REVIEW ARTICLE

Gene Therapy and CRISPR/Cas Technology in Dentistry: A Review

Arthi Marimuthu[1], Rathna P Subramani[2], Geeth Deepika[3], Azhagu A Alagianambi[4]

Received on: 23 December 2023; *Accepted on:* 02 February 2024; *Published on:* xx xx xxxx

Abstract

Background: The evolution of gene therapy, conceptualized in the 1960s, reached a pivotal moment in 1989–1990 with the approval of the first human clinical studies. Gene therapy, as defined by the US Food and Drug Administration (FDA), involves the administration of genetic material via nucleic acids, viruses, or genetically engineered microorganisms. This review explores the historical development and current landscape of gene therapy, focusing on its applications in dentistry.

Materials and methods: A comprehensive narrative literature search, utilizing PubMed, MEDLINE, Scopus, and Web of Science databases, was conducted. Keywords and MeSH terms related to gene therapy, CRISPR/Cas technology, and dentistry were employed. Inclusion criteria encompassed English-language publications from the last 10 years, specifically focusing on the gene therapy or CRISPR/Cas applications in dentistry. Data synthesis involved critical appraisal and extraction of relevant information.

Results: Gene transfer, a cornerstone of gene therapy, involves modifying defective genes through the injection of genetically modified vectors into target cells, either *in vivo* or *ex vivo*. Various methods, including physical (electroporation, microinjection) and chemical (calcium phosphate, liposome) approaches, facilitate gene modification. Dentistry applications range from addressing diseases such as squamous cell carcinoma and Sjogren's syndrome to enhancing bone regeneration, implants, and managing chronic pain.

Conclusion: The potential of gene therapy and CRISPR/Cas technology in dentistry is vast, offering innovative, personalized therapeutic interventions. However, challenges such as ethical considerations and the need for long-term efficacy studies must be addressed to ensure the transformative impact of these technologies on oral healthcare practices. The future promises a paradigm shift in dental care, with gene therapy leading the way towards more effective and targeted treatments.

Keywords: CRISPR/Cas technology, Dentistry genome editing, Gene therapy, Oral health.

Dental Journal of Advance Studies (2024): 10.5005/djas-11014-0033

Introduction

In the 1960s, pioneering work by Dr. Theodore Friedmann on genetically modified cells laid the groundwork for the emerging field of gene therapy. Between 1989 and 1990, first approved human clinical trials and potentially therapeutic studies were made.[1]

The US Food and Drug Administration defines gene therapy as a treatment technique that involves introducing therapeutic genetic material into a patient's cells, either by integrating it into their genome or through its expression.[2] This material can be delivered in various forms, including 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]

In this review, we shall discuss about the genes involved in gene therapy, types of gene transfer and various applications of gene therapy and CRISPR/Cas system in the field of dentistry.

Materials and Methods

This review article was conducted through a narrative literature search using the following databases: PubMed, MEDLINE, Scopus, and Web of Science. The search strategy utilized a combination of keywords and MeSH terms related to gene therapy, CRISPR/Cas technology, and their applications in dentistry. The search terms were combined using Boolean operators (AND, OR) to ensure a comprehensive but focused search. Specific terms included:

Gene therapy: gene transfer, gene delivery, vectors, adenoviral vectors, lentiviral vectors, non-viral vectors.

CRISPR/Cas technology: CRISPR, Cas9, gene editing, genome editing, targeted therapy.

Dentistry: oral health, dental disease, squamous cell carcinoma, Sjogren's syndrome, bone regeneration, implants, caries, chronic pain.

This review covered a 20-year span (2003–2023) and restricted its focus to English-language publications. Additional literature was discovered by examining the bibliographies of identified articles.

[1–4]Department of Conservative Dentistry and Endodontics, CSI College of Dental Sciences and Research, Madurai, Tamil Nadu, India

Corresponding Author: Rathna P Subramani, Department of Conservative Dentistry and Endodontics, CSI College of Dental Sciences and Research, Madurai, Tamil Nadu, India, Phone: +91 8015508427, e-mail: rathnapiriyanga14@gmail.com

How to cite this article: Marimuthu A, Subramani RP, Deepika G, *et al*. Gene Therapy and CRISPR/Cas Technology in Dentistry: A Review. Dent J Adv Stud 2024;x(x):xx–xx.

Source of support: Nil

Conflict of interest: None

Selection Criteria

Articles were Included if they Met the Following Criteria:

- Original research articles, review articles, or case reports.
- Focused on gene therapy or CRISPR/Cas technology applications in dentistry.
- Provided evidence-based information on the efficacy and safety of these technologies.
- Published in peer-reviewed journals.

Articles were Excluded if they

- Were not published in English.
- Focused on non-dental applications of gene therapy or CRISPR/Cas technology.
- Were not peer-reviewed.

Data Synthesis

The retrieved articles were critically appraised and the relevant information was extracted and synthesized (Table 1). The review narrative was structured to address the following key points:

- Current understanding of gene therapy and CRISPR/Cas technology in dentistry.
- Potential applications of these newer methods in the treatment of specific dental applications, (e.g., squamous cell carcinoma, Sjogren's syndrome, etc.).

Gene Transfer

The repair or replacement of the defective gene can be done by cleaving the human genetic coding for the therapeutic

Table 1: Summary of extracted data

Gene involved	*Protein involved*	*Vector involved*	*Type of approach*	*Result obtained*
SCCHN Immunomodulatory gene therapy: Lipid formulated murine interleukin 2 (mIL-2) and polymer formulated mIL12 gene[8]		Lipid	Nonviral	Significant antitumor effects due to increase activation of cytolytic T lymphocytes and natural killer cells
Gene addition therapy: Ad-p53 gene		Adenovirus vector expressing wild p53	Viral	Antitumor effect
Suicide gene therapy: HSV-TK gene	A viral enzyme which phosphorylates ganciclovir into a monophosphate form which is further phosphorylated into an active compound that terminates DNA synthesis	HSV-TK (herpes simplex virus-thymidine kinase)	Viral	Converts a non-toxic compound (prodrug) into an activated cytotoxic drug. This therapy targets the actively dividing tumor cell
ASO therapy and RNA interference:				The antisense RNA inhibit the activity of severe oncogenes. The phenotype of the tumor cell which depends on the expression of the particular oncogene is altered, hence the growth of the tumor is abrogated
Salivary gland gene transfer: Human aquaporin-1 gene (hAQP1)	Water channel protein	AAV2 vector	Viral	It increases the salivary flow from radiation damaged glands
Histatin-3 cDNA transfer: Histatins	Histidine rich cationic peptides		Nonviral	It has a natural salivary antifungal activity that acts against both azole activity and azole resists candidiasis in oral cavity.
CTLA4IgG in secondary Sjogren's syndrome: CTLA4IgG gene	Recombinant fusion protein CTLA4IgG consists of extracellular portion of murine CTLA-4 and the Fc portion of human IgG			Anti-inflammatory properties hence used to treat RA.
Bone repair: Ad-BMP7 cDNA Dental implant: titanium dental implant fixture with Ad/BMP7		Adenovirus, Cytomegalovirus, gingival or dermal fibroblasts (*ex vivo*) Adenoviral vector with a collagen matrix	Viral and nonviral	• Robust osteogenic response • Mature lamellar bone formation • Immobilize the transgene at the implant defect site delivers PDGF gene to tooth supporting structures

(Contd...)

Table 1: *(Contd...)*

Gene involved	*Protein involved*	*Vector involved*	*Type of approach*	*Result obtained*
Gene activated matrix (GAM)		Plasmid DNA encoding BMP-4 and parathyroid hormone fragment mixed with type I collagen carrier	Direct transfer (*in vivo*)	BMP-4 and parathyroid hormone fragment mixed with type I collagen carrier
MSCs derived from nonhematopoietic portion of marrow		Adenovirus containing BMP 2, 4 and 7 cDNA under CMV promotor	Viral	Participate in osteogenesis where the supply of endogenous osteogenic precursors are limited
Osteogenic transcription factor RUNX2 (bone related product of clefa1)		Adenovirus	Viral (*in vitro*)	Bone and hypertrophic cartilage differentiation induces alkaline phosphatase and osteocalcin in C3H10T1/2 cells with the stimulation of extracellular matrix mineralization
Cbfa1 involved in BSP gene expression			*In vivo*	Cell differentiation during bone repair and regeneration of osseous defect in periodontal alveolar bone
Periodontal defect repair: • PDGF gene • Ad-PDGF-B gene		Adenovirus	Viral	• Stimulate gingival fibroblast, PDL and cementoblast, promote bone repair of periodontal defects • Accelerates gingival soft tissue wound healing
Combinatorial gene therapy: • Ad-BMP2 + Ad- BMP7 or Ad- BMP4 + Ad-BMP7 • Combined transduction of C2C12 cells with consistently active ALK2 and ALK 3/6		Adenovirus		• Synergistic stimulation of osteoblast differentiation • Activate BMP signaling and osteoblast differentiation to a greater extent. Co-expression of angiogenic and osteo inductive factor enhance bone formation
Tooth regeneration therapy: Anti-USAG1 antibody administration			*Ex vivo*	• Inhibits uterine sensitization-associated gene-1 (USAG-1) which further inhibit Wnt and BMP signals which are essential for tooth development • Enhanced BMP signaling leading to supernumerary teeth formation
Gene transfer to keratinocytes: Gene is inserted into the keratinocyte and it expresses the protein/enzyme that will correct the defect in the keratinocyte		Retroviruses	*Ex vivo*	• Secrete factors such as human growth hormone and factor IX (hemophilia) into the systemic circulation • Normalization of tissue architecture epidermal function for conditions such as ichthyosis and epidermolysis bullosa

(Contd...)

Table 1: *(Contd...)*

Gene involved	*Protein involved*	*Vector involved*	*Type of approach*	*Result obtained*
Reparative dentin formation: • Dentin morphogen GDF11 gene • Enamelysin, a tooth specific matrix metalloproteinase • Phex, a cell surface metalloprotease			*Ex vivo* (electroporation)	• Differentiate into odontoblast to form tubular dentin • Expressed at the onset of predentin secretion in odontoblasts and after mineralization in mature odontoblast • Regulates phosphate homeostasis, a mineralization is highly expressed in secretory odontoblast layer at the end of the adult dental pulp
For orthodontic tooth movement: Receptor activator of the nuclear factor kappa B (RANK) or receptor activator of nuclear factor κ-B ligand (RANKL)			Locally transferred	Accelerates orthodontic tooth movement resulting in reduced treatment time and also moves the ankylosed tooth
In pain control: Opioid genes like beta-endorphin		Herpes simplex virus	Viral mediated	• Directly target specific regions of neuraxis involved in pain transmission • Encoding opiate peptides to peripheral and central neurons can lead to antinociceptive effects
Immunization: *P. fimbrial* gene *HagB* gene		Plasmid DNA		• Secrete the secretory IgA which could neutralize *P. gingivalis* • Any secreted fimbrial protein in saliva binds to pellicle components and inhibit the attachment of *P. gingivalis* further to develop plaque • Periodontal vaccine development

protein and insert it into the genome of the vector(carrier).[4] This genetically modified vector (Table 2) is injected into target cell resulting in the release of the DNA that integrated with the chromosome.[4,5] This modification can be transferred to the next generation by germ line gene therapy or concise to the individual alone by somatic gene therapy.[4,6] The genetic material can be either directly introduced into the body (*in vivo*) or the gene is delivered into the target cell and re-introduced into the body (*ex vivo*)[4,7] Genes can be transferred into cells through either physical techniques (electroporation, microinjection, ballistic material) or chemical techniques (calcium phosphate, liposome, and protein complexes).[8,9]

Genetic Approach toward SCCHN

Strategies for gene therapy: There are several general strategies utilized in a gene therapy approach to cancer, including:

- **Tumor suppressor gene addition:** Introduce functional copies of missing or mutated genes to actively suppress tumor growth.[5]
- **Defective gene excision:** Utilize enzymatic or other techniques to eradicate harmful genes from cancer cells, preventing their pro-tumor protein production.
- **Anti-tumor gene silencing:** Employ antisense RNA molecules to bind and block the expression of specific genes that drive tumorigenesis.
- **Immunostimulatory gene therapy:** Enhance the body's immune surveillance by introducing genes that increase cancer cell visibility and recognition by the immune system.
- **"Suicide Gene" therapy:** Deliver genes encoding enzymes that convert harmless prodrugs into potent chemotherapeutic agents within tumor cells, achieving targeted cytotoxicity.[10]
- **Oncolytic viral therapy:** Utilize genetically engineered viruses that selectively replicate and lyse cancer cells, propagating viral spread and tumor destruction.
- **Chemo-protective gene delivery:** Introduce genes into healthy tissues that confer resistance to chemotherapy side effects, enabling higher dosage tolerance for improved tumor control.

Table 2: Vectors commonly used in gene therapy

Vectors[1–5]	*Advantages*	*Disadvantages*
Non-viral		
Electroporation	No replication risk; transfect dividing and quiescent cells; less immunogenicity	Limited transfection efficiency
DEAE-Dextran		
Calcium phosphate		
Liposomes		
Naked DNA	No replication risk	Moderate efficiency
Viruses		
Retroviruses	Random integrate into host genome; long-term genetic alterations	Non-specific cell targeting
Adenoviruses	Infect both dividing and quiescent cells; high transduction efficiency	Infect only dividing cells; low transduction efficiency
Adeno-associated viruses	High transduction efficiency; low immunogenicity	Transient expression; anti-adenoviral immunity can lessen effect
Herpes viruses	Express thymidine kinase; high gene transfer efficiency; low immunogenicity	Difficult to manufacture, low titer
Murine leukemia viruses	Non-pathogenic in humans	Toxicity related to lytic infection

- **Anti-angiogenesis gene therapy:** Deliver genes that inhibit the formation of new blood vessels (angiogenesis), effectively starving the tumor and limiting its growth and metastasis.

CRISPR-Cas System

The CRISPR-Cas9, an RNA-guided genome editing tool, revolutionizes oral healthcare by diagnosing and treating diverse pathologies. It identifies disease-causing organisms or faulty genes and manipulates the genome for treatment, even holding the promise of pinpointing genes to counteract oral cancer's oncogenic drivers.[11–14]

Applications in Dentistry

Dental Plaque

Streptococcus Mutans

Researchers used CRISPR technology to target the production of extracellular polysaccharides (EPS), major contributors to dental plaque biofilm formation. By cloning CRISPR arrays onto plasmids and introducing them into UA159 bacteria, they successfully engineered mutants with significantly reduced EPS synthesis, leading to biofilm breakdown.[12,15]

Oral Squamous Cell Carcinoma (OSCC)

CRISPR technology exhibits promising applications in both identifying genes associated with oral cancer pathobiology and directly treating the disease through gene knockout approaches. Studies have demonstrated immunohistochemical expression of p75 neurotrophin receptor (p75NTR) in oral leukoplakia and human tongue squamous cell carcinoma (SCC) via CRISPR/Cas9 technology. Furthermore, deletion of p75NTR within SCC9 cells was shown to suppress their tumor-promoting properties.[15]

Salivary Dysfunction

The CRISPR/Cas9 system has demonstrated potential for treating salivary dysfunction associated with primary Sjögren's syndrome. Researchers utilized this technology to enhance AQP1 gene expression through precise engineering of a guide RNA sequence and a homology-directed repair template containing the cytomegalovirus (CMV) promoter. This approach led to the successful development of mesenchymal stem cell (MSC)-derived therapy for the disease.[15]

Tooth and Palate Development

The CRISPR/Cas9-mediated investigations unraveled the functional significance of the Msx1 gene's C-terminal domain for craniofacial development, specifically in tooth and palate morphogenesis.[15]

Temporomandibular Disorder

The CRISPR/Cas9 system holds promise for the treatment of temporomandibular disorders (TMD) through targeted gene editing. This approach can potentially activate epigenetic markers within the pain pathway, leading to altered signal transmission and ultimately pain relief. [15]

In Pedodontics

The CRISPR technology offers a promising avenue for targeted correction of orofacial anomalies with a genetic basis. This powerful tool allows for precise identification and modification of the relevant DNA sequence, potentially leading to restoration of normal development and function. Table 1 shows summary of extracted data.[5,7,16–27]

Limitation of CRISPR/Cas 9 Technology

- Serious immune storm occurs in patients with naturally present Cas 9 antibodies.
- "Genetic drive" – The alteration in gene can be transferred to next generation.
- This technique is very expensive.

Difficulties in Gene Transfer

Therapeutic gene delivery presents significant challenges, even for single-gene disorders. This complexity is amplified in multigene

disorders, rendering efficient treatment through gene therapy difficult. Viral vectors, a common delivery system, raise concerns due to potential toxicities, immune reactions, and even disease induction. Pre-existing or acquired immune responses further diminish gene therapy efficacy. Additionally, the rapid turnover of tumor cells presents a hurdle for achieving long-term therapeutic benefits.

Multiple rounds of gene transfer or stable integration of therapeutic genes into host DNA offer potential solutions for prolonged effects, but these approaches raise concerns about possible undesirable side effects and unpredictable genomic modifications.[7,26]

Conclusion

The field of gene therapy, particularly with the advancements brought about by the CRISPR/Cas system, holds significant promise for revolutionizing dentistry. Our review, conducted through a comprehensive literature search, highlighted the current understanding of gene therapy and CRISPR/Cas technology in dentistry. The applications span a wide range, from addressing dental diseases like squamous cell carcinoma, Sjogren's syndrome, and caries to enhancing bone regeneration, implant therapies, and managing chronic pain. The potential applications of CRISPR/Cas in dentistry are particularly noteworthy, with its ability to diagnose and treat oral pathologies by identifying causative organisms or faulty genes and manipulating gene expression.

References

1. Cotrim AP, Baum BJ. Gene therapy: Some history, applications, problems, and prospects. Toxicol pathol 2008;36(1):97–103. DOI: 10.1177/0192623307309925.
2. Prabhakar AR, Paul JB, Basappa N. Gene therapy and its implications in dentistry. Int J Clin Pediatr Dent 2011;4(2):85–92. DOI: 10.5005/jp-journals-10005-1088.
3. Goncalves GAR, de Melo Alves Paiva R. Gene therapy: Advances, challenges and perspectives. Einstein (Sao Paulo) 2017;15(3):369–375. DOI: 10.1590/S1679-45082017RB4024.
4. Siddique N, Raza H, Ahmed S, et al. Gene therapy: A paradigm shift in dentistry. Genes 2016;7(11):98. DOI: 10.3390/genes7110098.
5. Baum BJ, Kok M, Tran SM, et al. The impact of gene therapy on dentistry: A revisiting after six years. J Am Dent Assoc 2002;133(1):35–44. DOI: 10.14219/jada.archive.2002.0019.
6. Mammen B, Ramakrishnan T, Sudhakar U, et al. Principles of gene therapy. Indian J Dent Res 2007;18(4):196–200. DOI: 10.4103/0970-9290.35832.
7. Gupta K, Singh S, Garg KN. Gene therapy in dentistry: Tool of genetic engineering. Revisited. Arc Oral Biol 2014;60(3):439–446. DOI: 10.1016/j.archoralbio.2014.11.018.
8. Xi S, Grandis JR. Gene therapy for the treatment of oral squamous cell carcinoma. J Dent Res 2003;82(1):11–16. DOI: 10.1177/154405910308200104.
9. Kumar NA, Agrawal A, Sreedevi R. Novel therapy for oral cancer – Gene therapy: An Update. Br J Med Med Res 2015;9(3):1–8. DOI: 10.9734/BJMMR/2015/15317.
10. Denny WA. Prodrugs for gene-directed enzyme-prodrug therapy (suicide gene therapy). J Biomed Biotech 2003;2003(1):48–70. DOI: 10.1155/S1110724303209098.
11. Gholizadeh P, Aghazadeh M, Ghotaslou R, et al. CRISPR-cas system in the acquisition of virulence genes in dental-root canal and hospital-acquired isolates of Enterococcus faecalis. Virulence 2020;11(1):1257–1267. DOI: 10.1080/21505594.2020.1809329.
12. Gholizadeh P, Aghazadeh M, Ghotaslou R, et al. Role of CRISPR-Cas system on antibiotic resistance patterns of Enterococcus faecalis. Ann Clin Microbiol Antimicrob 2021;20(1):1–12. DOI: 10.1186/s12941-021-00455-6.
13. Kaushal K, Tyagi A, Karapurkar JK, et al. Genome-wide CRISPR/Cas9-based screening for deubiquitinase subfamily identifies ubiquitin-specific protease 11 as a novel regulator of osteogenic differentiation. Int J Mol Sci 2022;23(2):856. DOI: 10.3390/ijms23020856.
14. Gong T, Tang B, Zhou X, et al. Genome editing in Streptococcus mutans through self-targeting CRISPR arrays. Mol Oral Microbiol 2018;33(6):440–449. DOI: 10.1111/omi.12247.
15. Jangam DK, Talreja KM. Applications of crispr technology in dentistry: A review. Eur J Biomed Pharm Sci 2020;7(6):314–320. Available from: https://www.ejbps.com/ejbps/abstract_id/6901.
16. Baum BJ, Alevizos I, Chiorini JA, et al. Advances in salivary gland gene therapy – oral and systemic implications. Expert Opin Biol Ther 2015;15(10):1443–1454. DOI: 10.1517/14712598.2015.1064894.
17. Corden A, Handelman B, Yin H, et al. Neutralizing antibodies against adeno-associated viruses in Sjögren's patients: Implications for gene therapy. Gene Ther 2017;24(4):241–244. DOI: 10.1038/gt.2017.1.
18. Franceschi RT. Biological approaches to bone regeneration by gene therapy. J Dent Res 2005;84(12):1093–1103. DOI: 10.1177/154405910508401204.
19. Koh JT, Zhao Z, Wang Z, et al. Combinatorial gene therapy with BMP2/7 enhances cranial bone regeneration. J Dent Res 2008;87(9):845–849. DOI: 10.1177/154405910808700906.
20. Luk KDK, Chen Y, Cheung KMC, et al. Adeno-associated virus mediated bone morphogenetic protein-4 gene therapy for in vivo bone formation. Biochem Biophys Res Commun 2003;308(3):636–645. DOI: 10.1016/s0006-291x(03)01429-3.
21. Murashima-Suginami A, Kiso H, Tokita Y, et al. Anti-USAG-1 therapy for tooth regeneration through enhanced BMP signaling. Sci Adv 2021;7(7):eabf1798. DOI: 10.1126/sciadv.abf1798.
22. Nakashima M, Iohara K, Ishikawa M, et al. Stimulation of reparative dentin formation by ex vivo gene therapy using dental pulp stem cells electrotransfected with growth/differentiation factor 11 (Gdf11). Hum Gene Ther 2004;15(11):1045–1053. DOI: 10.1089/hum.2004.15.1045.
23. Iglesias-Linares A, Moreno-Fernandez AM, Yanez-Vico R, et al. The use of gene therapy vs. corticotomy surgery in accelerating orthodontic tooth movement. Orthod Craniofac Res 2011;14(3):138–148. DOI: 10.1111/j.1601-6343.2011.01519.x.
24. Kanzaki H, Chiba M, Arai K, et al. Local RANKL gene transfer to the periodontal tissue accelerates orthodontic tooth movement. Gene Ther 2006;13(8):678–685. DOI: 10.1038/sj.gt.3302707.
25. Finegold AA, Mannes AJ, Iadarola MJ. A paracrine paradigm for in vivo gene therapy in the central nervous system: Treatment of chronic pain. Hum Gene Ther 1999;10(7):1251–1257. DOI: 10.1089/10430349950018238.
26. Mahale S, Dani N, Ansari SS, et al. Gene therapy and its implications in periodontics. J Indian Soc Periodontol 2009;13(1):1–5. DOI: 10.4103/0972-124X.51886.
27. Dunn CA, Jin Q, Taba Jr M, et al. BMP gene delivery for alveolar bone engineering at dental implant defects. Mol Ther 2005;11(2):294–299. DOI: 10.1016/j.ymthe.2004.10.005.