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Nanoparticles as Delivery Vehicles for the Treatment of Retinal Degenerative Diseases

Yuhong Wang^{1,4}, Ammaji Rajala^{1,4}, and Raju V.S. Rajala^{1,2,3,4}

¹Department of Ophthalmology, University of Oklahoma Health Sciences Center, Oklahoma City, OK;

²Department of Physiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK;

³Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK;

⁴Department of Dean McGee Eye Institute, Oklahoma City, OK, USA

Abstract

Over the last few years, huge progress has been made regarding the understanding of molecular mechanisms underlying the pathogenesis of retinal degenerative diseases. Such knowledge has led to the development of gene therapy approaches to treat these devastating disorders. Non-viral gene delivery has been recognized as a prospective treatment for retinal degenerative diseases. In this review, we will summarize three representative nanoparticles (NPs) constituent characteristics and recent application in ocular therapy.

1. Introduction

Ocular diseases currently still lack effective treatments, thus, development of effective therapeutics is a primary research goal. So far, many delivery strategies have been explored, and gene delivery systems are the more promising approach in this field (Han et al., 2011; Walkey et al., 2015) (Wang et al., 2015). Gene delivery systems can broadly be classified into two groups, viral and non-viral delivery approaches. Each system comes with itself advantages and disadvantages. Although AAV viral vectors have high gene transduction efficiency, they face a major obstacle is biosafety issue for clinical applications. Thus, development of effective non-viral approaches for ocular disease is very importance.

Non-viral nanoparticles are the most popular and success category in non-viral delivery. They possess the advantage of biodegrade-ability, biocompatibility, minimal toxicity, relatively large capacity, simplicity of use (Adjianto and Naash, 2015; Silva et al., 2015). Functionally, nanoparticles can serve as intrinsic antioxidant or as carriers to deliver drugs and biological macromolecules to target regions.

Nanoparticles for molecular therapy could be classified into three groups: (1) metal-based nanoparticles (metal NPs), (2) polymer-based nanoparticles (polymer NPs), (3) Lipid based-

nanoparticles (LPD). The characteristic features of these particles are described in Table 1. They differ in size, charge, shape and structure, but all possess a mechanism to enter the cell, avoid or escape from endosomes, and finally enter the nucleus in order to deliver therapeutic agents (Adijanto and Naash, 2015; Wang et al., 2015). The eye has unique advantages for the study of therapeutic agents due to the immune privilege, the ability to directly visualize access, locally treat the target tissue, and the benefit of a simultaneous control provided by the other eye.

2. Metal-based nanoparticles

Over the past decade, several different types of metal nanoparticles have been characterized. For example, Cerium oxide (CeO₂) nanoparticles and yttrium oxide nanoparticles have been shown have a high redox scavenging capability with little or no toxicity after delivery to the eye (Cai and McGinnis, 2016b; Mitra et al., 2014; Wong and McGinnis, 2014). Cerium oxide (CeO₂) nanoparticles have intrinsic anti-oxidant properties (Chen et al., 2006). Cerium is a rare earth element and cerium oxide (CeO₂) is inorganic compounds and possess catalytic antioxidant activity (Kong et al., 2011; Walkey et al., 2015; Zhou et al., 2011). These nanoceria particles can act as antioxidants similar to vitamin C and E. In addition, these particles also possess catalytic antioxidant enzymatic activities of superoxide dismutase (SOD) and catalase (Kong et al., 2011; Walkey et al., 2015; Zhou et al., 2011).

3. Metal-based nanoparticle application in ocular disease:

The retina is exposed to chronic oxidative stress through several mechanisms, including constant exposure to light and reactive oxygen species generated by visual signal transduction pathways due to high oxygen consumption, oxidization of polyunsaturated fatty acids, and phagocytosis of photoreceptor cells. In the healthy state, all cell types in the retina are able to maintain homeostasis under conditions of oxidative stress. However, when the balance between pro- and antioxidative signaling is compromised, excessive oxidative stress induces dysregulation of functional networks and deleterious changes that result in visual impairment. Age-related macular degeneration, retinitis pigmentosa, diabetic retinopathy, and glaucoma are leading causes of visual impairment (Nita and Grzybowski, 2016). Nanoceria particles have been used as therapeutics to destroy reactive oxygen species (ROS) and prevent some of the retinal degenerative diseases mentioned above including inhibition of tumor growth of retinoblastoma (Cai et al., 2014a; Cai et al., 2014b; Cai and McGinnis, 2016a; Cai and McGinnis, 2016b; Kong et al., 2011; Wong and McGinnis, 2014). These studies suggest that nanoceria have the potential to provide effective protection in retinal diseases and opens a promising avenue for treatment of ocular diseases due to their antioxidative properties.

4. Polymer-based nanoparticles:

Researchers have used multiple polymer nanoparticles to deliver genes to ocular tissues; however, the most extensively characterized particle was CK30-PEG (Conley and Naash, 2010). The CK30-PEG polymer nanoparticles contain a single molecule of plasmid DNA compacted into nanoparticles (NPs) by 10kDa PEG-substituted lysine 30-mers (CK30-

PEG). As a nanoparticle therapy, CK30-PEG NPs can directly transport molecules to the nucleus, where gene expression occurs. CK30-NPs have advantages in driving long-term, safe gene expression and improving ocular disease based on several key characteristics (Conley and Naash, 2010). CK30-NPs have no theoretical limitation on plasmid size, and they do not provoke an immune response, and furthermore the compacted particle is efficiently taken up into dividing and non-dividing cells (Conley and Naash, 2010; Han et al., 2011).

5. Polymer nanoparticles application in ocular disease

CK30-PEG NPs have been successfully used for the delivery genes to rescue degenerations associated with *Rpe65* gene deletion and retinopathy mouse models (Koirala et al., 2013a; Koirala et al., 2013b). This approach has been shown to improve retinal morphology and function (Cai et al., 2010; Han et al., 2012a; Han et al., 2012b) (Koirala et al., 2013a; Koirala et al., 2013b; Koirala et al., 2014). CK30-PEG nanoparticles have also been used in packaging genomic DNA to rescue rhodopsin-associated retinitis pigmentosa phenotype (Han et al., 2015). MicroRNAs (miRNAs) are a class of naturally occurring small, non-coding RNA molecules. miRNA have been recognized as a key component in regulating cell biological development and may be involved in the pathogenesis of DR, therefore CK30-PEG nanoparticle-mediated miR200-b have been delivered for the treatment of diabetic retinopathy and lead to an effective anti-angiogenic therapy for DR (Mitra et al., 2016). Thus, Polymer NPs have a tolerable safety profile with a lack of immunogenicity in the retina, thus make CK30-PEG nanoparticles become a promising non-viral gene delivery vehicle for t ocular diseases.

6. Lipid based-nanoparticles

Lipids, with their hydrophilic and hydrophobic properties, provide a potent tool box for nanotechnology (Mashaghi et al., 2013). They can be self-assembled into nano-films and other nano-structures, including micelles, reverse micelles, and liposomes (Mashaghi et al., 2013). The use of lipid nanoparticles as part of a system delivering drugs and genes to the retina has been suggested (del Pozo-Rodriguez et al., 2013). Lipid-protamine-DNA (LPD) complexes have been characterized for *in vivo* gene delivery with reporter plasmid constructs (Li et al., 1998; Li and Huang, 1997). Liposome nanoparticles have been widely studied in molecular therapy due to their ease of use, commercial availability, efficient delivery and biocompatible advantages (Honda et al., 2013; Wang et al., 2015; Zhu et al., 1993). Although multiple different types of liposome nanoparticles have been used in molecular therapy, peptide-modified liposome protamine/DNA lipoplex (LPD) nanoparticles have been among the most successful in improving disease progression. LPD nanoparticles are electrostatically assembled from cationic liposomes and an anionic protamine-DNA complex with two peptides NLS (nuclear localization peptide) and TAT (transactivator of transcription), thereby peptide-modified LPD reach optimal transfer efficiency (Ma et al., 2013; Rajala et al., 2014; Wang et al., 2015). These peptide-modified LPD have many advantages for future clinical applications. First, liposome nanoparticles are able to deliver large molecular cargo. Second, the optimization of peptide-modified LPD nanoparticles

allows multiple mutant genes to be simultaneously co-delivered to a single vector. Third, peptide-modified LPD formulations are more biocompatible and safe than viral vectors.

7. Lipid nanoparticle application for ocular diseases

In the eye, Rpe65 is the key enzyme, this gene deficiency mice results in cone degeneration (Znoiko et al., 2005). Our laboratory tested LPD nanoparticle delivery of Rpe65 gene in Rpe65- knockout mice, leading to correction of blindness. The efficacy of this method in restoring vision is comparable to AAV and lentiviral gene transfer (Rajala et al., 2014). LPD nanoparticles also deliver MicroRNA-184 to the retina, and successfully repress Wnt-mediated ischemia-induced neovascularization (Takahashi Y et al., 2015). One of the disadvantages of lipid nanoparticles is that they cannot achieve cell specificity. The retina is composed of seven different kinds of neural cells layers, improper delivery can lead to potentially harmful ectopic expression. We recently overcome this limitation using cell specific promoters to delivery to specific cell types of retina, such as RPE, rod cell, cone cell and ganglion cell using respective cell specific promoters (Wang Y et al., 2016). These studies suggest that peptide-modified LPD nanoparticles are attractive gene therapy vehicles for ocular gene therapy for retinal diseases.

8. Conclusions

On the whole, a successful molecular therapy strategy should encapsulate and protect the molecular materials, escape endosomal degradation and reach the specific target site. The most challenging aspect of molecular therapy is delivering precisely the right quantity of therapy molecule to stimulate optimal levels of expression in a specific cell type, without stimulating an immunity response from the host. Despite rapid advances in molecular therapy during the last two decades, major obstacles to clinical applications for human diseases still exist. These impediments include managing the immune response, vector toxicity, and the lack of sustained therapeutic gene expression. The nanoparticles described above will overcome these barriers, and we can achieve safe and effective molecular therapy.

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Table 1-

Three Non-viral Nanoparticles Comparison in Eye

Nanoparticle	Type	Size	Shape	Charge	Carrier	Target Cell
Nanoceria	Metal NPs	3–5nm	Octahedral	Positive	Intrinsic	Photoreceptors
				Negative		RPE
				Neutral		
CK30	Polymer NPs	<25nm	Rod	Positive	cDNA	Photoreceptor s
					microRNA	RPE
					Genomic DNA	Ganglion cells
LPD	Lipid NPs	50–250nm	Sphere	Positive	cDNA	Photoreceptors
					microRNA	RPE
					Lipid	Ganglion cells