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Review Article

Current Concepts and Future Aspects of Gene Therapy in Periodontics

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Abstract

Periodontal disease is a chronic inflammatory disease of the supporting tissues of the teeth, having a multifactorial ethology. Genetic variance has been proved to be a major risk factor for periodontitis. Gene therapy involves the transfer of genetic material to cure a disease or at least to improve the clinical status of a patient. Gene therapy has emerged with newer dimensions to enhance the existing therapeutic modalities in biological science and in the management of periodontal diseases and reconstruction of dentoalveolar apparatus. Gene therapy is one of the recent advancements and its applications in the field of periodontics are reviewed in general here.

Keywords: Gene Therapy; Vectors; Periodontitis; Regeneration; Implantology

Abbreviations

BSP: Bone Sialoprotein; Bcl2: B Cell Lymphoma 2; BMP: Bone Morphogenetic Proteins; CRISPR-Cas 9: Clustered Regularly Interspaced Short Palindromic Repeats; DNA: Deoxyribonucleic Acid; FGF: Fibroblast Growth Factor; GAT: Gene Augmentation Therapy; HIV: Human Immunodeficiency Virus; HIF α: Hypoxia Inducible Factor α; HSV: Herpes Simplex Virus; HBD-2: Human Beta Defensin 2; ICTP: Bone Collagen Telopeptide; LMP: LIM Domain Mineralization Protein; mRNA: Messenger Ribonucleic Acid; NTF: Neurotrophic Factor; Osx: Osterix; PDGF: Platelet Derived Growth Factor; Runx2: Runt-Related Transcription Factor 2; rh: Recombinant; TGF- β: Transforming Growth Factor Beta; TALENs: Transcription Activator-Like Effector-Based Nucleases; VEGF: Vascular Endothelial Growth Factor; Zfn: Zinc Finger Nucleases

Introduction

Periodontal diseases are chronic inflammatory diseases of the supporting tissues of the teeth caused by specific microorganisms, resulting in progressive destruction of periodontal ligament, alveolar bone with pocket formation, recession or both [1]. Genetic variance has been reported as a risk factor for periodontitis. The host's response to the microbial challenge acts as a key determinant. Genetic factors play a role in modulating how individuals interact with many environmental agents, including biofilm, to determine susceptibility to periodontitis [2]. Gene therapy involves mechanisms which replace defective genes with their correct analogues to produce functional proteins. Studies have shown that gene therapy can be used to prevent, alleviate or treat underlying disorders, including cancers, infectious diseases, and genetic and autoimmune disorders [3]. Genetic approaches in periodontal tissue engineering show early progress in achieving delivery of growth factor genes to periodontal lesions. Currently gene-based therapy is at the preclinical level and it may be several years before it enters the clinical area. However, the gene therapy methods collectively interface and complement stem cell therapy and emerging scaffold technologies to enhance their potential to restore tissue function and structure in a predictable manner.

This article reviews the basics of gene therapy and its various modes of administration, with an insight into the role of gene therapy in periodontics.

History of gene therapy

Author	Event
Rogers, 1973 [4]	First gene therapy trial
Rosenberg., *et al*, 1989 [5]	Gene transfer in humans
Wikesjo., *et al*, 2004[6]	Effect of rhBMP-12 on periodontal regeneration
Peng Z.,2005 [7]	Gendicine™ - for squamous cell carcinoma
Wirth., *et al*, 2013[8]	Cerepro®- for brain tumours

Fundamentals of gene therapy

The basic principle of gene therapy is that the modification of the intrinsic expression of certain genes in body tissues to treat disease. The various gene therapeutic strategies include:

- Gene Augmentation Therapy (GAT)
- In gene augmentation therapy, inherited disorders caused by genetic deficiency of a gene product are treated by simple addition of functional alleles e.g. GAT has been applied to autosomal recessive disorders.
- Targeted Killing of Specific Cells
- Genes encoding toxic compounds (suicide genes), or prodrugs are used to kill the transfected/ transformed cells. This general approach is popular in cancer gene therapies.
- Targeted Inhibition of Gene Expression
- This involves blocking the expression of any diseased gene or a new gene expressing a protein which is harmful for a cell. This is particularly suitable for treating infectious diseases and some cancers
- Targeted Gene Mutation Correction
- It is used to correct a defective gene to restore its function which can be done at genetic level by homologous recombination or at mRNA level by using therapeutic ribozymes or therapeutic RNA editing [9].

Gene therapy involves the following 3 steps:

- Administration means introduction of the gene or a vector containing the gene into the body.
- Delivery means the transfer of the gene from the site of the administration to the nucleus of the target cell.
- Expression means the production of a therapeutic gene product in the cell [9].

Types of gene therapy

1. Somatic and Germline gene therapy: In germline therapy, germ cells, i.e., sperm or eggs are modified by the introduction of functional genes, which are ordinarily integrated into their genomes. Therefore, therapy induced changes would be heritable and would be passed on to later generations. In somatic gene therapy, therapeutic genes are transferred into the somatic cells of a patient and it is non-inheritable.
2. Permanent and temporary: In permanent gene therapy, target genes are enhanced or blocked indefinitely and in temporary gene therapy, target cells could produce a selected beneficial therapy over a limited period of time.
3. *Ex vivo* and *In vivo* gene therapy: In *ex vivo* gene therapy, genes are transferred to the cells grown in culture, transformed cells are selected, multiplied and then introduced into the patient, whereas in *in vivo* gene therapy, transfer of cloned genes are done directly into the tissues of the patient [10,11].

Gene delivery

To execute effective pharmacological action, the drug must reach the target site of action, otherwise it loses its therapeutic value and may even cause side effects through unintended interactions. In gene therapy applications, cellular compartments (i.e. in cytoplasm or nucleus) represent target sites for large, charged nucleic acid molecules, which must navigate across the plasma membrane to reach these sites. Several viral and non-viral gene carriers have been proposed and investigated for delivery of intracellular therapeutic bio actives [12].

Technical difficulties

1. Difficulty in delivery of gene.
2. Short-lived nature of gene therapy.
3. Activation of immune response.
4. Chance of inducing a tumour - Insertion mutagenesis.
5. Safety of vectors.
6. Difficulty to treat multigene disorders.
7. Durability and integration.
8. Expensive.

Gene therapy in clinical medicine

Gene therapy is one of the most promising and active research fields in medicine, although it has experienced significant setbacks and limited success. Interest in this therapeutic modality is based on the potential for treatment and cure of some of the most malignant and devastating diseases affecting humans like cystic fibrosis, Duchenne muscular dystrophy, Alzheimer's disease, cancer, atherosclerosis, human immunodeficiency virus etc. The basic mechanisms for gene delivery has not been modified for some time, *in vivo* and *ex vivo*. However, new, insightful techniques to deliver genetic material emerge continuously. Currently gene therapy-based clinic trials are being conducted in many of the major disease processes and in rare, usually well defined, genetic disorders. As basic science continues to progress within the field, more effective clinical trials will be launched and eventually will successfully treat or even cure patients. As this occurs, it will be essential for all physicians to understand the basic concepts encompassing a complex field [19]. The non-therapeutic use of cells, genes, genetic elements, or of the modulation of gene expression, having the capacity to improve athletic performance is defined as gene doping by the World Antidoping Agency [16].

Gene therapy in dentistry

Gene therapy has started to make a mark in the field of dentistry and are utilized for dental applications like treatment of orofacial pain, oral carcinomas, salivary gland dysfunctions, orthodontic tooth movements, tooth repair and regeneration and dentinal hypersensitivity [20]. The successful outcomes of human clinical tri-

als in recent years have paved way for clinicians for the progression of gene therapy to practical applications very soon. Even though there are innumerable number 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. Also, the transfer of a large number of genes into many cells is crucial to achieve the desired therapeutic effect and may not be cost-effective. In addition, there are ethical and safety issues for using gene therapies in humans; only somatic cell gene therapy is allowed at present.

Gene therapy in Periodontics

Periodontal tissue regeneration

Growth factor mediated tissue regeneration

Periodontal regeneration has long been the ultimate goal in periodontal therapy. However, treating and re-establishing the diseased periodontium's original structure, properties, and function constitute a significant challenge. A number of different approaches have been proposed, but the amount of regenerated tissue is oftentimes limited and difficult to predict. Periodontal regeneration, by definition, implies the regeneration of the cementum, periodontal ligament, alveolar bone in a specific temporal sequence and spatial distribution is based on a number of essential factors:

1. Protein based approach - Growth and differentiation factors are used for regeneration of periodontal tissues like PDGF, BMP-2,6,7,12, b FGF, VEGF and TGF- β etc.

2. Cell based approach - Evidence using mesenchymal stem cell have demonstrated efficient reconstruction of bone defect that are too large to heal spontaneously.

3. Gene delivery approach - Gene therapy that uses a vector that encodes the growth factor is utilized to stimulate tissue regeneration and to overcome the short half-lives of growth factor peptides *in vivo.*

Platelet derived growth factor

Platelet derived growth factor (PDGF) is a member of a family of multifunctional polypeptide growth factors, considered a critical switch to initiate tissue repair process. It is considered a potent recruiter of and strong mitogenic factor for cells crucial to musculoskeletal tissue repair, including mesenchymal stem cells, osteogenic cells, and tenocytes, and it also upregulates angiogenesis. Recombinant human PDGFBB in a synthetic scaffold matrix (beta tricalcium phosphate) promotes long term stable clinical and radiographic improvements for patients with localized periodontal defects [21,22].

Bone morphogenetic proteins

Bone morphogenetic proteins (BMPs) are multifunctional growth factors belonging to the transforming growth factor β (TGF-β) superfamily. BMPs are powerful regulators of cartilage and bone formation during embryonic development and regeneration in post-natal life. Some BMPs also participate in the development and repair of extraskeletal tissues and organs such as the brain, kidney, and nerves. Bone morphogenetic proteins (BMP). BMPs -2-4, -7 and -12 have all been evaluated for peri¬odontal and peri-implant bone regeneration. Studies suggests that human recombinant bone morphogenic protein-2 (rhBMP-2) can accelerate the regeneration of not only bone but also the cementum and the insertion of periodontal ligament fibres [23].

Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF), the best-characterized angiogenic factor, plays an important role in bone growth via the endochondral ossification pathway. Blocking VEGF leads to a decrease in trabecular bone formation at the growth plate secondary to suppression of blood vessel invasion and impairment of cartilage resorption. The involvement of VEGF in bone formation also is suggested by its interaction with humoral factors that regulate bone homeostasis. Despite the close association between VEGF and bone development, it remains unknown whether VEGF function is essential for bone formation induced by osteogenic BMPs [24].

Transforming growth factor beta

Transforming growth factor beta (TGF-β) is a multi¬functional growth factor structurally related to bone morphogenetic protein but is functionally quite differ¬ent. However, TGF-β can control gene expression either positively or negatively, a factor that can interfere with its therapeutic use [25]. TGF-β1, the most abundant iso¬form of the TGF-β family and found primarily in the platelets and osseous tissue, has been used for this ap¬plication. Clokie., et al. suggested that TGF-β1 increased the amount of bone healing adjacent to dental implants in mini pigs. TGF-β1 seems to play an important role in inducing fibroblastic differentiation of PDL stem/progenitor cells and in maintaining the PDL apparatus under physiological conditions.

Table 2 enumerates a few studies using growth factors for periodontal tissue engineering using gene therapy.

Viral	Nonviral

a) Retrovirus
b) Lentivirus
c) Adenovirus
d) Adenoassociated virus
e) Herpes simplex virus
f) Amplicon based HSV vectors
g) Sindbis virus
h) Hybrid vectors
i) Alphaviruses
j) Vaccinia viruses
k) Baculovirus
	A) Physical vectors: (Microseeding gene therapy)
	• Electroporation
	• Gene gun
	• Ballistic particle delivery
	• Ultrasound
	• Hydrodynamic injection
	• Molecular vibration
	• Laser irradiation
	• Magnetoception
	B) **Chemical vectors**:
	• Cationic polymer diethylaminomethyl-dextran
	• Calcium phosphate co-precipitation
	• Lipid mediated
	  • Cationic liposomes
	  • Lipoplexes
	• Polymer mediated (Polyplexes)
	  • Polyethyleneimine
	  • Poly-L-lysine
	  • Polyallylamine
	  • Chitosan
	  • Dendrimers
	• Peptide mediated
	  • Poly (ethylene glycol) peptides
	  • Glycopeptides
	• DNA/cationic polymer/cationic lipid (lipopolyplexes)

Gene editing tools: 1. Zinc finger nucleases(ZFNs) 2. Transcription Activator-Like Effector-based Nucleases (TALENS) 3. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR-Cas9) [16]	

Table 1: *Vectors of gene delivery.*

(References: a,b[13], c[14], d[15], e,f,g,h,i,j[16], k[17], A,B[18]).

Other methods in periodontal tissue regeneration using gene therapy

Transcription factors and regulators

The genes that are critical transcription factors and regulators of osteogenesis, such as Runt-related transcription factor 2 (Runx2), Osterix (Osx), and LIM domain mineralization protein (LMP), may hold promise in periodontal tissue engineering, especially in alveolar bone augmentation. Runx2 is a master transcription activator of osteoblast differentiation. Osx is a zinc-finger-containing transcription factor that works downstream of Runx2 in osteoblast differentiation. LMP-1 is an intracellular protein that is highly upregulated at the early stage of osteoblast differentiation [33].

Wnts

In 1987, the fly Wingless gene, which controls segment polarity during larval development in Drosophila melanogaster, was shown to be a homolog of integration site 1 (int-1), seen in

virally induced breast tumours. Because of the homology between int-1 and Wingless, the gene was named Wnt-1. Wnts are a family of 19 secreted glycoproteins that are crucial for embryonic development and post-developmental physiology through regulation of cell proliferation, differentiation, and apoptosis. In the last several years, the role of Wnt signalling in bone development, postnatal maintenance of bone mass, and tooth morphogenesis has been investigated, and several pharmaceutical targets in Wnt signalling pathway for skeletal diseases have been identified, such as leucine-responsive regulatory protein and sclerostin [34].

Growth factor	Gene encoding	Vector	Mode of delivery	Model	Conclusion	Reference
BMP 2	Rh BMP2	Polylactic acid polyglycolic acid copolymer	Gelatin	In vivo (Animal models)	On histometric analysis, the amount of new bone, new cementum and connective tissue attachment was greater in rhBMP-2 treated group	Kinoshita., *et al*, 1997 [26]
PDGF	Growth arrest specific gene (Gas gene)	Recombinant adenovirus	Collagen	In vitro	Demonstrated the prolonged effects of adenoviral delivery of PDGF	Chen., *et al*, 2002[27]
BMP- 7	BMP 7	Adenovirus	Collagen matrix	In vivo (animal model)	Sustained, targeted transgene expression for up to 10 days at the osteotomy sites providing enhancement of alveolar bone defect fill, coronal new bone formation, and new bone-to-implant contact	Dunn., *et al*,2005[28]
PFGF	rhPDDGFBB	Local	Grafts	In vivo (Humans)	Demonstrate contrasting inducible expression patterns of PDGF-AB, VEGF, and ICTP during periodontal wound healing	Cooke., *et al*, 2006[29]
PDGF	PDGF-B gene	Adenovirus	Collagen matrix	Animal model	AdPDGF-B delivered in a collagen matrix exhibits acceptable safety profiles for possible use in human clinical studies.	Po-Chun Chang., *et al*, 2009 [30]
HIFα	Hypoxia inducible factor α	Adenovirus	Gelatin sponge scaffold	In vivo (animal model)	Scaffolds loaded with AdHIF-1α were able to sustain the release of AdHIF-1α for up to 21 days and alveolar bone defects treated with scaffolds containing AdHIF-1α significantly induced new bone and new vessel formation in vivo.	Yang Zhang., *et al*, 2016[31]
BMP- 4	BMP - 4 cDNA	Electroporaton	Plasmid	In vitro and In vivo (animal model)	In vitro-transfected rat PDL cells exhibited production and secretion of the mature-form BMP-4. After in vivo electroporation of pCAGGS-BMP4, site-specific BMP-4 expression peaked on day 3, gradually decreased until day 14, and was absent by day 21.	Tsuchiya., *et al*,2017[32]

Table 2: *Growth factor mediated periodontal tissue regeneration using gene therapy.*

Bone sialoprotein

Bone sialoprotein (BSP), a glycoprotein containing arginine -glycine-aspartic acid sequence, mediates attachment of cells to extracellular matrix proteins. BSP is a major no collagenous protein in mineralizing connective tissues such as dentin, cementum and calcified cartilage tissues. As a member of the SIBLING (Small Integrin-Binding Ligand, N-linked Glycoprotein) gene family of glycoproteins, BSP is involved in regulating hydroxyapatite crystal formation in bones and teeth and has long been used as a marker gene for osteogenic differentiation. Cbfa1 is a "master gene" in osteogenesis and is involved in BSP gene expression which controls the cell differentiation during bone repair and regeneration. By the in vivo delivery of a BSP-gene into an osseous defect, it has been shown to regenerate periodontal alveolar bone [35].

NTF-hydrogel therapy

Neurotrophic factors (NTFs) are polypeptides primarily known to regulate the survival and differentiation of nerve cells during

the development of the peripheral and central nervous systems, thus effective for various neurodegenerative diseases. One among the widely studied NTFs include glial cell line-derived neurotrophic factor (belonging to transforming growth factor growth factor-β superfamily). NTFs have been immobilized in hydrogels, microspheres, electrospun nanofibers and combined systems, which serve as depots for sustained local release of protein NTF-hydrogel therapy is a novel, innovative method of regenerating bone. A number of pre-clinical trials have shown that injecting an NTF gene (non-viral non-immunogenic gene) together with a synthetic, non-immunogenic hydrogel made from hyaluronic acid into the site of bone degeneration or loss, induces neigh boring cells to produce new bone tissue. This therapy represents a significant improvement over conventional treatment [36].

Anti-apoptosis gene

The Bcl2 family of proteins are considered as gatekeepers to the apoptotic response and comprises proapoptotic and antiapoptotic members. It is used in regulation of tissue dynamics and is specifically thought to induce apoptosis in terminally differentiated cells, including inflammatory cells. Utilization of Bcl2 gene (anti apoptosis gene) with gene activated matrix technology (GAM) when introduced into a highly localized tissue injury site like those found in periodontal disease will improve the clinical outcome of the tissue injury by means of tissue repair and/or tissue regeneration [37].

DNA devices

"DNA devices," has been introduced for the first time using selective genetics for the fulfilment of mechanical targeting (one of the forms of gene transfer). This technology employs proprietary formulations incorporating intact DNA into polymers capable of being used as coatings on implantable devices such as periodontal implants creating a new class of site-specific gene therapy products. The use of these devices has shown to improve the biocompatibility between the implanted device and human tissue [33].

Gene therapy in implantology

Hall., *et al.* in 2007, evaluated local bone formation at titanium porous oxide implant surfaces adsorbed with recombinant human BMP 2 [38]. Lutz., *et al.* in 2008 evaluated rate of bone formation and osseointegration after topical gene delivery with a liposomal vector system carrying BMP 2cDNA in combination with collagen carrier in freshly created peri implant bone defects [39]. Luo., *et al.*in 2011 evaluated synergistic effect of BMP -2 and VEGF on the repair of bone defects around dental implants by conducting an invitro study with a scaffold loaded with adenoviruses expressing BMP 2 and VEGF [40]. Park., *et al.* in 2015 evaluated ex vivo BMP 2 gene delivery using canine periodontal ligament stem cells for regeneration of per-impactites defects [41].

Antimicrobial Gene Therapy to Control Disease Progression

Gene medicines or nucleic acid drugs can be categorized on the basis of their therapeutic relevance as gene inhibitors, gene vaccines and gene substitutes. Gene inhibitors (oligonucleotides, siRNA) are potent drugs that silence defective genes, by and large at mRNA level. Gene vaccines are antigens of specific pathogens encoding either the genes or RNA that activate cell-mediated and humoral immune response and production of antibodies. Gene substitutes are transcriptionally fully competent genes introduced into cells to reimburse deficiency of a specific protein or its insufficient protein production. The results promise an alternative method for developing novel DNA-based medicines for incurable disorders. Gene therapy is now an important part of pharmaceutical development as evidenced by number of clinical trials undertaken globally [42]. One way to enhance host defines mechanism against infection is by transfecting host cells with an antimicrobial peptide/protein- encoding gene. Researchers have shown that 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 defences [43].

Periodontal Vaccination

The salivary gland of a mouse when immunized using plasmid DNA encoding the Prohormones gingival is (*P gingival*) fimbrial gene produces fimbrial protein locally in the salivary gland tissue resulting in the sub sequent production of specific salivary immunoglobulins A, or Ig A and immunoglobulin G, or IgG, antibodies and serum IgG antibodies. This secreted IgA could neutralize *P. gingival* sand limit its ability to participate in plaque formation. Scientists have also demonstrated the efficacy of immunization with genetically engineered *Streptococci gordonii* vectors expressing *P. gingival* is fimbrial antigens vaccine against *P. gingival* is associated periodontitis in rats. The gene hemagglutinin which is an important virulence factor of *P. gingival* is has been identified, cloned and expressed in *Escherichia coli.* The recombinant hemagglutinin B when injected subcutaneously in. Fischer rats infected with *P. gingival* is showed serum IgG antibody and interleukin-2 (IL-2), IL-10, and the IL-4 production which gave protection against *P. gingival* is induced bone loss [44].

Genetic Approach to Biofilm Antibiotic Resistance

Researchers have found bacteria growing in biofilms become up to 1,000-fold more resistant to antibiotics as compared to a planktonic counterpart making them hard to control. Study by Mah., *et al*. identified gene ndv B encoding for glycosyltransferase required for the synthesis of periplasmic glucans in wild form of Pseudomonas aeruginosa RA14 strain. This remarkably protected them from the effects of antibiotics biocides, and disinfectant. Using a genetic approach. Researchers have isolated ndv B mutant of Pseudomonas aeruginosa still capable of forming biofilm but

lacking the characteristic of periplasmic glucans there by rendering microbial communities in biofilm more susceptible to conventional antibiotic therapy [45].

An *In vivo* Gene Transfer by Electroporation for Alveolar Remodelling

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 predict able alveolar bone remodelling [46].

Future Perspectives

Major advances have been made over the past decade in the reconstruction of complex periodontal and alveolar bone wounds that have resulted from disease or injury. Developments in scaffolding matrices for cell, protein, and gene delivery have demonstrated significant potential to provide "smart" biomaterials that can interact with the matrix, cells, and bioactive factors. The targeting of signalling molecules or growth factors (via proteins or genes) to periodontal has led to significant new knowledge generation using factors that promote cell replication, differentiation, matrix biosynthesis, and angiogenesis. Utilization of exosomes as carriers for gene therapy have also been studied. 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 A major challenge that has been less studied is the modulation of the exuberant host response to microbial contamination that plagues the periodontal wound microenvironment. For improvements in the outcomes in periodontal regenerative medicine, scientists must examine dual delivery of host modifiers or anti-infective agents to optimize the results of therapy. Further advancements in the field will continue to rely heavily on multidisciplinary approaches that combine engineering, dentistry, medicine, and infectious disease specialists in repairing the complex periodontal wound environment [46].

Conclusions

Gene therapy has a promising role in the field of periodontics, but it does encompass serious ethical issue to be dealt with. It is evident that gene therapy has emerged from its stage of infancy of mere theoretical and hypothetical quotations to factual scientific researches, which reveals potential hopes. There are still lots of research and details of mechanisms to be understood to include these practically in day to day treatment modalities.

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