified gene ndvB encoding for glycosyltransferase required for the synthesis of periplasmic glucans in wild form of Pseudomonas aeuroginosa RA14 strain. This remarkably protected them from the effects of antibiotics, biocides, and disinfectant. Using a genetic approach researchers have isolated ndvB mutant of Pseudomonas aeuroginosa, still capable of forming biofilm but lacking the characteristic of periplasmic glucans, thereby, rendering microbial communities in biofilm more susceptible to conventional antibiotic therapy.

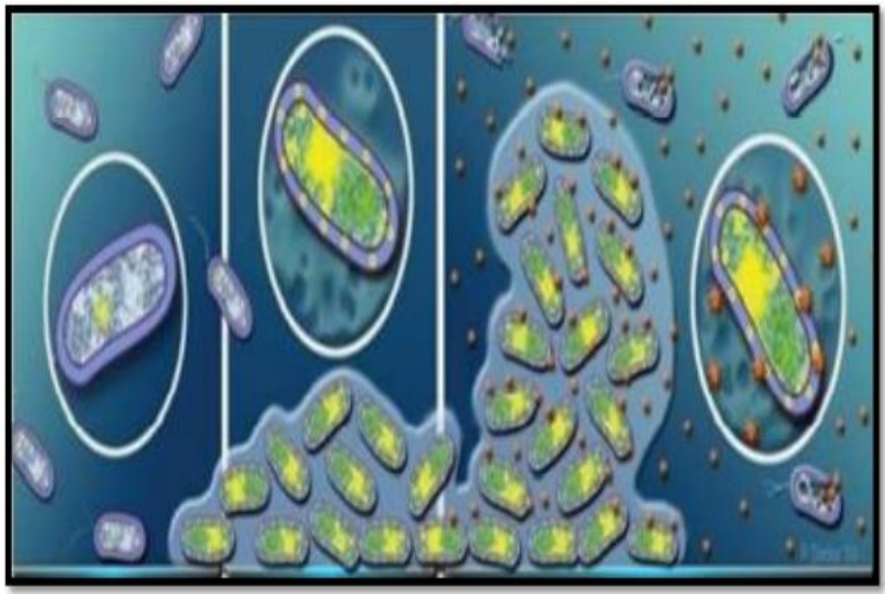

Figure 3: Genetic approach to biofilm antibiotic resistance

An in vivo Gene Transfer by Electroporation for Alveolar

Remodelling periodontal tissue reacts to stimuli such as mechanical stress and inflammation by active remodelling with the expression of various molecules. Using an in vivo transfer of LacZ gene (gene encoding for various remodelling molecules) into the periodontium and using plasmid DNA as a vector along with electroporation (electric impulse) for driving the gene into cell, has shown predictable alveolar bone remodelling.

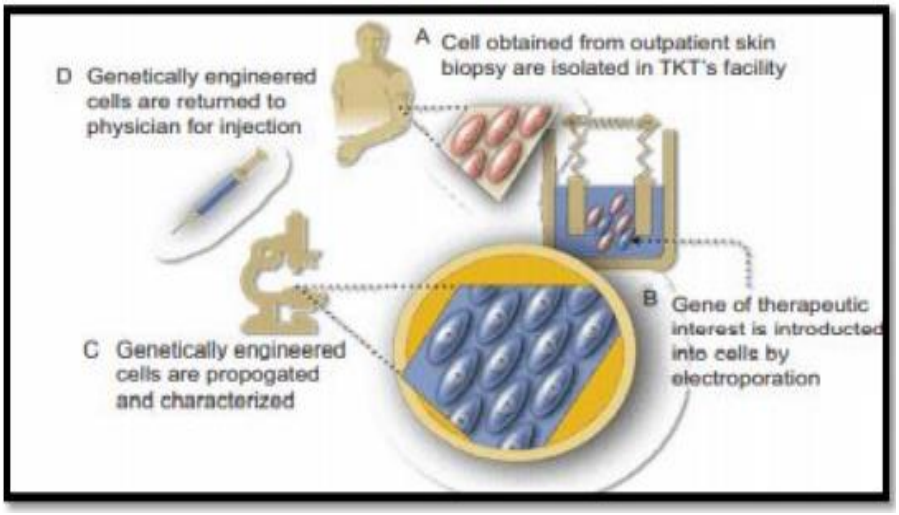

Figure 4: Electroporation for alveolar bone

Antimicrobial Gene Therapy to Control Disease Progression

One way to enhance host defense mechanism against infection is by transfecting host cells with an antimicrobial peptide/protein-encoding gene. Researchers have shown when host cells were infected in vivo with β defensin-2 (HBD-2) gene via retroviral vector; there was a potent antimicrobial activity which enhanced host antimicrobial defense.

Designer Drug Therapy in Treating Periodontal Disease

If genes necessary for normal development are known, then "designer drug therapies" aimed at one area of the gene or the other can be developed. These designer drugs will be safer than today's medicines because they would only affect the defect in a gene clearly identified through genetic research.

Tight Adherence Gene for the Control of Periodontal Disease

Progression Colonization of target tissue by a periodontal pathogen like Actinobacillus actinomycetemcomitans is an essential first step involved in the pathogenesis of localized aggressive periodontitis. It has been shown a "tight adherence gene" of Actinobacillus actinomycetemcomitans is required for its adherence and virulence. Researchers have developed mutant strains lacking the "tight adherence gene" which could predictably control periodontal disease progression by limiting colonization and pathogenesis of Actinobacillus actinomycetemcomitans.

Gene Therapy to Regenerate New Teeth

Dental researchers hope to regenerate 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 has found PAX 9, a master gene critical for tooth development. The hope is we will be able to bioengineer human teeth for replacement in the future[20].

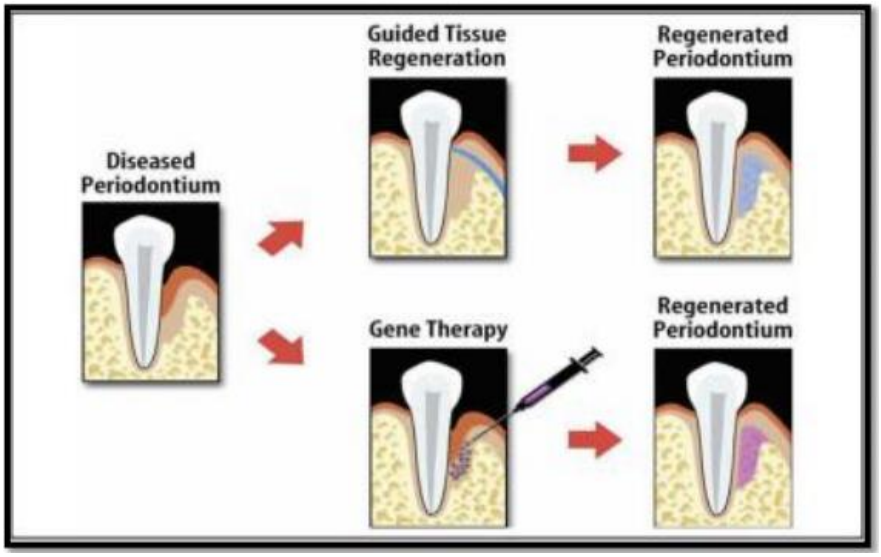

Figure 5: Approaches for regenerating a tooth supporting structure

Conclusion

Gene therapy essentially consists of introducing specific gene material into target cells without producing toxic effect on surrounding tissue. Gene therapy has a promising role in the field of dentistry. Gene therapy has the potential to disrupt existing therapies or create therapies where none previously exist and undoubtedly will revolutionize dentistry in the years to come.

References

1. Chatterjee, et al. Gene therapy in periodontics. Journal of Indian society of periodontology,2013;vol 17: 2
2. Kinane DF, Shiba H, Hart TC. The genetic basis of periodontitis. Periodontol 2000 2005;39:91-117.
3. Indhulekha Vimalakshan, et al. Applications of gene therapy in dentistry- a review. Drug Invention Today,2018; vol 10;6
4. B. V. Karthikeyan. Gene Therapy in Periodontics: A Review and Future Implications. The Journal of contemporary dental practice,2006;vol 7:3
5. Goker et al. Gene Delivery Therapeutics in the Treatment of Periodontitis and Peri-Implantitis: A State of the Art Review. International Journal of Molecule Science,2019;20,3551
6. Human Genome Project Information. Available from: http// www.genomics.energy.gov. Clinical Genetics-Kaya Lahiri. [Last accessed on 2012 Aug 2].
7. Yin H, Kanasty RL, Eltoukhy AA, Vegas AJ, Dorkin JR, Anderson DG. Non-viral vectors for gene-based therapy. Nat Rev Genet 2014;15:541.
8. Lee JY, Peng H, Usas A, Musgrave D, Cummins J, Pelinkovic D, et al. Enhancement of bone healing based on ex vivo gene therapy using human muscle derived cells expressing BMP-2. Hum Gene Ther 2002;13:1201-11.
9. Baum BJ, O'Connell BC. The impact of gene therapy on dentistry. J Am Dent assoc 1995;126:179-89.
10. Crombleholme TM. Adenoviral-mediated gene transfer in wound healing. Wound Repair Regen 2000;8:460-72.
11. Phillips, J.E.; Gersbach, C.A.; Garcia, A.J. Virus-based gene therapy strategies for bone regeneration. Biomaterials 2007, 28, 211–229.

12. Anusaksathien O, Webb SA, Jin QM, Giannobile WV. Platelet- derived growth factor gene delivery stimulates ex vivo gingival repair. Tissue Eng 2003;9:745-56.
13. Kaiger D, Cirelli JA, Giannobile WV. Growth factor delivery for oral and periodontal tissue engineering. Expert Opin Drug Deliv 2006;3:647-62.
14. Franceschi RT, Wang D, Krebsbach PH, Rutherford RB. Gene therapy for bone formation: In vitro and in vivo osteogenic activity of an adenovirus expressing BMP-7. J Cell Biochem 2000; 78:476-86.
15. Dunn CA, J A, Jin QM, Taba MJr, Franceshi RT, Bruce Rutherford R, Giannobile WV. BMP gene delivery for alveolar bone engineering at dental implant defects. Mol Ther 2005:11:294-9
16. SharmaA, HonmaK, EvansTR, HrubyDE. Oral immunization with recombinant Streptococcus gordonii expressing Porphyromonas gingivalis Fim A Domains. Infect Immun 2001;69:2928-34.
17. Gupta K, Singh S, Garg KN. Gene therapy in dentistry: Tool of genetic engineering. Rev Arch Oral Biol 2015;60:439-46.
18. Katz J, Black KP, Michalek MS. Host response to recombinant hemagglutinin B of Porphyromonas gingivalis in an experiment rat model. Infect Immun 1999;67:4352-59.
19. Mah TF, Pitts B, Pellock B, Walker GC, Stewart PS, O'Toole GA. A genetic basis for Pseudomonas aeruginosa biofilm antibiotic resistance. Nature 2003;426:306-10.
20. Cathy AJ. Teeth - We're going to grow them back! www.Filipinodentist.Com. April 17, 2005.