Review Article

JRAD Journal of Research and Advancement in Dentistry

J Res Adv Dent 2019;9:2s:128-134.

Clinical Implication of Gene Therapy in Dentistry

Kavita Verma[1*] Shrish Charan Srivastava[2] Kavan Patel[3] Sareen Duseja[4]

[1]Reader, Department of Endodontics and Conservative Dentistry, Narsinbhai Patel Dental College, Visnagar, Gujarat, India.
[2]Professor, Department of Orthodontics and Dentofacial Orthopedics, Narsinbhai Patel Dental College, Visnagar, Gujarat, India.
[3]Reader, Department of Prosthodontics Crown and Bridge, Narsinbhai Patel Dental College, Visnagar, Gujarat, India.
[4]Reader, Department of Prosthodontics Crown and Bridge, Narsinbhai Patel Dental College, Visnagar, Gujarat, India.

ABSTRACT

Background: In search of a better health care to the patients, gene therapy has found place as a recent advancement in treatment options. Gene therapy has been applied in various medical and dental treatments to get a favourable outcome. The idea of gene therapy is unique as it works by the addition of a healthy gene in place of defective genes so that the function in the patient's body is restored. It represents a means to treat a disease by use of genetic materials leading to expression of proteins in the cells which interfere with the synthesis of proteins to treat diseases. Different vector systems are presently in a trial state to perform gene transfer with certain advantages and disadvantages this article therefore aims to describe gene therapy and outlines its various applications in dental field.

Keywords: Bone regeneration, gene therapy, viral vector.

INTRODUCTION

With development in the technology, the manipulation at the genetic level has become possible. This has led to marked advancement in the field of gene therapy and its applications in dentistry have increased. Gene therapy has found place in various fields of dentistry like in the treatment of cancer, DNA vaccinations, salivary glands, treatment of autoimmune diseases, treatment of pain, bone repair, orthodontics, keratinocytes and more recently treatment of dental caries.

A gene is a distinct portion of a DNA which is responsible for the development and function of all organisms. It determines the structure of different proteins and controls the production of proteins. Genes are the smallest functional unit of the genetic system. Broadly gene therapy is the genetic modification of cells for therapeutic purposes.[1] In gene therapy the genes are used as medicines to treat a disease either knocking out the defective gene or by interfering with its function. First of all the defective gene is identified and the then the working or therapeutic copy of a gene, acquired by restrictive endonuclease enzyme(cutting and splicing) , is then transferred to a cell with clinical condition, with the help of a vector, for the repair of the faulty gene copy in that cell. The techniques include the replacement of a faulty gene, or introduction of a new gene whose function is to cure or to favourably modify the clinical course of a condition. [2, 3]

There are two types of gene therapy – the somatic gene therapy and the germ line gene therapy .Somatic gene therapy involves the transfer of the therapeutic genes in the somatic cells of the patient and the gene is not inherited by the next generation where as in the germ line gene therapy, the functional genes are transferred into the genomes of the sperms or eggs, thus the therapy is transferred to the next generation. [4] (Table 1)

The therapeutic genes are delivered to the target site with the help of a vector. The vector should be non immunologic, stable, easy to reproduce and should have longevity of expression. [5]The vector is prepared in two steps. The vector is isolated,

Received: Jan. 11, 2019**: Accepted**: Mar. 3, 2019
***Correspondence Dr. Kavita Verma.**
Department of Endodontics and Conservative Dentistry, Narsinbhai Patel Dental College, Visnagar, Gujarat India.
Email: kavitaverma@gmail.com

pISSN 2278-0076
eISSN 2321-9270

purified and cleaved.DNA fragment is then joined to cleaved ends of the vector and thus the molecule is closed and DNA chimera is formed. This vector is then introduced into the cell and genetically identical cells containing same DNA are produced. Thus a mass of cells with same genetic makeup is produced. [6]

The vectors can be either viral or non viral. The viral vectors are altered genetically and made harmless. These viruses can replicate gene of interest. Various virus used in gene therapy include adenovirus, retrovirus, adeovirus associated virus, lentivirus, vaccinia virus, herpes simplex virus. [7]

Among the viral vectors, adenovirus is commonly used, as it can be cultured easily and because of its lower pathogenecity. (Table 2) amongst the non-viral vectors most commonly used are the Lipid complex, liposomes, peptide/protein, polymers, mechanical, electroporation, and gene gun. [7]non-viral vectors are comparatively simpler and safer alternative for viral vectors. The details of the of the nonviral vector are given in the table three .(Table 3)

Plasmid DNA can be delivered to tissues in vivo by intravenous infection at high hydrodynamic pressure. It ispossible to restrict bloodflow to increase the pressure by use of a blood-pressure cuff in the limbs to deliver plasmid DNA. The delivery of DNA by coated metal microparticles by particle bombardment into cutaneous tissues has been useful. However, attendant issues include heat generation and transfer at the site of penetration of the microparticles [16]

Application of regulated electric pulses in delivering genes to cells is known as electroporation. Electroporation is commonly used to deliver DNA to bacteria,yeast and mammalian cells in culture. Electroporation uses electric fields to create transient pores to facilitate entry of plasmid DNA. The major limitations of electroporation is tissue damage and moreover it is an invasive method [16] The application of ultrasound leads to acoustic cavitation and produces cell membrane permeabilization, the use of a combination of ultrasound and electro-poration was found to be better than either of these methods alone.The recent advances in the uses of ultrasound to drug and gene delivery has multiple therapeutic applications including regenerative medicine [16, 17]

APPLICATIONS OF GENE THERAPY IN DENTISTRY

Bone regeneration

Gene therapy can be used in bone regeneration. There are 4 methods to bring about bone repair-they are osteoinduction , differentiation of osteoblasts which will give rise to osteoid matrix, osteoconduction and mechanical stimulation. Gene therapy can help in osteoconduction in cases of single gene defects; this will lead to differentiation of osteoblasts and then osteoconduction. Gene therapy is helpful in single defect cases as it can be delivered to a specific anatomic location and discrete site and can lead to bone regeneration there. Transient expression is beneficial for bone regeneration because of gene transfer techniques.[18]

BMP's are the growth factors which can induce bone formation specifically, BMP 2, 4 and 7. BMP 2 has shown to repair mandibular osseous defects. [19] BMPs can induce bone formation both individually and in combination with other BMPs. For example BMPs 2, 3a, 4, 7 and 8 are involved in fracture healing. [20]. VITAPEX is also helpful in the expression of BMP 2 and induces bone regeneration in periapical lesions. [21] Platelet derived growth factors can also help in bone regeneration or formation. [22]

Bone sialoprotien also controls the cell differentiation in bone repair and regeneration. bfa1 is the master gene which is involved in BSP gene expression.BSP is a non collagenous protein in bone and other mineralised tissue.BSP gene can be delivered in vivo to regenerate periodontal alveolar bone. [22]

Gene therapy is also helpful in reducing the periapical bone resorption, inhibition of endodontic disease development and inflammation. It has been demonstrated that adeno- associated virus (AAV) -mediated Atp6iRNA (RNAi) helps to knock down Atp6i/ TIRC7 gene. This therapy impairs the osteoclast function in vitro; it reduces the number of Tcells in periapical lesions and also reduces the bone resorption caused by bacterial infection in endodontics.[23] Genetically engineered mesenchymal stem cells (MSCs) harboring a tetracycline-regulated expression vector encoding the

Table 1: Difference between somatic gene therapy and Germ line gene therapy.

	Somatic gene therapy	Germ line gene therapy
1	Therapeutic genes transferred to somatic cells	Therapeutic genes transferred to germ cells
2	Will not be inherited in the later generation	It will be inherited in the later generation

Table 2: Table 2 vectors used in gene therapy

	Viral vectors	**Non viral vectors**
1	Retroirus [8]	Lipid complex,
2	Recombinant virus encoding BMP-7 [9]	liposomes,
3	Lentivirus vector encoding BMP-2[10]	peptide/protein,
4	Adenovirus encoding BMP-2 gene[11]	polymers,
5	Adenovirus encoding BMP-7[12]	mechanical,
6	Adenovirus encoding PDGF beta[13]	electroporation,gene gun. [7]
7	Retrovirus encoding active form of beta catenin[14]	
8	Adenovirus encoding BMP-7, PDGF beta or combination of both[15]	

Table 3: Advantages and disadvantages of gene therapy.

Table 3 Advantages and disadvantages of gene therapy	
Advantages	Disadvantages
It has the ability to replace defective cells	Technique sensitive
It can treat genetic disorders	Selection of appropriate vector
It can treat even before the onset of disease	Risk of pathogenicity by the vectors
	Costly

osteogenic growth factor human BMP-2 can help in the osteogenic differentiation of the mesenchymal stem cells and lead to bone formation and healing.[24]

Gene therapy for caries prevention

Gene therapy can be applied in dental treatment for caries prevention by regeneration of enamel.Ctip2 (gene which is a transcription factor) is responsible for development of immune system, skin and nervous system. This transcription factor was also found to have a role in the formation and maturation of ameloblasts. It was researched in 2009 that when the researches knocked out this gene the mice could not live after birth and had rudimentary teeth and lacked enamel coating. Thus it was concluded that gene Ctip2 control the production of tooth enamel and can be helpful in the repair of damaged enamel.[25]

A similar study was published in developmental biology where the researchers concluded that the deletion of tbx1 was shown to be involved in the maintainance of dental epithelial stem cells which form ameloblasts.[26]

S mutans have affinity to tooth surface therefore their pathogenic potential is related to their mechanical adherence.[27]In replacement therapy a genetically modified S.mutans is colonized onto the host surface, it might block the attachment site and will compete for nutrients. The genetically modified S mutans strain (BCS3-L1)is used in replacement therapy to prevent dental caries.[28] In this strain BCS3-L1gene encoding lactate dehydrogenase was deleted therefore lactic acid production was stopped.When analysed for fermentation end product it was found that lactic acid was not detectable in BCS3-L1.[29] Replacement therapy will

provide life time protection after single application.[30]

In the year 2000 Burne and Marquis brought about a process in which alkali was generated from ammonia which was produed from aginine and urea. This process occurred from association of genetically altered strain of S.Mutans with dental plaque. Long term studies and RCTs are still required for clinical application.[31]

Gene therapy for Orthodontic tooth movement

Tooth movement in orthodontic treatment depends upon osteoclastic and osteoblastic activity.osteoclastic activity depends upon periodontal ligament cells which is required to support osteoclastogenesis.the receptor activator of the NF- Kappa B (RANK)ligand or RANKL is the molecule which supports this interaction.Osteoclastic precursors express RANK, the receptor for RANKL.OPG osteoprotegerin is produced by periodontal ligament cells and osteoblastic cells.

RANKL acts as a ligand for OPG. OPG also acts as a decoy receptor for RANKL, preventing RANKL – RANK binding. Therefore excessive suppression of osteoclastic formation.It has been demonstrated in studies where gene therapy is used with OPG and RANKL to accelerate or inhibit tooth movement for normal as well as in cases where ankylosis is present.[32, 33]

Gene therapy for Reparative dentine formation

It has been found that gene therapy can be helpful in regeneration of reparative dentin where BMP11 and GDF II when transferred to amputated pulp stimulated reparative dentine formation in vivo.Isolated progenitor stem cells in the pulp were transfected with BMP 11/GDF II by electroporation and implanted onto the amputated pulp.[34]

When biomolecules like bone sialoprotien , bone morphogenetic protein-7, dentonin (a fragment from matrix extracellular phosphoglycoprotien) and two small amelogenin gene splice products (A+4and A-4)were implanted onto the exposed pulp induced formation of reparative dentinal bridge. Cells which were formed in this process differentiated into osteoblasts and odontoblast like cells. Odontoblast progenitor cells if implanted can also get same results.[35,36]

Gene Therapy in Oral Cancer

Gene therapy in cancer treatment is done to bring about cancer cell death which can be done in several ways. Gene addition therapy is where addition of tumour suppressor gene can be done. Gene excision therapy is where deletion of a defective tumour gene is done. The expression of gene that stimulates tumour growth can be down regulated and tumor angiogenesis can be inhibited by introduction of particular genes.[37, 38]

Gene Therapy for Salivary Glands

Salivary glands produce large amount of proteins and are encapsulated[39], therefore they can be a good site for gene transfer. This minimises undesirable access of vectors and trans genes into other tissues[40]The delivery of vectors whether viral or non viral is done through the main duct opening of salivary gland in the oral cavity. It is done by retrograde injection.[41]

Advantages and challenges

Inspite of plenty of active research and ongoing clinical trials, there are a number of limiting factors such as technique sensitivity, identification of related genes and vectors, delivery of genes at the site of action and duration of action. The transfer of a large amount of genes into many cells is required to achieve the desired therapeutic effect and may not be costly Gene therapy has emerged as one of the greatest technical challenges in modern medicine. It is very hard to introduce new genes into cells of the body and keep them working. And there are financial concerns as the treatment might not be cost effective. (Table 3)

CONCLUSION

Gene therapy has developed as an active area of research for a range of biomedical and dental applications. Considering the exponential rise in the quantity and quality of research in the field of gene therapy it is expected to become a very useful tool for the management of oral diseases. The successful outcomes of human clinical trials in recent years have given hopes for clinicians for the practical applications of gene therapy very soon.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. Misra S. Human gene therapy: A brief overview of the genetic revolution. J. Assoc. Physicians India. 2013;61:127–133..

2. Produced by the Centre for Genetics Education. Internet: http://www.genetics.edu.au www.genetics.edu.au © Centre for Genetics Education 1 produced by the Centre for Genetics Education. Internet: http://www.genetics.edu.auProduced au

3. Durai S,Mani M, Kandavelou K, Wu J, Porteus MH, Chandrasegaran S. Zinc finger nucleases: custom-designed molecular scissors for genome engineering of plant and mammalian cells. Nucleic Acids Res 2005:33(18): 5978–90.

4. Baum BJ, O'Connell BC. The impact of gene therapy on dentistry. JADA 1995; 126:179–89.

5. Kay MA, Glorioso JC, Naldini L. Viral vectors for gene therapy: the art of turning infectious agents into vehicles of therapeutics. Nat Med 2001; 7:33–40.

6. Breau L.B. Clay man LG gene replacement therapy In Harrison BL, Sessions BR., Hong KW., eds. Head and neck cancer, A Multidisciplinary approach (6th) edition Lippincott Williams and wilkkins. 2004:1018-28.

7. Iskander A, Yoo HG.Gene therapy for patients with head and neck cancer.Adelstein JD Ed Squamous cell head and neck cancer.Humuna press,Totowa, New Jercy 2005:263-78

8. Naldini L, Blomer U, Gallay P, Ory D, Mulligan R, Gage FH, et al. In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. Science 1996; 272:263-7.

9. Rutherford, R Bruce. BMP-7 gene transfer to inflamed ferret dental pulps. Eur J Oral Sci 2001; 109:422-4.

10. Sugiyama O, An DS, Kung SP, Feeley BT, Gamradt S, Liu NQ, et al. In vivo Lentivirus-mediated gene transfer induces long-term transgene expression of BMP-2 in vitro and new bone formation. Mol Ther 2005; 11:390-8.

11. Yang X, van der Kraan PM, van den Dolder J, Walboomers XF, Bian Z, Fan M, et al. STRO-1 selected rat dental pulp stem cells transfected with adenoviral-mediated human bone morphogenetic protein 2 gene show enhanced odontogenic differentiation. Tissue Eng 2007; 13:2803-12.

12. Zhang Y, Song J, Shi B, Wang Y, Chen X, Huang C, et al. Combination of scaffold and adenovirus vectors expressing bone morphogenetic protein-7 for alveolar bone regeneration at dental implant defects. Biomater 2007; 28:4635-42.

13. Shang SH, Zhang YF, Shi B, Cheng XR. Construction and identification of recombinant human platelet-derived growth factor-B adenoviral vector and transfection into periodontal ligament stem cells. Zhonghua Kou Qiang Yi Xue Za Zhi 2008; 43:584-8.

14. Scheller EL, Chang J, Wang CY. Wnt/â-catenin inhibits dental pulp stem cell differentiation. J Dent Res 2008; 87:126-30.

15. Zhang Y, Shi B, Li C, Wang Y, Chen Y, Zhang W, et al. The synergetic bone-forming effects of combinations of growth factors expressed by adenovirus vectors on chitosan/collagen scaffolds. J Control Release 2009; 136:172-8.

16. Nakashima M[1], Iohara K, Zheng L. Gene therapy for dentin regeneration with bone morphogenetic proteins. Curr Gene Ther. 2006 Oct; 6(5):551-60.

17. Anil Dhingra, Viresh Chopra and Shalya Raj. Challenges of bioengineering and endodontics.Journal of Developmental Biology and Tissue Engineering Vol. 3(7) pp. 80-84, July 2011

18. Luo J et al. Gene therapy for bone regeneration. Current gene therapy 2005; 84(12): 1093-1103.

19. Baum JB., Kok M, Tran Ds, Yamano S. , The impact of gene therapy on dentistry JADA 2002;133:35-44.

20. Cho TJ[1], Gerstenfeld LC, Einhorn TA. Differential temporal expression of members of the transforming growth factor beta superfamily during murine fracture healing. J Bone Miner Res. 2002 Mar;17(3):513-20

21. Xia X, Man Z, Jin H, Du R, Sun W, Wang X. Vitapex can promote the expression of BMP-2 during the bone regeneration of periapical lesions in rats. J Indian Soc Pedod Prev Dent 2013;31:249-53

22. Karthikeyan VB, Pradeep R.A. Gene therapy in periodontics. A review and future implications. The J contemporary dental practice 2006 :7(3)83-91.

23. Junqing Ma[a,b],Wei Chen[a], Lijie Zhang[c], Byron Tucker[a,d], Guochun Zhu[a], Hajime Sasaki[c], Liang Hao[a], Lin Wang[b], Hongliang Ci[a], Hongbing Jiang[a,b], Philip Stashenko[c] and Yi-Ping Li[a]. RNA Interference-Mediated Silencing of Atp6i Prevents Both Periapical Bone Erosion and Inflammation in the Mouse Model of Endodontic Disease. Infect. Immun. April 2013 vol. 81 no. 4 1021-1030

24. Exogenously regulated stem cell-mediated gene therapy for bone regeneration.I K Moutsatsos, G Turgeman, S Zhou, B G Kurkalli, G Pelled, L Tzur, P Kelley, N Stumm, S Mi, R Müller, Y Zilberman, D Gazit; Mol Ther.2001 Apr;3(4):449-61

25. Feb2, 2009, genetic discovery could lead to advances in dental treatment. url

26. "Dentistry of future? gene responsible for formation of enamel discovered"March 3,2009-team of researchers lead by Dr. Thimios Mitsiadis, university of zurich, Switzerland

27. Van houte J, Gibbons RJ,pulkkihen AJ. Ecology of human oral lactobacilli. Infect Immun 1972;16(5):1723-9

28. Hillman JD, Brooks TA, Mchalek SM, Harmon CC, SnoepJL, Van der weijden cc. construction and characterization of an effector strain of streptococcusmutans for replacement therapy of dental caries.Infect immune 2000;68(2):543-9.

29. Hillman JD.Genetically modified streptococcus mutans for the prevention of dental caries.antonie van leuwenhoek 2002;82(1-4):361-6.

30. Kenneth J Anusavice. Journal of dental education present and future approach for the control of caries.vol 69, number 5

31. BURNE RA, marquis RE, alkali production by oral bacteria and protection against dental caries. FEMS Microbiol lect 2000 193(1):1-6.

32. Havens B , wadhwas , nanda r .orthodontics in the year 2047:genetically driven treatment plans JCO. 2007; 41(9):549-56.)

33. AR Prabhakar, jibbi m paul, n basappa. International journal of clinical pediatric dentistry, May – august 2011; 4(2):85-92.

34. "Tissue engineering in endodontics"nakashima M; Aust Endod J, 31(3):111-3.2005.

35. Ref – the impact of bioactive molecules to stimulate tooth repair and regeneration as part of restorative dentistry. Goldberg M, Lacerda pinhero S, Jegat N, Six N, Septier D, Priam F, Bonnefoix M, Tompkins K, Chardin H, Denbesten P, Veis A, Poliard A; dent clin north am , 50(2):277-98, 2006.)

36. Molecular biology and preservation of tooth vitality current implications. Sowmya shetty, M. Umar Farooq, B suresh chandra, sucharitha nanjappa. Endodontology, (42-43).

37. Heise C, Sampson – Johannes A, Williams A, Mc Corrnick F, Von Hoff DD, Kim DH, ONYX-015, an EIB gene attenuated adenonilus causes tumor-specific cytolisis and antitumoral efficacy that can be augmented by standard chemotherapeutic agents. Nat Med 1997; 3:639-45.

38. Dental surgeons as gene therapist. Dr sunil PM, Dr Ramachandran CR, Dr. Jaisanghah, Dr. Santosh Kumar C.

39. Baum J.B., Kok M, Tran DS, Yamano S, The impact of gene therapy in dentistry.JADA 1995; 126:179-89.

40. Simmons KR, Baum JM., Transferring genes to salivary glands. J Dental Education 2001; 11:1425-26.

41. Zufferey R. Aebischer P. Salivary glands and gene therapy: the mouth waters. Gene therapy 2004; 11:1425-26.

42. Rutherford RB: BMP-7 gene transfer to inflamed ferret dental pulps. European Journal of Oral Sciences, 2001;109(6):422-424.