Ocular Gene Delivery

Subjects: Genetics & Heredity

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The eye is at the forefront of developing therapies for genetic diseases. With the FDA approval of the first gene-therapy drug for a form of congenital blindness, numerous studies have been initiated to develop gene therapies for other forms of eye diseases. These examinations have revealed new information about the benefits as well as restrictions to using drug-delivery routes to the different parts of the eye. In this entry, authors will discuss the ocular delivery landscape that is currently being investigated and provide insights into their advantages and disadvantages. Efficient delivery routes and vehicle are crucial for an effective, safer, and longer-lasting therapy.

retina eye gene therapy

1. Introduction

The complexity of eyes has perplexed scientists of the likes of Charles Darwin. The eye is considered one of the greatest leaps in evolution. Fossil evidence has revealed that eyes appeared ~500 million years ago and became an indispensable tool for survival [1].

The human eye is a camera-type sense organ that allows external visual cues to be transmitted to the brain. The light enters through the anterior chamber of the eye and passes through the cornea, aqueous humor, and the lens before entering the vitreous humor and traversing the inner retina to reach the retina in the posterior compartment. Here, the light signal is converted into an electrical impulse that communicates with the inner retinal neurons and eventually transports to the optic nerve. The signal is then sent to the processing centers in the central nervous system [2].

The vertebrate retina is a light-sensitive tissue containing five major types of neurons (photoreceptors, bipolar cells, amacrine cells, horizontal cells, and retinal ganglion cells) and three types of glial cells (Muller glia, microglia, and astroglia) organized in three distinct layers of cell bodies separated by two synaptic layers. The photoreceptors (rods and cones) account for >70% of the cell types in the retina and are the first responders to light. They contain the photopigment opsin that isomerizes in response to light and generates action potential. The rods are sensitive to lower-intensity light and help us see in starlight (at night). Cones, on the other hand, respond to brighter light and help us see during the day. Commensurately, we depend upon our cones for our day-to-day activities. The importance of cones in maintaining our quality of life is also exemplified by the presence of a cone-rich and rodless central area in the primate retina called the fovea. This structure is part of the macula, which contains the highest density of rods and cones in the central region [2].

Given the importance of visual input for human survival, vision disabilities are one of the top ten disabilities in humans. According to the Centers for Disease Control and Prevention, >3 million people in the United States have vision impairment. By 2050, this number is expected to double to ~6 million people (https://www.cdc.gov/visionhealth/risk/burden.htm ; accessed on 6 July 2021). Although the most prevalent eye disorders include complex genetic diseases such as age-related macular degeneration, diabetic retinopathy, cataracts, and glaucoma, the rare forms of inherited retinal degenerations (IRDs) have presented unique challenges in management and treatment.

2. Gene Therapy

The subretinal injection is an invasive surgical procedure in which the therapeutics are delivered between the photoreceptors and the RPE [3] (Figure 1). It requires an operating room, is usually performed under general anesthesia, and carries the risk of retinal tears, detachments, and macular holes. Intravitreal injections (IVIs) on the other hand, are relatively safer and can be performed in the doctor's office [3]. It is currently used clinically for injecting anti-angiogenic agents for age-related macular degeneration and diabetic retinopathy. Thus, IVI is a preferable procedure for ocular injections.

Suprachoroidal injections are a recent breakthrough in the retinal gene-delivery landscape [4] (Figure 1). These are less invasive than subretinal injection and involve accessing the retina by injecting into the space between the choroid (overlaying the RPE) and the sclera [5]. This method has been successful in large-animal models and was demonstrated to be a safer approach in a phase 3 clinical trial to treat uveitis with macular edema [6]. Some disadvantages of this method include the use of specialized needles, inaccessibility in smaller animals and difficulty of the AAV vectors to traverse the choroid layer to reach the RPE and the photoreceptors. Nonetheless, the suprachoroidal delivery route provides a unique opportunity to perform less invasive surgeries for retinal gene delivery.

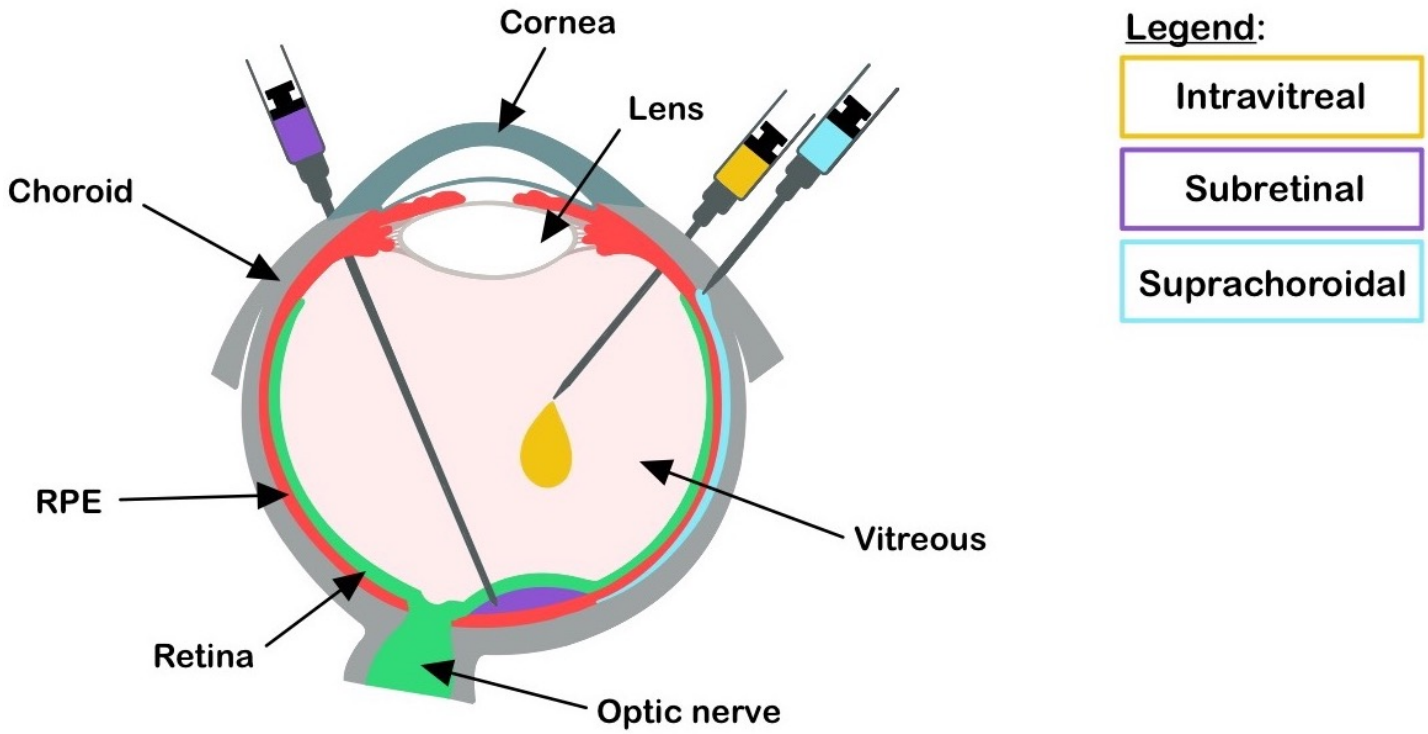

Figure 1. Drug delivery routes. Ocular delivery routes at the indicated locations are depicted. RPE: retinal pigment epithelium.

2.1. Vectors for Gene Delivery

Vectors for gene therapy are vehicles that carry the gene of interest to the host cells. There are two major subclasses of vectors: nonviral and viral vectors (Figure 2).

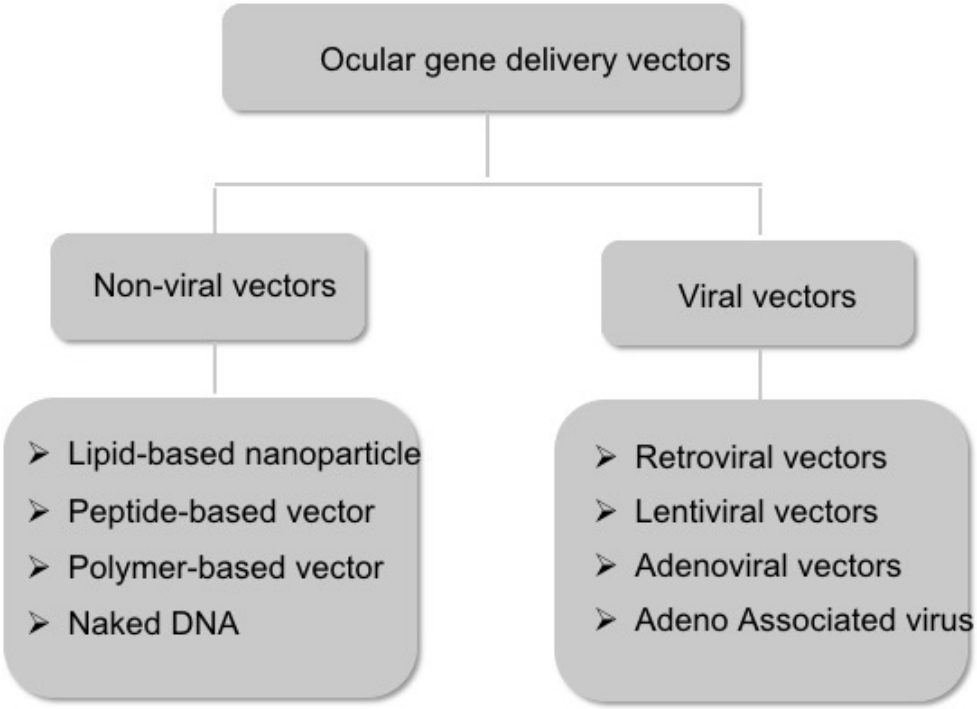

Figure 2. Schematic representation of the major subtypes of the ocular gene delivery vectors.

2.1.1. Non-Viral Vectors

Non-viral gene delivery involves delivering a circular, double-stranded plasmid DNA encoding the gene of interest directly into the target cell type [7]. The nanoparticles (NPs) that are used for such delivery can accommodate large sizes of the plasmid DNA, are relatively safer and less immunogenic, maintain long-term protein expression, and carry no risk of insertional mutagenesis [8]. The other advantage associated with NPs is low production cost [9]. The three major criteria for selecting or synthesizing the optimal NP are: cellular uptake, NP composition, and plasmid design.

NPs are usually engulfed by the target cells via phagocytosis or endocytosis. In the eye, the RPE shows both phagocytic and endocytic capacities [10]. In addition to phagocytosing photoreceptor outer segments in vivo, RPE can take up large naked DNA or DNA NPs by phagocytosis [11]. Photoreceptors, on the other hand, are predominantly endocytic [12]. The NPs that undergo clathrin-mediated endocytosis can end up in endosomes, which mature to endolysosomes and undergo degradation. However, caveolin-mediated endocytosis encapsulates the NPs in caveolae vesicles that enter early endosomes or endoplasmic reticulum and escape degradation [13].

NP uptake by target cells also depends upon their composition and net charge. The major types of NPs that are being tested for ocular delivery are as follows:

a. Lipid-based NPs: The naked DNA is further packaged into synthetic compounds to improve DNA transfection efficiency and stability. Lipid-based NPs are composed of a cationic lipid (with a positive charge, a hydrophilic head, and a hydrophobic tail such as DOTAP) and a helper lipid (such as cholesterol) [14]. The positively charged head binds to a negatively charged phosphate group in the DNA to form a compact structure of lipoplexes [15]. When DNA is enclosed in lipoplexes, it is protected from degradation. The lipid-DNA complex enters the cell by

endocytosis. Several liposome formulations have been tested for DNA delivery to ocular tissues using intravitreal and subretinal routes [16]. Although ganglion cells and RPE were transfected with high efficiency, the photoreceptors did not exhibit successful DNA transfection [17]. However, it was not until recently that non-viral gene transfer through liposomes could achieve tissue or cell type-specific sustained expression. Liposomes-protamine-DNA (LPD), in combination with a nuclear localization signal (NLS) peptide and a transactivator of transcription (TAT) peptide, make cell-specific and efficient gene delivery with sustained gene expression [18]. Lipid-based compaction of DNA using multifunctional lipids, such as (1-aminoethyl) N-oleicylcysteinyl-1-amino-ethyl) propionamide (ECO), results in a smart escape from endosomal lysis in the cytoplasm and trafficking to the nucleus. ECO nano lipids consist of an ethylenediamine (E) head group, two cysteine (C) functional linkers, and two oleoyl (O) lipophilic tails integrated into DNA for efficient gene delivery to the retina. Sun et al. showed that ECO can efficiently deliver the RPE65 gene into the retinal pigmented epithelium in the Leber congenital amaurosis (LCA) model of $Rpe65^{-/-}$ mice to restore vision [19].

b. Peptide-based NPs: Peptides are used in compaction of DNA for gene delivery and can be considered the best non-viral gene therapy modality. A cationic peptide, enriched in lysine/arginine, makes a tight, compact structure with the DNA. This has an advantage over other non-viral vectors, as it targets specific cell receptors, disrupts the endosomal membrane, and delivers the cargo to the nucleus. It induces minimal immune response and has the capability to be delivered in higher doses [20].

c. Polymer-based NPs: Along with peptide- and phospholipid-based vectors, polymer-based vectors are also used for the compaction of DNA. In this case, the cationic polymer is mixed with DNA to form nanosized polyplexes. Some examples of polymer-based vectors are polyethylene (PEI), dendrimers, and polyphosphoesters. Polymeric nanoparticles are now gaining importance in gene delivery due to their versatility of structural confirmation, biodegradability, and easy synthesis. Some outstanding synthetic polymers include Poly (L-ornithine), polyethyleneimine, and poly(amidoamine) dendrimers. Some natural polymers are chitosan, dextran, and gelatin [21]. Outstanding studies from Naash and colleagues showed that rod-shaped CK30-PEG (polyethylene glycol)-compacted NPs effectively transfected both RPE and photoreceptors and showed efficacious gene therapy of *Rpe65*$^{-/-}$ (retinal pigment epithelium 65; model of LCA) and *Abca4*$^{-/-}$ (Stargardt Disease) mice [22].

d. Naked DNA: In a naked plasmid vector delivery, a clinical-grade plasmid DNA is prepared to transfer the gene to the tissue. Clinical trials have been initiated for non-infectious uveitis (NIU) disease of the eye using naked DNA delivery. NIU is an inflammatory symptom in the eye that develops due to eye injury. Systemic anti-TNF (Tumor Necrosis Factor) administration has been approved for NIU to reduce inflammation. The clinical grade plasmid pEYS606 currently in the clinical trial is electro-transferred to the ciliary muscle of the eye [23].

Another criterion for efficient non-viral gene delivery is plasmid design. After the DNA enters the cell, it has to reach the nucleus for transcription initiation. In a dividing cell, nuclear envelope breakage during cell division allows the plasmid DNA to enter the nucleus. However, post-mitotic cells such as photoreceptors present unique challenges to access the nucleus. In such cases, the plasmid DNA is modified by addition of regulatory sequences, including promoters, anti-repressor and epigenetic elements as well as nuclear localization signal. The scaffold matrix

attachment region (S/MAR)-containing sequence has been shown to maintain the plasmid in an episomal state and bind to nuclear scaffold proteins [24]. This allows efficient attachment to the nuclear matrix and DNA entry into the nucleus. These plasmid modifications have been shown to be effective in gene delivery in *Rpe65*$^{-/-}$, *Abca4*$^{-/-}$ and *Rhodopsin*$^{-/-}$ [25].

2.1.2. Viral Vectors

Viral vectors are the delivery system where the genetic materials are introduced in vivo and in vitro to the cell by replication deficient virus. Recombinant replication-deficient adenoviruses have been used in several clinical manifestations to achieve longer-lasting therapeutic effects. In addition, retroviruses, lentiviruses, and adeno associated virus (AAV) are also being used in gene therapy. Each virus type has unique advantages and limitations to transfer the genetic material into host cells.

a. Retroviral vectors: Retroviruses are enveloped single-stranded positive-sense RNA viruses. They reverse transcribe their RNA into double-stranded DNA, which can integrate into the genome of the host cell [26]. It has broad tropism and low immunogenicity, with a packaging ability of 8 kb DNA. The main advantages of this delivery method are persistent integration and expression of transgene in the dividing cell. Some of the drugs that use retroviral gene delivery include strimvelis for SCID and Yescarta for large B cell lymphoma [27]. The disadvantages of this vector are the random integrations of genes into the host genome that raise the possibility of insertional mutagenesis and oncogene activation. This delivery is not suitable for non-dividing post-mitotic cells, including the retina.

b. Lentiviral vectors: Lentiviruses are single-stranded positive-strand RNA viruses and belong to the retrovirus family. The packaging capacity of this viral vector ranges from 8–9 kb. The main advantage of this viral vector is the persistent gene transfer in the transduced tissue and the preferential integration at the 3′ region of the host gene [28]. This virus inserts the genetic material to both the dividing and non-dividing cells and is suitable for ex vivo application. As the lentivirus can accommodate a larger DNA fragment, some of the retinal diseases caused by mutations in larger genes can be delivered with this viral vector. Currently, the non-pathogenic equine infectious anemia virus (EIAV) is in clinical trials to treat Usher Syndrome [29] and Stargardt disease [30]. Moreover, Kymriah is another lentiviral vector-based product in clinical trials for ex vivo gene therapy to treat acute lymphoblastic leukemia [31]. The disadvantages of lentiviruses are similar to those of the retrovirus, as they can integrate to the genome and have limited photoreceptor transduction capability.

c. Adenoviral vectors: Adenoviruses (Ad) are non-enveloped, double-stranded DNA viruses with a packaging ability of 30 to 40 kb DNA [32]. Ads are an attractive delivery system because of their broader tropism, grown as high-titer recombinant viruses present in an episomal state, and their ability to transduce dividing and non-dividing cells [33]. As the transduction of the Ad activates innate immune signaling pathways and stimulates immune cells to secrete pro-inflammatory cytokines for robust adaptive immune response, these properties make the adenoviral vector useful for a vaccine vehicle [34]. As it selectively infects cancer cells and induces the expression of pro-inflammatory cytokines to kill tumor cells, Ad is mainly used for gene therapy for cancer cells. About 18.5% of clinical trials use this vector for gene therapy [35]. A major disadvantage of the adenoviral vector is its lengthy

production protocol, risk of infection to off-target cells, and severe immune response. There is a high prevalence of serotypes such as Ad5 in the human population, thus increasing the number of neutralizing antibodies against this virus [36]. Therefore, adenoviral vectors are uncommon for gene therapy in the retina.

d. Adeno Associated Virus (AAV): Adeno associated viruses (AAVs) are small (with an icosahederal capsid of ~26 nm diameter), non-pathogenic, non-enveloped, single-stranded linear DNA-containing viruses. They belong to the family Parvoviridae and genus Dependovirus because they can infect only in the presence of a helper virus [8 44]. The AAVs were discovered as a satellite virus in the adenovirus (Ad) preparation during an electron microscopic examination by the groups of M. David Hoggan and Robert W. Atchison [37]. As it associates with Ad and needs it for its replication, this virus was named “adeno associated virus” (AAV).

The capsid determines the cell and tissue tropism of the rAAV. Through capsid development, novel rAAV capsids have been discovered or developed that have new and favorable characteristics. Over the years, the AAV virus capsid was modified to infect diverse cell types in the retina. AAV2 and AAV5 were isolated from humans, while AAV4 and AAV8 were isolated from monkeys.

The prevalence of neutralizing antibodies and immunological response in humans against viral capsids is an important consideration when selecting rAAVs for gene delivery. Although rAAVs are non-integrating and efficiently transduce retinal populations, intraocular inflammation and loss of efficacy have been associated with rAAV-gene delivery. This response was observed in both intravitreal and subretinal delivery routes and is linked to the capsid and the dose of the AAV. AAV activates innate immune response, which releases the inflammatory cytokines and type-1 interferons. Neutralizing antibodies against the capsid can also reduce the therapeutic potential of the gene delivery [38]. To evade the immune system, George Church and colleagues recently reported the generation of engineered AAV vectors that are intrinsically less immunogenic. They achieved this by incorporating immunomodulatory noncoding sequences to “cloak” the vector from immune responses [47].

3. Conclusions

Non-viral delivery systems offer distinct advantages, including unlimited payload size, low immunogenicity, and minimal side effects. Although the efficacy of this approach has been reported earlier, such as in Abca4 −/− mice, the non-viral gene delivery strategy has not yet been used in any clinical trials for ocular diseases. Anatomical barriers in the retina and pH sensitivity of the nanoparticles are some of the many environmental challenges for efficient non-viral gene delivery and prolonged gene expression.

Based on the considerable research on the different viral vectors for gene delivery to date, rAAVs seem to be the safest and the most reliable vehicles for gene delivery. Advances in capsid identification, safety, and transduction are needed for their robust, successful, and wider clinical applications. Viral gene therapies are promising tools to transfer genetic material. The insights of precise gene therapy increase the speed for the discovery to restore vision that is destroyed by blinding diseases.

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