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Review

# Gene delivery to cornea

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ABSTRACT

This paper reviews the strategies of *in vivo* gene delivery to the cornea. A number of studies have demonstrated the feasibility of targeted delivery of oligonucleotides, small interfering RNA (siRNA), plasmid DNA, and viral vectors to the corneal cells *in vivo*, specifically stromal keratocytes and corneal epithelial cells, via intrastromal injection, iontophoresis, electroporation, and gene gun. Intrastromal injection of plasmid DNA and adenovirus each can result in efficient transgene expression to stromal keratocytes. The introduction of foreign genes into intact corneal epithelium specifically requires more invasive procedures such as gene gun to disrupt the tight junction barrier and/or cell membranes. The combination of iontophoresis and electroporation was found to be effective in delivering siRNA but not plasmid DNA into the corneal epithelium. Nanocarriers such as polymeric micelles are promising methods of corneal gene delivery.



**Contents**



## 1. Introduction

The transparent and avascular cornea is an ideal tissue for examining the efficacy of gene therapy of congenital and/or acquired diseases. Since the first attempt of corneal gene therapy reported in 1994 [27], gene therapy has been explored for many corneal diseases such as allograft rejection, herpes simplex keratitis, corneal neovascularization, corneal haze, and corneal dystrophies [23,28,20]. Current gene delivery methods can be divided into viral and non-viral carrier systems. Despite high expression efficiency of viral vectors, inherent immunogenicity and toxicity limit their uses in human eyes. Non-viral vectors can overcome

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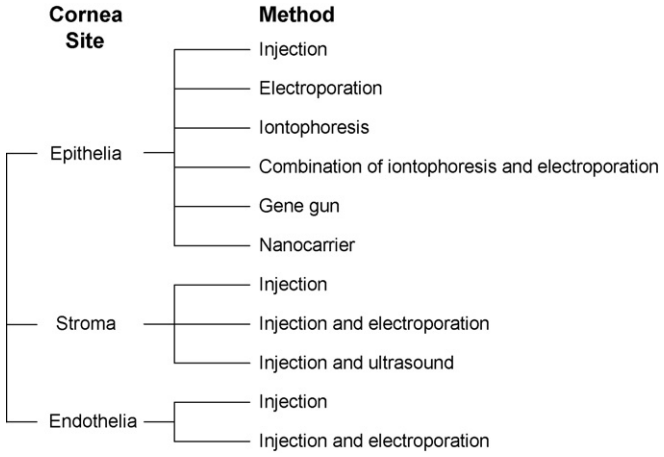


**Fig. 1.** Methods of gene delivery targeting the epithelia, stroma, and endothelia of the cornea reviewed in this paper.

the immunogenicity and toxicity problems but their expression efficiency is relatively low. Physical and chemical enhancement methods have been investigated to increase gene delivery to corneal cells. However, successful delivery of genes into the target corneal cells and the retention of genes in the cells remain challenging. Readers are encouraged to go to a number of recently published reviews on the topic of corneal gene therapy [23,28]. The focus of the present review is to provide an overview on the strategies of gene delivery to the cornea *in vivo* and particularly recent advances in gene delivery methods (Fig. 1).

## 2. Anatomy of the cornea

The cornea primarily consists of a stratified epithelium, a thick collagenous stroma, and a monolayer of endothelium [23]. The thickness of the human cornea varies from 0.5 mm at the center to 0.7 mm at the periphery. The tear film of 4–9 μm covers the epithelium. The corneal epithelium consists of approximately 6–7 layers of stratified epithelial cells that migrate from the inner layer to the outer surface in about 2 weeks. Tight junctions exist between the epithelial cells, forming the major barrier to topical drug delivery. The stroma is mainly composed of an extracellular matrix and the predominant stromal cells are the keratocytes. The thick stroma (>90% of the corneal thickness in human) plays an important role in maintaining corneal transparency. The endothelium with a thickness of 5 μm is a non-regenerated monolayer cells, forming a leaky barrier, and regulates the hydration of the stroma.

## 3. Gene delivery to the corneal epithelium

Although the outermost corneal epithelium is the most accessible, e.g., to the administration of eye drops, the tight junctions in the epithelium create a major barrier to topical gene delivery [14]. For the delivery of hydrophilic molecules, the paracellular route of the epithelial layer is the predominant pathway and the permeability is a function of molecular size. It is generally accepted that macromolecules such as genes cannot be effectively delivered into the corneal epithelium by passive diffusion mechanism using eye drops in the absence of permeation enhancers. Even topical applications of viral vectors failed to deliver and express transgenes in the epithelial layer without the removal of tear film [41] or/and scarification of the cornea [37]. Various enhancers capable of opening the tight junctions in mucosal membranes such as ethylenediamine tetraacetic acid (EDTA) and cytochalasin B have been tested in corneal epithelial cells [25]. For example, a novel cell-penetrating peptide for ocular delivery was found to enter the corneal epithelium after topical application in mice and was suggested to have potential for gene delivery [19]. Excimer laser ablation procedure was used to ablate the basal epithelial rabbit eyes before topical application of viral vectors expressing green fluorescent protein (GFP), which allowed transduction of genes into the basal epithelial cells and the stromal fibroblasts [26]. Nanocarriers such as polymeric micelles [40] and nanoparticles [9] were also investigated to examine the efficacy of corneal gene delivery. This section provides a summary of common injection and physical enhancement methods reported in the literature that allow and/or enhance gene delivery in the epithelial layer.

### 3.1. Injection

Intrastromal injection has been used to deliver naked DNA plasmids into the cornea for gene expression in the corneal epithelium of mice [38,1]. The expression of *Lac*Z reporter gene driven by cytomegalovirus (CMV) promoter for the protein β-galactosidase (β-gal) occurred as early as 1 h after intrastromal injection and lasted for 10 days [38]. It is believed that the rapid gene expression was related to the ability of the injected DNA plasmids to enter the epithelial cells by both endocytosis and intrastromal pressure effect due to the volume change from the injection of isotonic buffer. When plasmids that express small interfering RNA (siRNA) against vascular endothelial growth factor (VEGF) were injected into the corneal stroma of mouse at 8 h before alkaline-induced corneal trauma or 1 week after the injury, expression of VEGF was effectively suppressed compared to the control group [35]. Intrastromal injection was suggested to be suitable mainly for treating acute corneal diseases [38] because the durations of gene retention and expression in the cornea after intrastromal injection were relatively short irrespective of the rapid expression.

### 3.2. Electroporation

Electroporation uses electric pulses to create transient pores in a cell membrane [13]. The permeant is transported through these pores mainly by diffusion with some contributions from electrophoresis and electroosmosis. The pores would then reseal over time after the termination of the electric field [5]. Electroporation has been widely used in the transfection of cells *in vitro*, but *in vivo* electroporation studies on gene delivery to the cornea are limited. A previous study has investigated the delivery of cyanine 3 (Cy3) labeled glyceraldehydes-3-phosphate dehydrogenase (GAPDH) siRNA (MW ~ 13 kDa) to the epithelium of mice with electroporation of the siRNA solution on the eye using a custom-made applicator [15]. However, the use of electroporation alone to deliver siRNA into the cornea epithelium was considered not very effective. For the delivery of macromolecules greater than 1 kb pairs such as plasmid DNA into the cornea, plasmid delivery can be significantly enhanced when the DNA plasmid is first injected into eyes and electric pulses are subsequently applied to the eyes. Application of electric pulses to mouse eyes to deliver plasmids expressing luciferase or GFP after intrastromal or subconjunctival injection of the plasmids resulted in gene expression over the entire surface of the cornea in both epithelial and stromal layers [7]. The amount of protein expressed was about 1000 fold higher than that with the injection of DNA alone. At optimal field strength of 200 V/cm, no trauma, corneal edema, or inflammation was observed. Electroporation parameters such as electric field, pulse number, pulse length, and pulse interval are critical for effective gene delivery without causing damages to the cornea.