Gene therapy of corneal diseases

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قال تعالى: 
إِوَّا خَلَقْنَا الإِوَّاسَانَ مِهْ وُطْفَةٍ أَمْشَاجٍ وَبْتَلِيوِ فَجَعَلْنَاهُ سَمِيعًا بَصِيرًا
Anatomy and physiology

- The cornea is the avascular tissue on the surface of the eye that is directly exposed to the external milieu.

- The cornea refracts light (with the lens), provides protection from microscopic pathogens (with the conjunctiva and tear film) and confers mechanical strength (with the sclera) thereby shielding the eye from physical injuries.

- Regulated hydration and the precise architecture of the cornea contribute to its unique transparency that is essential for transmittance of incident visible light through the lens to the retina thereby enabling vision.
Structure of the cornea

From anterior to posterior the cornea is composed of 5 layers: epithelium, Bowman's membrane, stroma, Descemet's membrane, and endothelium.
The immune response of the eye entails interactions between: which include macrophages, lymphocytes, eosinophils and antigen-presenting cells; and soluble mediators of the immune system, which encompass complement, cytokines and immunoglobulins.

Despite the availability of various components for an immune response, the ability of the eye to tolerate inflammation is very limited, since a conventional immune response, as observed for instance after the penetration of an antigen or a pathogen through the skin, might destroy the microanatomy of the visual axis needed for accurate vision. This explains the need for a special type of immunity in the eye, termed immune privilege.

(حصانة قوية)
Another aspect of immune privilege is the eye's ability to regulate the systemic immune response to eye-derived antigens through a mechanism termed anterior chamber-associated immune deviation. In this case the immune response induced by penetrating antigens is deficient in B cells that secrete complement-fixing antibodies and in T cells that mediate delayed hypersensitivity.

The result of ocular immune privilege is that immune defense takes place with no inflammation.

The cornea itself takes an active role in immune protection of the structure and function of the eye surface in that corneal epithelial cells and keratocytes secrete cytokines. Together with the lacrimal gland, tear film and the conjunctiva, the cornea constitutes the ocular mucosal-associated lymphoid tissue.
Gene Therapy

- Gene therapy to the cornea can potentially correct inherited and acquired diseases of the cornea.
- Factors that facilitate corneal gene delivery are the accessibility and transparency of the cornea, its stability ex vivo and the immune privilege of the eye.
- Initial corneal gene delivery studies characterized the relationship between intraocular modes of administration and location of reporter gene expression. The challenge of achieving effective topical gene transfer, presumably due to tear flow, blinking and low penetration of the vector through epithelial tight junctions left no alternative but invasive administration to the anterior chamber and corneal stroma. DNA vaccination, RNA interference and gene transfer of cytokines, growth factors and enzymes modulated the corneal microenvironment.
Positive results were obtained in:

- Corneal gene therapy initially emerged in 1994 when its potential in correcting acquired corneal inflammatory diseases was noted following successful transduction of corneal tissues using replication-deficient adenovirus.

- Inherited corneal diseases such as corneal endothelial dystrophies are natural candidates for corneal gene therapy. Local corneal gene delivery has the potential to achieve low and continuous concentrations of biologically active proteins thereby improving the efficacy and safety of the treatment.

- Minimal inflammation might impair vision.

- Prevention and treatment of corneal graft rejection, neovascularization, haze and herpetic stromal keratitis.
Vectors and methods of corneal gene therapy

Many of the prominent gene vectors, knock-down methods and physical techniques for gene therapy have been used in corneal gene delivery. We briefly describe these vectors and methods.

1. Viral vectors.
2. Non-viral vectors.
3. Physical methods.
Viral vectors

- Adenovirus. Adenoviral vectors, commonly used in gene therapy, have a broad range of host cells and can infect both dividing and non-dividing cells.
- Adeno-associated virus. Adeno-associated viruses have a small (4.7 kb) genome, are non-pathogenic and can infect both dividing and non-dividing cells.
- Retroviruses. Retroviral vectors, commonly used in gene therapy, integrate into the host genome and therefore offer long term transgene expression.
- Lentiviruses. Lentiviruses are a subtype of retroviruses that have the ability to infect both dividing and nondividing cells, which was one advantage of constructing gene vectors from them.
- Herpes simplex virus-1 (HSV-1). HSV-1 is strongly neuro-tropic and can replicate in epithelial cells. The genome size of HSV-1 is 152 kb and this enables HSV-1 vectors to accommodate relatively large and/or multiple transgenes.
Non-viral vectors

- Naked DNA. Naked plasmid DNA is a negatively charged, large macromolecule with a molecular weight of ≥2000 kDa.
- Polyethylenimines. Polyethylenimines, which appear as linear or branched isomers, are the most extensively used cationic polymers for gene delivery.
- Polyamidoamine dendrimers. Dendrimers are branched polymers with a well-defined spherical shape and very low polydispersity.
Physical methods

- Electroporation. Electroporation is the enhancement of DNA penetration through the cell membrane by creating transient and localized membrane pores using electric fields.

- Gene gun. The term, gene gun, refers to the ballistic delivery of DNA particles laden with heavy metal, usually gold, to cells using pressure and speed.

- Sonoporation. Sonoporation is the enhancement of DNA penetration through the cell membrane by creating transient and localized membrane pores using ultrasound.
Techniques for gene transfer to the cornea

- **Epithelium**: The accessibility of the corneal epithelium is a clear practical advantage for potential gene therapy applications.

- Furthermore, the central role of epithelial cells in the well-defined keratoepithelin dystrophies makes this layer an appealing target for genetic modification.

- Earliest attempts to genetically modify corneal epithelium used the physical method of bombardment with gold microparticles coated with DNA encoding green fluorescent protein (GFP) marker.

- This approach successfully delivered the marker gene to the target cells without corneal damage or ocular irritation.
In an alternative approach, the commonly used surgical technique of stromal hydration was used to inject a saline solution containing naked plasmid DNA directly into corneal stroma.

Long-term genetic modification of keratocytes may be a useful therapy for keratoconus and inherited stromal disorders.
Short-term expression of the marker gene in donor corneal endothelial cells was demonstrated in vivo, without significantly increased clinical or histopathological evidence of ocular inflammation. Moreover, stable corneal thickness measurements postgrafting indicated that endothelial function remained satisfactory, excluding significant cytopathic effects of the virus or the tissue manipulation.

Long-term genetic modification of corneal endothelial cells would be a significant therapeutic advance for endothelial disorders such as Fuchs’ dystrophy and inherited diseases.

Nonviral gene transfer vectors have several potential advantages over viral vectors. These avoid the potential for viral cytopathogenicity and induced immunogenicity, and are comparatively easy to produce.
Corneal diseases and manifestations of metabolic diseases treatable by gene therapy

- Corneal transplantation and graft rejection.
- Prevention and treatment of herpetic stromal keratitis.
- Corneal haze.
- Keratoconus.
The seminal works of gene delivery to the cornea appeared in 1994–1995 and concentrated on the transfer of reporter genes. George et al. were the first to study the prevention of graft rejection by ex vivo genetic manipulations of the cornea prior to transplantation.

In 1996, additional seminal works involving corneal gene therapy were conducted by Rouse et al. to treat HSK (hirpis simplex keratitis).

(1997) and Rakoczy et al. to correct corneal neovascularization.
Haze is the term used to depict corneal opacity following excimer laser procedures. Following photorefractive keratectomy most patients develop a mild haze that in the majority of cases resolves over time.

Corneal haze occurs during the wound healing process when stromal keratocytes transform to activated fibroblasts and subsequently proliferate and migrate to the anterior stromal compartment where they synthesize new collagen and extracellular matrix, thus causing opacity.

The current treatment for corneal haze is topical mitomycin C, which presumably induces activated keratocyte apoptosis.

Clearly, there is a need for additional therapies.

It should be noted that corneal opacity (or opacification) is the term used to describe cloudiness due to other reasons than excimer laser procedures, for instance corneal cauterization.
Corneal transplantation, the sole treatment for many corneal diseases that often lead to blindness, is the most commonly transplanted solid tissue with approximately 40,000 annual transplants in the United States. While corneal transplantation success rates of 75% survival through 5 years and 60% survival through 10 years are relatively high, there is no tendency of improvement with the passage of time, thereby emphasizing the need for additional clinical solutions.
Allograft rejection occurs when a cell-mediated immune response that involves T lymphocytes stimulates a cascade of events that lead to graft destruction.

Damage to the endothelium is crucial during this process.

Risk factors for immunologic allograft rejection, which is the most common reason for graft failure, include neovascularization, inflammation, glaucoma and a history of previous graft rejection.

Factors that facilitate successful corneal transplantation include the immune privilege of the tissue as well as the site, which obviates the necessity for systemic immunosuppression or human leukocyte antigen matching, and the fact that the donor cornea can be stored ex vivo for up to a month before transplantation. The latter is particularly appealing for gene therapy approaches as it enables modulation of the donor cornea prior to transplantation.
The fact that immunologic graft rejection is the major reason for transplant failure may explain the high prevalence of corneal gene therapy studies, which aim to inhibit the cascade of immunological events that lead to graft rejection.

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Recommendation

- Future for treat corneal diseases by gene therapy.
- Recommend to do most research for this subject.
- Therapy studies for control of neovascularization aim to reduce neovascularization.
- The potential of gene therapy by the administration of naked DNA to improve the treatment of corneal diseases.
- Corneal neovascularization using DNA encoding.
Thank you