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Gene Therapy: A Review

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ABSTRACT

The goal of gene-enhanced tissue engineering is to regenerate lost tissue by the local delivery of cells that have been genetically-enhanced to deliver physiologic levels of specific growth factors. The basis for this approach lies in the presence of a population of progenitor cells that can be induced, under the influence of these growth factors, to differentiate into the specific cells required for tissue regeneration, with guidance from local clues in the wound environment.

Key Words: Gene therapy, Growth factor, Tissue engineering, Regeneration

INTRODUCTION

From a tissue engineering approach, the oral cavity has significant advantages compared to other sites in the body, including easy access and observability. Potential applications for gene-based tissue engineering therapies in the oral and maxillofacial complex include the delivery of growth factors for periodontal regeneration, pulp capping/dentin regeneration, treatment of malignant neoplasms of the head and neck.

Potential applicability of any dental hard tissue regenerative protocol could include the regeneration of an entire missing tooth or the regeneration of specific component of an otherwise viable tooth (e.g. a decayed tooth with early pulpal involvement). The lack of any enamel forming cells in the enamel of fully developed erupted teeth precludes the potential for cell-based approach for enamel regeneration.

Following tooth eruption, the secretary activity of these cells is down-regulated, although they continue to produce secondary dentine at a low level. Pulpal tissue retains a limited potential to repair itself following various insults. These healing stages in the pulp resemble those of other hard tissues. Depending on a number of poorly defined factors, surviving post-mitotic Odontoblastic cells can secrete tertiary dentin, a process known as reactionary or reparative dentinogenesis, or, alternatively, perivascular progenitor cells in the pulp can be triggered to differentiate into Odontoblastic-like cells under the influence of specific growth factors.

GENE DELIVERY

The application of gene therapy to treat exposed pulp by delivering DNA, RNA, or antisense sequences alters gene expression within a target cell population in pulp tissue. The gene therapy manipulates cellular processes and responses. The transfected genes stimulate immune response, modify cellular information or developmental program, or produce a therapeutic protein with specific functions.[1]

1. Vectors for Gene Transfer

Gene transfer should achieve a stable expression of transgene in a target cell in an appropriate form without side effects, such as interaction with host genome, toxicity, carcinogenic

transformation and insertional mutagenesis. Therefore, development of safe and effective vectors has been one of the biggest challenges, which has been facilitated by understanding of the transduction process through studies of vector uptake, intracellular trafficking, and gene regulation.

An ideal vector should be as follows:

1) Produced and purified in large amounts and at high concentrations using a convenient and reproducible production procedure.
2) Targeting the most suitable cell for the disease.
3) Achieve stable, sustained gene expression either by integration of the vector DNA into the host DNA or maintenance as an episome.
4) Regulate expression of the therapeutic gene exquisitely.
5) Protected from degradation and sequestration.
6) Elicit no pathogenic or adverse effects, including immune responses
7) Control the effective distribution of the transfected gene, access to the target cell, and/or recognition by a cell-surface receptor followed by intracellular uptake and nuclear translocation.

The vectors used in gene therapy include genetic elements such as introns, polyadenylation sequences, and stabilizer for transcription, translation and secretion from the target cells to regulate the function of gene within the target cells.

2. Viral and Non-Viral Gene Therapy

Both viral vectors and non-viral vectors have been employed for gene transfer.[2,3]

Viral vectors are derived from viruses with either RNA or DNA genomes, such as Retrovirus, Lentivirus, Adenovirus, Adeno-associated virus, Herpes simplex virus. Integrating vectors (Retrovirus and Lentivirus) have the potential to express the gene product over long periods. On the other hand, non-integrating vectors (Adenovirus and Herpes simplex virus) that are maintained as episome can be useful for efficient gene transduction in nondividing cells.

Non-viral methods represent a simple and safer alternative to viral vectors. Simple quantitative production, low host immunogenicity and further recent advances in sustained gene expression and efficient and long-term gene expression are now making non-viral gene therapy more reality for human clinical medicine. Traditional non-viral vectors include various liposome based formulations and cationic basic proteins which facilitate DNA entry into the cell by electrostatic interactions.

Recent advances include ligands for receptor-mediated endocytosis, specific peptide sequences for DNA compaction and nuclear import signals. These designer domains permit entry into the nucleus and are biomimetic of the viral delivery of DNA. A variety of physical methods such as hydrodynamic pressure, particle bombardments, electroporation and sonoporation by ultrasound are used for gene delivery.

1. **Intravenous Infection at high hydrodynamic pressure:** Plasmid DNA can be delivered to tissues *in vivo* by intravenous infection at high hydrodynamic pressure. It is possible in a practical sense to use a blood-pressure cuff in the limbs to achieve high pressures 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.
2. **Electroporation:** Application of regulated electric pulses to deliver genes to cells is electroporation. Electroporation is routinely 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. Electroporation *in vivo*

was successfully used in muscle, skin, brain, and liver. Sustained long-term expression of the plasmid DNA has been shown especially in skeletal muscle and cardiac muscle. In several investigations, electroporation increased the gene expression over one hundred fold compared to infection of the plasmid DNA.

One of the limitations of electroporation is the tissue damage. Although electroporation is an efficient technique, it is an invasive method.

3. **Ultrasound:** The application of ultrasound leads to acoustic cavitation and produces cell membrane permeabilization promoting the delivery of plasmid DNA. Ultrasound contrast agents can improve cavitation. Microbubbles, Optison which are coated by albumin and contain octafluoro-propane gas, were found to be superior for cavitation using ultrasound. The use of a combination of ultrasound and electroporation 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.

Thus, the evolving methods in non-viral gene therapy holds great promise in future clinical applications minimizing certain risks associated with viral gene delivery.

Of the numerous growth factors normally expressed during primary odontogenesis, members of the transforming growth factor beta (TGF-beta) superfamily, including several members of the bone morphogenetic protein family (e.g. BMP-2, BMP-7), and insulin-like growth factor-1 (IGF-1) appear to play a key part in the induction of odontoblast-like cell differentiation from progenitor pulpal cells. A number of these growth factors are incorporated into the developing dentin matrix during initial tooth formation, forming a reservoir from which they can be released following dentin breakdown.

Bone morphogenetic proteins (BMPs) are multifunctional cytokines and widely distributed both in skeletal and non-skeletal tissues and have a major role in organogenesis. BMPs have actions beyond bone in neural, renal and cardiac development.1, 2 BMPs also play a role in differentiation of dentin3–8 in teeth. The recent progress in molecular developmental biology permits the delivery of BMPs by gene therapy using optimal delivery

A detailed description of the various BMP's used are as follows;[4-7]

***In Vivo* BMP Gene Therapy for Dentin Regeneration**

Protein therapy with some BMPs such as recombinant human BMP2, BMP4 and BMP7 has been established to induce reparative/regenerative dentin formation but the half life of BMP's is limiting and the high concentrations are required in addition to an optimal scaffold during the local application to the exposed pulp for reparative dentin formation.

Gene therapy of BMPs in dentin regeneration was developed to overcome the limitations of protein therapy. The main components for developing the efficient and safety gene delivery system in dentin regeneration are:

1) Higher transduction rates into targeted cells,
2) Sufficient long-term, time controlled and localized expression of transgene and
3) Safety ensured, minimal attendant immune responses and/or toxicity.

The use of both viral and non-viral vectors has been reported to express transgene and produce endogenous BMPs in dental pulp tissue. *In vivo* direct infection with adenovirus containing a full-length *Bmp7* gene induced only small amounts of poorly organized mineralizing masses in ferret dental pulp tissue of Reversible pulpitis experimentally produced by injection of bacterial lipopolysaccharide. The efficiency of gene transduction however is relatively low. Therefore

ultrasound-mediated gene delivery with Microbubbles was performed next. Non-invasiveness, non-toxic, safety, extremely localized transduction, simple operation are advantages for gene therapy with sonoporation in dentin regeneration. Ultrasound-mediated *Gdf11* plasmid gene transfer induced differentiation of pulp stem cells into odontoblasts *in vitro* and homogenous and complete reparative dentin *in vivo* in dogs without any tissue damage and cell necrosis unlike electroporation.

***Ex Vivo* BMP Gene Therapy for Dentin Regeneration**

In the clinical point of view when the dental pulp tissue is accidentally exposed during removing caries and/or inflammation is localized in coronal part, the ultrasound mediated *in vivo* gene therapy with *BMP* might be effective on dentin regeneration. Thus, an alternative approach is *ex vivo* gene therapy: pulp stem/progenitor cells are transduced with *BMP* gene to differentiate into odontoblasts *in vitro* and are transplanted into the amputated/exposed pulp tissue. The autogenous transplanted cells had the potential to integrate seamlessly into the exposed pulp tissue without disrupting normal function. The regenerative response of host pulp cells was observed after transplantation of *BMP*-transduced cells even under pulpitis, suggesting the efficacy of the *ex vivo* gene therapy in dentin regeneration for clinical endodontic treatment.

Another Growth/differentiation factor 11 (*Gdf11*),a novel member of the BMP/TGF family was found to be expressed in terminally differentiating odontoblasts, implying a role in the differentiation of dental pulp stem cells into odontoblasts. Therefore Gdf11 was investigated for prospects of gene transfer. *Gdf11* might stimulate odontoblast differentiation and reparative dentin formation *in vitro* and *in vivo*.

The results revealed the potential utility of *Gdf11* gene therapy in endodontic treatment in dentistry. Viral vectors and non-viral techniques can be used for gene transfer in gene therapy. The gene transfer of *Gdf11* to pulp cells was initiated to differentiate odontoblasts *in vitro* and reparative dentin formation *in vivo* by electroporation for the endodontic treatment of pulp tissue regeneration and dentin repair. During terminal differentiation of odontoblasts, the expression of *Gdf11* mRNA by *in situ* hybridization in mouse tooth germ11 and by RT-PCR in the primary dental pulp cell culture was demonstrated. The human recombinant GDF11 protein was used to explore the function of *Gdf11* in dental pulp cells. Differentiation of dentin-forming odontoblasts was monitored by three differentiation markers, dentin matrix protein1 (Dmp1), dentin sialoproteins (DSP) and osteocalcin. The expressions of these genes are known to increase during differentiation of pulp cells into odontoblast-like cells in pulp cell culture.

Gdf11 was found to be expressed during differentiation of pulp cells into odontoblasts.

PITFALLS

Despite the recent advances in gene therapy approaches to the dentin regeneration there remain several challenges.[2,3,7]

1. The source of the stem/progenitor cells has to be optimized.
2. The immunosuppressive properties.
3. The regulated coordination of the critical stages of neurogenesis and angiogenesis by gene therapy with BMPs and VEGFs bodes well for regeneration of dentin and dental pulp.
4. The potential dangers of using viruses including immunogenicity, cytotoxicity have been poignantly highlighted in recent clinical trials.
5. Insertional mutagenesis might be additional cause of concern, in which the ectopic chromosomal integration of viral DNA disrupts the expression of a tumor-suppressor gene or activates an

oncogene, leading to the malignant transformation of cells.

6. These formulations work optimally *in vitro*, they are not efficient *in vivo*.

CONCLUSION

Gene therapy has emerged as an active area of research for a range of biomedical and dental applications. Although we still consider current gene transfer methods to be fairly primitive, and associated with significant problems, gene therapy's acceptance as part of the routine clinical armamentarium seems very close.

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