REVIEW

Gene therapy in oral diseases – an insight

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ABSTRACT

The oral cavity is one of the most accessible areas for the clinical applications of gene therapy for various oral tissues. The idea of genetic engineering has become more exciting due to its advantages over other treatment modalities. The concept of transferring genes to tissues for clinical applications has been discussed for nearly half a century. With the development of the researches, genetic engineering has evolved with greater capability to determine etiopathogenesis, the processes, the disorders caused by gene alterations, the genes responsible and the actions in modulations of metabolic defects for the prevention and treatment of the pathologies. With the diversity of microorganisms and bacterial proliferation, it is difficult to control pathologies and homeostasis in the oral environment. Odontology has been employing technological resources in treatments through gene therapy and recombinant DNA to repair the genome, manipulating and substituting defective genes for healthy genes and providing a certain relief of the symptoms or the cure of oral diseases. This review is presented to highlight various applications of gene therapy in dentistry in the areas such as salivary gland disorders, chronic pain, DNA vaccines, bone repair, implantology, head and neck cancer, orthodontic therapy, periodontal repair and tooth regrowth.

KEYWORDS: Gene Therapy, Oral immune Diseases, Bone tissue engineering

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INTRODUCTION

Genes are considered the building blocks of life. Editing these to treat a disease at its very source or to modify the response to a host of illnesses is, therefore, an exciting scientific challenge. Gene therapy is a promising new field in molecular medicine. Harnessing its potential in dentistry calls for understanding the principle of gene therapy and its possible applications in the treatment of various conditions involving the maxillofacial region.

While scholarly studies on the hybridization of plants had been published first in 1761 by Joseph Kölreuter, research in the field began in earnest in 1900 with the publication of an analysis of Gregor Mendel's 1866 research paper by deVries, Correns and Von Tschermak-Seysenegg which reignited work on genetics. Identifying genes as the reason behind heritable traits has been a scholarly work strangely reminiscent of Mendeleev's quest for the atomic number. When Mendeleev built his periodic table of elements on the basis of atomic masses of elements, he was cognizant of a more elementary factor influencing the factors- the atomic number which was unfortunately not discovered in his times (Van Spronsen,1981). Similarly, research in genetics has precedence in Charles Darwin, mentioning 'infinitesimal variations' yet unable to pin down the underlying mechanism for the same. Bateson suggested 'discontinuous variations' while supporting Mendel's pea hybridization experiments and in 1905 coined the term 'Genetics'.

After that, a flurry of activity began in this new and exciting field and in 1963 Demerec proposed that research in genetics could be seen to have six major epochs in the 60 years of its existence until his time; beginning from the rediscovery of Mendel's work and recognition of its scientific merit followed by acceptance of the Drosophila as a model genetic system for experiments on chromosomes. The third major discovery was Muller's report that X-rays induced mutations in 1927 (Haynes,1998). This was succeeded by the discovery of bands in polytene chromosomes of Drosophila and position of the genetic locus in the same. Next microbes were identified for analyzing genetics and the discovery of the link between genes and enzymes. The sixth and final great shift was the discovery of DNA macromolecular structure by J.D. Watson and F.H.C. Crick in 1953 (Watson and Crick, 1953).

With the discovery of the molecular basis of genes, attempts began at modifying the genome of a living organism for both treatment or cure of diseases and, controversially also for Eugenics. Research in genetic engineering began in the 1990s and primarily focused on two types of gene targeting: Homologous recombination (HR) and Conditional targeting. HR could be used for treating disease in humans. Inducing double-strand breaks (DSBs) can increase the efficiency of HR. Nuclease enzymes, like Zinc Finger Nucleases (ZFNs), Transcription Activator-like Nucleases (TALENs) and engineered mega nucleases, can generate site-specific DSBs. This is a potent tool to edit genomes. In 2015, with the advent of CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats) system for gene editing, research became affordable and more dynamic than before (Bak Gomez-Ospina Porteus, 2018). Just 5 years later, in February 2020, CRISPR gene editing was done on three patients of cancer in the USA in the first such clinical trial (AFP, 2020).

BASIC MECHANISM OF GENE THERAPY

The main aim of genetic editing is to induce double-strand breaks and edit DNA base pairs such that a base pair sequence is generated which either promotes or suppresses the gene expression of choice. This is done with the help of virus or non-virus vectors (Kay, 2011). The three classes of viruses used for this purpose are Retroviruses, Adenoviruses and Adeno-Associated Viruses (AAV). Two approaches for gene therapy are available- in vivo and ex vivo. In the first one, the active agent is injected directly into the tissue

of interest in the patient. In the later, cells are removed from the patient and cultured and genetically engineered to code for the therapeutic molecule of choice and then reintroduced into the patient. The ex vivo procedure is time-consuming and expensive but shows a long-term therapeutic effect (several years). Additionally, the autologous cells used prevent the possibility of immune rejection and most importantly allow freedom to use any vector system, without risking direct exposure of the patient to it (Emery, 2011, Jasin and Haber, 2016).

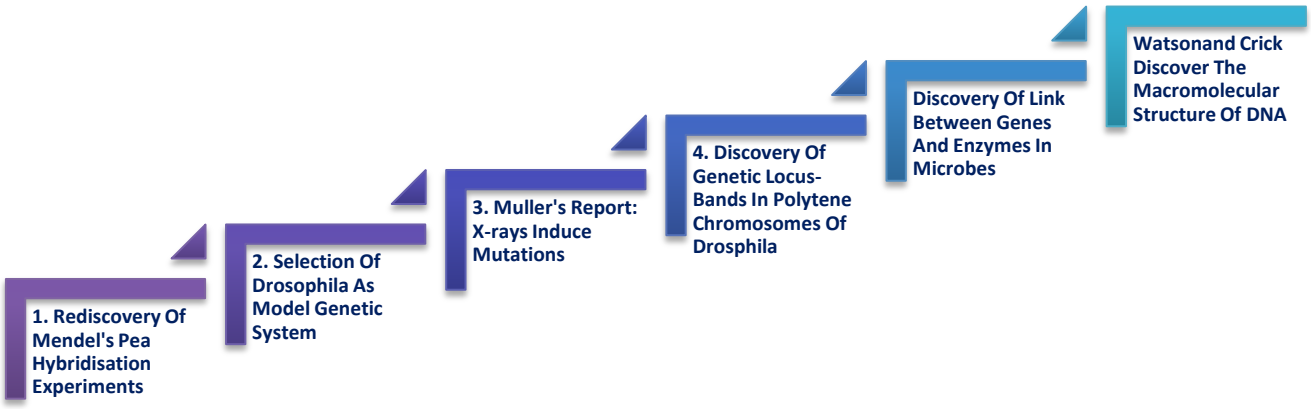

Fig 1: Milestones in Research on Genetics; the precursor to studies on gene therapy.

IMPACT IN DENTISTRY

In infectious diseases

Gene therapy aims at sustained stimulation of a targeted immune response or in vivo secretion of inhibitory factors by specifically altering the expression of gene or function of its products, to interfere with replication of infectious agent. Additionally, it can be used to prevent the spread of the infectious agent at the extracellular level. As of now, HIV infection is the one being studied extensively (US Gov NCT04378244, 2020). On 7 May 2020, a study using DeltaRex-G Gene Therapy for symptomatic COVID-19(CORONA) was listed at clinical trials gov. in the USA. Reportedly though, until 9 July 2020, it hasn't begun recruiting volunteers.

In autoimmune diseases

Overexpression of pro-inflammatory cytokines, like IL-2, TNF etc. is the hallmark of many autoimmune diseases.

1. Osteoarthritis: WHO reports that symptomatic osteoarthritis affects 9.6% men and 18% women (Kalladka, Quek, Heir, 2014). In India, osteoarthritis is the most common disease of joints affecting between 22% to 39% of the population surveyed. Temporomandibular joint osteoarthritis in about 8-16% patients is associated with symptoms. Gene therapy shows great promise in regenerative medicine because of the potential to restore the function of diseased or damaged tissue. Now here is its impact as profound as in

debilitating osteoarthritis. Proinflammatory cytokines notoriously disrupt stem cell therapy of this disease. But Brunger et al. showed that this disruption could be circumvented by using CRISPR-Cas9 engineering in stem cells which were notably inflammation resistant. In their murine model, on deleting the IL-1 receptor gene in homozygous clones, cartilage generated showed resistance to cytokine related tissue damage (Bruger, Zutshi, Willard, 2017).

2. Rheumatoid Arthritis: It has a 0.3-1.0% prevalence in developed countries (WHO, 2014). In India, the prevalence of rheumatoid arthritis is pegged at 0.28-0.7% (Handa, Rao, Lewis, 2016). In a study, 7.4% of patients with TMJ involvement showed joint to be affected and about 18.5% patients showed both muscular and joint involvement (Kurtoglu, Kurkcu, Sertdemir 2016). Evans et al. studied the intraarticular transfer of cDNA coding for IL-1 receptor antagonist(IL-1Ra), which had been shown to reduce disease in mouse models. Phase I clinical trial of 9 postmenopausal women with severe disease showed the possibility of transferring a potentially therapeutic gene into affected joints and obtaining a positive therapeutic benefit. Efficacy remains to be seen (Evans, Robbins, Ghivizzani et al. 2005). Local gene therapy is cumbersome in a polyarticular disease like RA. A variant approach of using genetically modified lymphocytes with the ability to selectively act on affected joints offers ease of use (Chernajovsky, Adama, Podhajcer et al. 1995).

3. Pemphigus vulgaris: Ellebrecht, Bhoj, Nace et al. (2016) used modified T-cells for the treatment of Pemphigus vulgaris in a murine model. The rationale behind the study was to identify and eliminate pathogenic autoreactive B-cell clones while preserving the remaining B cell compartment. The genetically modified T-cells were able to treat the disease in animal models, including a humanized mouse model. These T-cells could infiltrate epidermis even in a human xenograft model and showed a cytotoxic effect on cells expressing anti-DSG3 receptors with almost negligible toxic effects to other cells.

4. Sjögren's syndrome: Aquaporin 5 is found to be down-regulated and BMP6 overexpressed in patients of this disease. Aquaporin1 gene therapy was shown to not only reduce fluid movement in both salivary and lacrimal gland movement, it also reduced both systemic and local inflammation thereby correcting Sjögren's phenotype in mice (Lai, Lin, Cabrera-Perez 2016).

5. Type-I Diabetes: While diabetes in itself is not a disease of maxillofacial region, it affects the health of tissues in oral cavity most notably periodontium and affects the management of oral diseases. Gene therapy offers hope in the regeneration of damaged beta cells of islets of Langerhans in the pancreas. Adenovirus-mediated transfer of genes to induce neurogenic differentiation of beta cells and pancreatic and duodenal homeobox protein in mouse models has been shown to reverse symptoms of diabetes (Ferber, 2000). Kojima and his team (2003) showed that NeuroD with Betacellulin (islet growth factor) completely reverses diabetes in their mouse model.

In chronic pain

Chronic pain is one of the most challenging problems clinicians face in dentistry. When associated with erosion of cartilage in arthritic disease, pain can be severe and debilitating. Gene therapy for chronic pain targets pre pro-Encephalin A using the neurotropic HSV vector to localize therapeutic agent within the affected nerve. Chuang et al. (2004) using mouse model showed that both hyperalgesia and paw swelling were notably reduced on intramuscular injection of plasmid DNA coding for pro-opiomelanocortin. Pain due to ischemia in diabetes was similarly successfully ameliorated by targeting genes coding for kallikrein in the tissue.

In head and neck cancer

1. Primary tumor of HNSCC: In a clinical trial in 2009, twenty patients of un-resectable head and neck cancer who had failed conventional therapy were enrolled in gene therapy using an immune-based gene therapy strategy. Gene therapy product of HLA-B gene and beta2 microglobulin gene were directly injected into the tumor of patients. The rationale was to induce expression of class-I MHC on the cell surface of tumor cells to induce anti-tumor response by restoring antigen-presenting mechanism. Median survival was noted to be 54 weeks with apoptosis noted in responding tumors (Gleich 2000).
2. Radiation damage: The adenoviral vector delivering aquaporin 5 gene delivered directly into the ducts of irradiated rat submandibular glands showed near-complete recovery of salivary glands to pre-irradiation saliva output (Delporte, 2000).

In Tooth and Periodontal Regeneration

Sonoyama, Liu, Yamaza et al. (2006) showed functional tooth regeneration in a mini pig model with stem cells isolated from the root apical papilla of human teeth. They were able to generate a root-periodontal complex which could support a prosthetic crown. Tan, Zhao, Gong et al. (2009) showed successful production of bioactive basic Fibroblast growth factor-mediated by a recombinant plasmid which promoted periodontal regeneration in dogs.
In vivo transfer of platelet-derived growth factor-B gene stimulated periodontal tissue regeneration specifically of alveolar bone and cementum in a rat model (Jin, Anusasakthien, Webb et al., 2004). Stem cell-based tissue engineering involving dental pulp stem cells, periodontal ligament stem cells, stem cells from the dental apical papilla and stem cells derived from human exfoliated deciduous teeth dental follicle or epithelium or adipose tissue have been shown to help in regeneration of dentin-pulp, entire tooth or its root and even in the regeneration of periodontium (Hu, Liu, Wang, 2018).

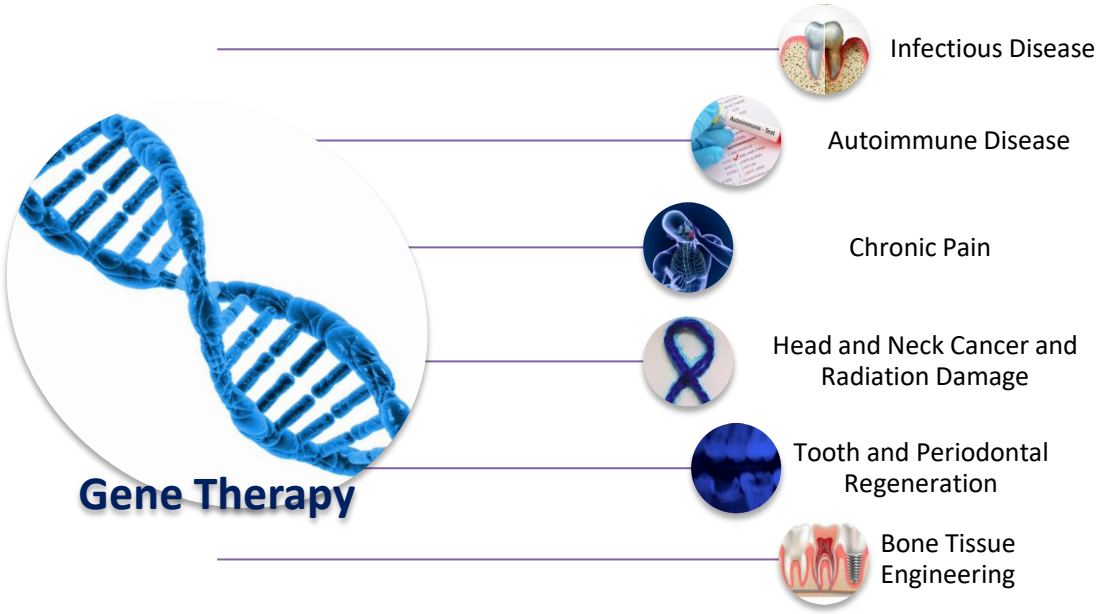

Fig 2: Potential Applications of Gene Therapy in Dentistry

Bone Tissue Engineering

1. Healing of bone fractures: Although epidemiologic data on pan Indian experience of jaw bone fractures is wanting, it is a common clinical experience to not only manage jaw fractures but also observe many patients

presenting with delayed union or non-union of fractured segments. Healing of bone is a complex orchestration of osteoconduction, osteoinduction, osteogenic signal responsiveness of cells, and an optimum vascular response. However, studies have shown that innate protective immune responses may also delay bone healing. In light of all of this, it has been of interest to study the role of gene therapy in bone healing.

Increased levels of terminally differentiated CD8+ effectors memory cells in peripheral blood, secrete interferon-gamma and tumor necrosis factor, thereby hindering differentiation of osteoblasts. It has been noted that the reduction of these two pro-inflammatory cytokines by local administration of aspirin markedly improves healing of fractured bone. An alternative pathway to achieve it is by using gene therapy delivered by viral or non-viral vectors locally or systemically. Osteogenesis and angiogenesis can be promoted with conventional anabolic factors for genes coding for Bone morphogenic protein or vascular endothelial growth factor. Recent studies also show a promising future for micro RNAs and novel genes like Hypoxia-Inducible Factor-1 α (HFI-1α) and Nell-1 (Lu, Chang, Lin et al., 2013)

2. Healing of dental implant defects: Courtney et al. in 2005 published their experience within vivo gene delivery of BMP-7 in the healing of titanium dental implants. Treatment of dental-implants with adenoviral vector-based BMP-7 gene or luciferase gene was done. Among the 44 Sprague-Dawley rats so studied those receiving adenoviral BMP-7 showed increased bone formation in alveolar bone defect and in osseointegration of the implant ((Dunn, Jin, Taba Jr et al., 2010).

Chang, Seol, Cirelli, et al. (2009) showed that the Platelet-derived Growth Factor enhanced implant osseointegration in jaws. Additionally, it stimulated healing in non-healing wounds like diabetic ulcers and periodontal lesions.

CONCLUSION

Present Concerns and Future Trends

Off-target effects are a rate-limiting factor in research on gene therapy. Viral vectors are fraught with unintended consequences because they may insert at sites other than those for intended therapy. This has the potential to disrupt essential genes and induce unwanted mutations (Birkeland, Ludwig, Spectre, Brenner, 2016). The famous case of Jesse Gelsinger is a grim reminder of the dangers the field of molecular medicine is fraught with. Using a viral vector for treating his inherited ornithine transcarbylamase deficiency, led to massive immune rejection and eventual death of the patient. Just three years later, two of the youngest patients being treated in a trial for severe combined immunodeficiency showed the integration of retroviral vector gene near LMO2 proto-oncogene promoter, leading to malignant T-cell proliferation (Hacien-Bey-Abina, von Kale, Schmidt, et al., 2003).

Since 1967, when MW Nirenberg first raised the question if the world was ready for the challenges of surgery of the genes (Nirenberg, 1967) until the approval of the first gene therapy product Glybera 45 years later, we have come a long way. Today gene therapy is a promising new line of treatment of disease. Extensive research is already underway to improve both the safety and efficacy of this novel treatment. As of November 2017, across 38 nations, there were about 2597 trials on gene therapy mainly focused on cancer and inheritable monogenic diseases (Gin, Amaya, Alexander, et al., 2017). It is highly likely that gene therapy will become a mainstream treatment modality in future by offering scientists a chance to edit out disease at its very beginning in the DNA.

The aim of reversion of disease by modifying the most upstream target, i.e. DNA, however, is a slippery slope. Until we have a minute knowledge of detailed molecular pathways in cell biology, we face a roadblock in fine-tuning genetic modification to minimize unwanted sequelae. As in the study quoted earlier in the text where CRISPR gene editing was done on three patients of cancer

in the USA, none of the patients showed any significant benefit. Safety concerns like generalized immunosuppression, deleterious off-target effects of transgenes and vectors and the fate of genetically modified cells in the human body have to be addressed before a translational shift from bench to bedside occurs (Chernajovsky, Gould, Podhajcer 2004).

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Conflict of interest

The authors have declared to have no conflict of interest or competing interest

Authors' contributions

SI designed the concept and manuscript writing, AC collected data and analyzed articles, RP edited the manuscript and proofread it.

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