Nanotechnology Approaches for Ocular Drug Delivery

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Abstract

Blindness is a major health concern worldwide that has a powerful impact on afflicted individuals and their families, and is associated with enormous socio-economic consequences. The Middle East is heavily impacted by blindness, and the problem there is augmented by an increasing incidence of diabetes in the population. An appropriate drug/gene delivery system that can sustain and deliver therapeutics to the target tissues and cells is a key need for ocular therapies. The application of nanotechnology in medicine is undergoing rapid progress, and the recent developments in nanomedicine-based therapeutic approaches may bring significant benefits to address the leading causes of blindness associated with cataract, glaucoma, diabetic retinopathy and retinal degeneration. In this brief review, we highlight some promising nanomedicine-based therapeutic approaches for drug and gene delivery to the anterior and posterior segments.

Keywords: Age-Related Macular Degeneration, Dendrimers, Nanoparticles, Ocular Gene Therapy, Targeted Therapies

INTRODUCTION

There is a relatively high prevalence of blinding eye diseases in the developing nations, which by some estimates are 10- to 40-fold higher than that in the developed countries. For example, in Saudi Arabia, over 1.5% of the total population, and over 20% of the population over 60 years of age are estimated to be blind by the World Health Organization criteria. The leading causes of blindness in Saudi Arabia include cataracts, trachoma, corneal scars, glaucoma, retinal degenerative diseases such as diabetic retinopathy (DR), age-related macular degeneration (AMD) and congenital anomalies. Some of these are related to, and exacerbated by, the high incidence of Diabetes Mellitus, with an overall prevalence of over 23% of the population. A report by the Saudi Ministry of Health suggests that diabetes and obesity may be on the increase. Therefore, there is an increased recognition, especially in Saudi Arabia and the Middle East, that eye diseases are a major health problem, leading to a high socio-economic burden. Major Saudi Arabian government research and technological initiatives are underway, both in medicine and nanotechnology that may pave the way for novel therapeutic applications through multi-disciplinary collaborations between governments, researchers from many countries and industries.

The field of ‘nanomedicine’ uses nanoscale technologies (≤100 nm, typically) for the diagnosis, treatment and/or prevention of diseases, as well as to gain an understanding of the pathophysiology of various diseases, with an ultimate goal of improving quality of life. In this respect, nanomedicine offers numerous advantages compared to treatments with drugs alone. These include sustained
delivery of therapeutic agents, targeted delivery of drugs to specific cells or tissue, improved delivery of both water-insoluble drugs and large biomolecule drugs, and reduced side effects.\textsuperscript{6–8} Liposomes are small spherical vesicles, typically composed of lipid bilayers surrounding aqueous inner phase. The bilayers and the inner aqueous phase can act as the reservoir for hydrophobic drugs and hydrophilic drugs, respectively.\textsuperscript{9–10} Liposomes are one example of nanomedicines that have been previously used in ocular drug delivery.\textsuperscript{9,11–13} Liposome-based ocular drug-delivery systems were described as early as in 1981 by Smolin \textit{et al.}\textsuperscript{14} The encapsulation of idoxuridine into liposomes increased the corneal penetration of the drug,\textsuperscript{15} and showed improved efficacy in controlling Herpetic keratitis in comparison to the free drug solution.\textsuperscript{14} Subsequently, a variety of other biomaterials for ocular drug delivery have been developed, including polymeric nanoparticles,\textsuperscript{16–17} dendrimers,\textsuperscript{18} and hydrogels\textsuperscript{19} [\textbf{Figure 1}], that may be administered in a versatile manner. In this mini-review, we highlight recent developments in polymer and nanotechnology based drug-delivery systems for the anterior and posterior segments of the eye. Successful translation of these promising approaches would require a careful assessment of both the risk and reward arising from the use of the nanomedicines and we discuss both these aspects in this review.

\section*{NANOMEDICINE APPLICATIONS FOR THERAPIES TO THE ANTERIOR SEGMENT DISEASES}

The incidence of topical and corneal infections have increased in recent years owing to an increased number of corneal surgeries for cataracts, glaucoma and corneal transplantations, and the increased use of contact lenses. Even though the cornea is protected from the external environment by a continuous tear fluid film that turns over rapidly, a variety of microorganisms can invade the cornea, leading to keratitis or conjunctivitis. Such ocular infections can cause major health problems if untreated, causing corneal reddening, opacification, rupture, irritation and inflammation, leading to obscure vision and even permanent blindness.\textsuperscript{40} Drug-delivery systems that improve drug residence time on the corneal surface can have a significantly positive impact. Improved pre-corneal residence time can enhance the absorption of drugs through periocular tissue to even reach the inner ocular tissues. The improved drug bioavailability can decrease the frequency of drug administration, and increase patient compliance. Various nanomedicine strategies, such as the mucoadhesive nanoparticles and drug-eluting contact lenses have been explored, and are highlighted here.

\subsection*{Improving corneal residence time}

Even though eye drops are the most conventional formulation for ocular drug delivery, they typically provide low bioavailability (less than 5\%) owing to poor pre-corneal retention and penetration. The factors affecting pre-corneal retention include rapid tear turnover, blinking, and solution drainage, which result in the loss of drug after topical administration. Therefore, frequent instillations of eye drops are required to maintain a therapeutic drug level on the pre-corneal surface. Frequent use of concentrated eye drops can induce toxicity, corneal dryness and possible severe systemic side effects.\textsuperscript{21,22}

Tear liquid contains secreted mucins and surface associated mucins. The mucins form a hydrophobic blanket that moves over the ocular surface to clear debris and pathogens.\textsuperscript{23} Prior studies have suggested that charged polymers (both cationic and anionic) could have hydrogen bonding and electrostatic interactions with mucins, rendering them ‘mucoadhesive’.\textsuperscript{24} Mucoadhesive forces between an anionic polymer surfactant (Carbopol\textsuperscript{®}) and mucins, attributable to hydrogen bonding interaction between them, were significantly higher than the forces between other neutral polymers and mucins.\textsuperscript{24} Such polymers have been used to design ocular drug-delivery systems for topical administration in order to increase the ocular residence time.\textsuperscript{22} Poly(lactic-co-glycolic acid) (PLGA) nanoparticles could be blended with a cationic polymer surfactant (Eudragit\textsuperscript{®}RL), or coated with anionic Carbopol\textsuperscript{®}to improve interactions with mucins. Carbopol\textsuperscript{®}-coated, cyclosporine A (CsA)-encapsulated-PLGA nanoparticles with negative surface charge showed higher tear film CsA concentration after instillation on healthy rabbit eyes in comparison to non-coated PLGA nanoparticles. Positively charged Eudragit\textsuperscript{®}RL-PLGA nanoparticles can further increase the tear film concentration (C\textsubscript{max}) and the area under the concentration-time curve between 0 and 24 h (AUC\textsubscript{0-24})
on healthy rabbit eyes, in comparison to both Carbopol®-PLGA nanoparticles and non-coated nanoparticles. It was suggested that negatively-charged mucins on the preocular surface interact with these positively-charged formulations, to increase the residence time and enhance the cellular uptake of nanoparticles. Thiolation of polymers can also improve interactions with ocular mucins by the covalent binding. Thiolated quaternary ammonium-chitosan (TCS) conjugates, synthesized from chitosan (CS), were used to prepare TCS-sodium alginate nanoparticles (TCS-SA). The thiol groups on TCS-SA nanoparticles could form a disulfide bond between TCS and the thiol groups on ocular mucins, which in turn could increase the adhesive interactions of nanoparticles on the preocular surface. A higher amount of a model drug was delivered to rat cornea after the instillation, as confirmed by confocal fluorescence microscopy. Therefore, prolonged ocular retention can be achieved by appropriate balancing of the surface chemistry of nanoparticles with mucoadhesive properties.

Efflux proteins in the cornea can be a barrier for the effective retention of drugs. Various efflux proteins exist in the cornea, such as P-glycoproteins (P-gp) and multidrug resistance associated proteins (MRPs), can pump drug molecules out from the corneal epithelium. Mitra et al. studied these efflux proteins, with an aim to reduce drug efflux and enhance corneal absorption of drugs after the topical administration. In an in vitro cultured rabbit primary corneal epithelial cell model for rabbit cornea, the efflux of erythromycin by P-gp and MRPs was significantly inhibited by co-administration of steroids, leading to enhanced corneal cell uptake of erythromycin. Furthermore, in rat models, topical co-administration of erythromycin with steroid prednisolone resulted in 4-fold increased uptake in rat cornea compared to erythromycin alone. The co-administration of inhibitors for efflux proteins could lead to an increase in drug retention.

A number of membrane transporters were discovered in various ocular tissues including the cornea, conjunctiva and retina. These transporters are involved in the translocation of nutrients and xenobiotics. Therefore, transporter-targeted prodrug strategy can improve drug delivery to ocular tissue by enhanced absorption of poorly permeating parent drugs. Acyclovir (ACV) is an anti-viral drug, with a poor aqueous solubility and low corneal permeability. Therefore, a prodrug strategy was adopted to improve corneal absorption of ACV. L-aspartate ester prodrug form of acyclovir (L-Asp-ACV) acted as a substrate of an amino acid transporter, corneal B⁰⁺, resulting in a 4-fold increase in the transcorneal permeability of ACV through the healthy rabbit cornea. Mitra et al. also showed that amino acid prodrugs of ACV (L-serine-ACV), exhibited a 3-fold increase in aqueous humor concentration compared to ACV, 8 h after topical administration.

Fresta et al. have explored poly(ethylene glycol)-coated poly(lactic acid) (PEG-PLA) nanoparticle strategies to deliver acyclovir. Both PLA and PEG-coated PLA nanoparticles improved the corneal residence time of ACV by 7- and 12-fold respectively, compared to ACV alone with no indication of toxicity or ocular irritancy in rabbit eyes. The encapsulation of ACV into PEG-coated nanoparticles provided sustained drug release, improved pharmacokinetics in the cornea, and increased drug levels in the aqueous humor, suggesting significantly improved bioavailability. Mitra et al. have encapsulated dipeptide prodrugs of ACV into PLGA nanoparticles and suggested that these nanoparticles may slow the degradation of prodrugs, resulting in an improved therapeutic effect upon topical administration. Thus, transporter-targeted prodrug delivery strategy could be further improved and sustained through the use of nanoparticles. The combination of prolonged residence time, a sustained release formulation and the inhibition of efflux proteins on the cornea could improve the therapeutic efficacy of ophthalmic drugs.

Drug eluting contact lenses for sustained delivery

Extended wear soft contact lenses are increasingly preferred by both younger and older generations. Therefore, nanoparticle-drug formulations could be incorporated into contact lenses for sustained therapy. Contact lenses loaded with drugs have been studied by Chauhan et al. Increased corneal bioavailability achieved by the drug-laden contact lenses can improve patient compliance and provide extended drug delivery. Glaucomatous dogs with inherited open angle glaucoma were successfully treated with timolol, with NIGHT&DAY™ silicone hydrogel contact lenses. Higher bioavailability of timolol was achieved by contact lenses with only one-third of the loaded drug
compared to eye drops, to achieve similar intraocular pressure (IOP) reduction.\(^\text{35}\) The inclusion of Vitamin-E (VE) into the contact lenses prolonged the release of timolol.\(^\text{36}\) Even though the addition of VE did not improve the IOP reduction in this study, it was suggested that the sustained release of timolol enabled by VE could lead to sustained IOP reduction through the use of the drug-laden contact lenses. Extended CsA delivery for the treatment of chronic dry eyes was also realized by the VE-loaded contact lenses.\(^\text{37}\) The inclusion of VE into silicone hydrogel contact lenses could maintain the therapeutic level of CsA released from the contact lenses for one month. In comparison, the duration of CsA released from commercial contact lenses and silicone contact lenses was only about 1 day and 2 weeks, respectively. Similarly, the inclusion of Brij 78 (a non-ionic polyoxyethylene-based surfactant) to poly-hydroxy ethyl methacrylate (p-HEMA) contact lenses led to an extended delivery of CsA up to 1-2 months.\(^\text{38}\) Dispersing ethylene glycol dimethacrylate and propoxylated glyceryl triacrylate cross-linked nanoparticles into HEMA contact lenses increased the duration of drug release at a therapeutic dose from the 1-2 h for commercial contact lenses to about 2-4 weeks for these nanoparticles-laden contact lenses.\(^\text{34}\)

Byrne \textit{et al.} synthesized molecularly-imprinted silicone hydrogel contact lenses, which cannot only release a small molecule such as ketotifen fumarate,\(^\text{38}\) but also comfort large molecules such as high molecular weight hyaluronic acid (HA)\(^\text{39}\) and high molecular weight hydroxypropyl methylcellulose.\(^\text{40}\) Molecular imprinting involves the self-assembly between the functional monomers and the template through non-covalent interactions and hydrogen bonding.\(^\text{41}\) This approach offers the potential to design and construct silicone hydrogel lenses to release comfort molecules, leading to decreased contact lenses-induced dry eye.

**Dendrimer-based topical delivery systems for cornea**

Dendrimers are globular, nanostructured polymers (~3-20 nm) with a well-defined shape, narrow polydispersity. Some dendrimers possess antimicrobial properties, and can be used as surface coating agents and drug carriers.\(^\text{42}\) The large number of surface functional groups on dendrimers can lead to multivalent interactions, with a potential for mucoadhesive properties that could lead to a reduction in tear washing and dilution, and improved pre-corneal residence. \textit{In vitro} studies of the interactions between ‘negatively-charged’ ocular mucins and poly (amido amine) (PAMAM) dendrimers revealed strong interactions between the dendrimers and the mucins in the tear film. Interestingly, both cationic (–NH\(_2\)) and neutral (–OH) PAMAM dendrimers showed similar mucoadhesive interactions, which were stronger, especially at pathological pH (~5.5) (cationic > neutral).\(^\text{25}\) At pathological pH, the primary amines of –NH\(_2\) dendrimers and the tertiary amines in the inner cores of both –NH\(_2\) and –OH dendrimers are partially protonated, which further contributes to stronger associations.\(^\text{25}\) The above study suggests that dendrimers could improve corneal residence times, through electrostatic interactions with the ocular mucins.

PAMAM dendrimers, by themselves, could have significant antibacterial activity, comparable to a potent antibiotic (ampicillin), by destabilizing the bacterial cell wall and exposing the bacterial contents for denaturation.\(^\text{43}\) Another study explored the encapsulation of amoxicillin into the internal cores of PAMAM dendrimers, which were further cross-linked with an 8-arm star polyethylene glycol (PEG) to form a transparent hydrogel matrix via disulfide bonds. Such hydrogels are injectable and can provide sustained release of drugs.\(^\text{44}\) Quinolones have been explored as bactericidal agents for ocular applications, but have major drawbacks such as low solubility and being destructive to corneal epithelial layers.\(^\text{45}\) Cheng \textit{et al.}\(^\text{46}\) reported that encapsulating the fluoroquinolones such as nadifloxacin and prulifloxacin within PAMAM dendrimers not only overcame the solubility issues but also enhanced their potency against \textit{E. coli} 2-fold better than free drug. Such systems can be potentially used as topical eye drops that can form a gel layer over the cornea, and providing ‘sustained’ delivery of antimicrobial agents without affecting the vision, and reducing toxicity to corneal cells due to frequent instillation, thereby improving patient compliance.

Corneal wounds (full- or partial-thickness lacerations), resulting from various conditions such as trauma, infections and corneal thinning disorders, cataract, glaucoma infiltration, and corneal transplantation surgeries, require sutures to fasten the corneal flaps. These sutures can sometimes
lead to infections, penetrating keratoplasties, corneal scarring, leaking and post-surgical cataracts. In recent years, sutureless procedures using biocompatible polymeric corneal glues are being explored. These polymer glues can be engineered with the desired physiochemical and biological properties to restore the integrity of cornea and decrease the risk of surgical complications when applied. Various polymeric glues such as cyanoacrylate and fibrin are reported to have beneficial effects but often cause problems such as non-flexibility, stiffness and require autologous blood components for polymerization respectively. To address this, Grinstaff et al. have developed polyester, polyester-ether, and polyamide dendrimers that are biodegradable and contain biocompatible entities such as succinic acid or lactic acid. These hydrogels are being explored in a wide range of applications from drug delivery to wound healing. One of the advantages of using these dendrimers in a hydrogel is that they offer numerous surface groups that enable high cross-linking densities at very low polymer concentrations. The photocurable dendrimer-based hydrogel bioadhesives were reported to efficiently and rapidly secure large central corneal lacerations and fasten allografts in porcine enucleated eyes. Interestingly, the porcine eyes sealed with bioadhesives could withstand high leaking pressures (well above 200 mmHg), compared to sutures (~85 mmHg). Moreover, a combination treatment of both sutures and bioadhesives could also be useful, as shown in a porcine allograft model. The allografts with minimal sutures (8 sutures) combined with bioadhesives could also be beneficial, as shown in a porcine allograft model. The allografts with minimal suture (8 sutures) combined with bioadhesives could withstand leaking pressures (80-85 mmHg) significantly higher than those with only 16 sutures (~45 mmHg).

**Corneal gene delivery**

Cornea is readily accessible and somewhat separated from the general circulation and the systemic immune system, which make it a good candidate for gene therapy. The goal of corneal gene therapy is to deliver and transfer a gene to the cornea itself or the nearby ocular tissue by various vector systems. The expressed transgenic proteins could have a structural function (such as collagen) or be active in modulating a pathological condition (such as cytokines and growth factors). Furthermore, RNA interference could be used to silence a gene expression in the cornea. In preclinical studies, corneal gene therapy was successfully applied to prevent the cornea rejection, cornea neovascularization and herpetic stromal keratitis. Intrastromal injection of nanoparticles has become a feasible method for corneal gene therapy in animal models. Komppa et al. utilized PLGA nanoparticles encapsulated with the plasmid containing a small hairpin RNA expression cassette against vascular endothelial growth factor A (shRNA.VEGF-A) by intrastromal injection for the treatment of corneal neovascularization induced by the mechanical-alkali trauma in BALB/c mice. Five days after a single dose of 2 μg plasmid/eye of this nanoparticle formulation, there was a significant reduction in the total corneal VEGF-A protein expression (80%) relative to the control eye without corneal neovascularization. However, at the same dose, the naked plasmid exhibited only a 20% reduction of corneal VEGF-A protein expression, compared to control. Nanoparticles were retained within the stroma after intrastromal injection and were not cleared rapidly from the tear liquid, therefore, may have facilitated long-term gene expression. After 4 weeks, the nanoparticle formulation was more than 2-fold better than naked plasmid at the same dose, based on the mean fractional area of corneal neovascularization.

Subconjunctival injections were tested for the delivery of plasmids expressing Flt23k (an anti-VEGF intrareceptor) to inhibit the murine cornea transplant rejection. In this work, Flt23k plasmid was formulated into 220 nm PLGA nanoparticles which were administered by subconjunctival injection. In a corneal graft mouse model with BALB/c mice as graft recipients and C57BL/6 mice as donors, Flt23k-loaded nanoparticles exhibited anti-VEGF effect and significantly decreased the fractional area of corneal neovascularization compared to control and fractional lymph angiogenesis area. Compared to the PBS group, Flt23k-loaded PLGA nanoparticles showed better graft survival until 8
post-operative weeks. The co-administration of steroid (triamcinolone) further enhanced the therapeutic effect by reducing the cornea transplant rejection rate due to the immunosuppressive effect. 2-month graft survival rate was 92% for co-administration group, whereas the graft survival rate was 48%, 20% and 0% for triamcinolone group, Flt23k-PLGA nanoparticle group and PBS group, respectively. Nanoparticles administered by subconjunctival injection were observed not only in the subconjunctival tissue, but also in the cornea up to 4 weeks after injection.

Nanoparticle-based gene therapy offers sustained delivery of therapeutic genes and enhanced transfection efficiencies as a result of improved cellular uptake, endosomal escape and transport to the nucleus.

NANOMEDICINE APPLICATIONS FOR TREATMENTS TO THE POSTERIOR SEGMENT

Neovascular and neurodegenerative retinal diseases are the leading cause of vision impairment both in developed and developing countries. Particularly, the incidence of diabetes and DR is a major concern in Saudi Arabia and other Middle Eastern countries. Neuroinflammation and ocular neovascularization are the common features of many retinal diseases such as DR, wet-AMD and retinitis pigmentosa. The anatomical features greatly prevent the penetration of drugs from the front of eye to the posterior segments of the eye (vitreous humor, retina and choroid), and the direction of drug penetration is opposite to the direction of the intraocular liquid circulation. Furthermore, the corneal epithelium, corneal endothelium, retinal endothelial cells and retinal pigmented epithelium are composed of tight junctions, which limit the diffusion of drug molecules. The delivery of the drugs to the back of eye by systemic administration is greatly restricted by the blood-retinal barrier. Various administration routes are available for delivery of drugs to the back of eye: intravitreal injection, subretinal injection, transscleral administration, subconjunctival injection, and topical instillation. All of the above mentioned methods are invasive and can sometimes lead to post-administrative complications such as retinal toxicity, retinal detachment and intraocular infections (endophthalmitis). Nanoparticle drug-delivery systems may decrease the frequency of injections, and improve efficacy, leading to reduced side effects and improved patient compliance.

Biodistribution of nanoparticles in the retina

Investigating the ocular biodistribution of nanoparticles can provide insights into the bioavailability, cellular uptake, duration of drug action, and toxicity. Many factors such as particle size, composition, surface charge and mode of administration influence the biodistribution in the retinal structures and also their drainage from the ocular tissues. Ocular biodistribution of well-defined fluorescently-labeled polystyrene nanoparticles (50 nm, 200 nm and 2 μm) was investigated by Sakurai et al. in pigmented rabbit model. The nanoparticles were administered intravitreally. Larger particles (2 μm) were found to remain in vitreous cavity near the trabecular meshwork from which they are discharged out from the ocular tissue within ~6 days, whereas the 200 nm particles were found evenly distributed in the vitreous cavity, and the inner limiting membrane. The smaller 50 nm particles crossed the retinal barriers, and were detected in the retina even after 2 months post injection. Intravenous administration of gold nanoparticles also showed size-dependent ocular biodistribution in C57BL/6 mice model, as reported by Yu et al. Two different sizes (20 nm and 100 nm) of gold nanoparticles were administered intravenously and the biodistribution differences were assessed by transmission electron microscopy. Within 24 h, 20 nm nanoparticles were found in the retinal cellular structures with a biodistribution of 75 ± 5% in retinal neurons, 17 ± 6% in endothelial cells and 8 ± 3% in peri-endothelial glial cells. Histological studies further confirmed that these nanoparticles did not cause any toxic or structural damages to the retinal structures. In contrast, 100 nm particles were not found anywhere in retina suggesting the preferential influence of size of nanoparticles on crossing the blood-retinal barriers (BRB). Kompella et al. have investigated the combined effect of particle size and mode of administration on the biodistribution in retinal and other intraocular tissues in a Sprague-Dawley rat model. They used 20 nm and 200 nm carboxylate-modified polystyrene nanoparticles with fluorescent dye. Upon subconjunctival and transscleral administration, 20 nm particles were rapidly cleared from the site of injection, and were not found in the pericellular or intraocular tissues, whereas the 200 nm particles were found accumulated only at the site of injection.
The rapid clearance of 20 nm particles could be due to periocular blood or lymphatic circulation. The surface chemistry can also affect nanoparticle distribution. Positively-charged nanoparticles can adhere to the anionic vitreous network components and aggregate within the vitreous. Anionic nanoparticles were found to diffuse through the vitreous and could even penetrate the retinal layers to be taken up by Müller cells. Vitreous was regarded as the barrier for non-viral ocular gene therapy because of the strong interaction of conventional cationic nature of non-viral gene vectors with the anionic vitreous. The inclusion of PEG (also called PEO) on the surface of nanoparticles enabled them to diffuse within vitreous, and offered the potential for these nanoparticles to reach the retina. Poly(ethylene oxide)-polyspermine (PEO-PSP) block copolymer were complexed with antisense oligonucleotide to form 12 nm particles with neutral surface charge, and these nanoparticles acted as carriers for delivery of oligonucleotide after intravitreal injection to healthy rat eyes. Fluorescein-labeled oligonucleotides were detected in retinal vascular cells at 24 h post-injection, and persisted up to 6 days, as shown by confocal fluorescence microscopy.

Kim et al. synthesized self-assembled amphiphilic nanoparticles with different surface chemistries and these nanoparticles were administered to healthy rat eyes by intravitreal injection. The cationic polyethyleneimine (PEI) nanoparticles aggregated within vitreous and were prevented from distributing to the retina by the vitreal barrier. In contrast, cationic glycol-chitosan (GC) nanoparticles and GC/PEI blended nanoparticles could penetrate the vitreal barrier and even reach at the inner limiting membrane because of the existence of glycol groups on nanoparticles (preventing the adhesive interactions with vitreous components). Anionic HA nanoparticles and human serum albumin nanoparticles penetrated the whole retina to the retinal pigment epithelium (RPE). Thus, the design and engineering of nanoparticles with suitable surface chemistry can enable them to be used as drug or gene carriers for retinal disorders.

**Topical and subconjunctival delivery to the retina**

Subconjunctival administration of drug-loaded nanoparticles and microparticles can enable sustained release of therapeutic level of drugs to the retina. Subconjunctival administration of triamcinolone acetonide (TA)-PLA microparticles (~2 μm) achieved sustained delivery of drugs for 2 months in both the normal and laser-induced choroidal neovascularization (CNV) Brown Norway rat models. In contrast, no drug levels were detected for triamcinolone acetonide-PLA nanoparticles (551 nm) or free drug. It may be possible that the differences in the clearance rates between the micro and the nanoparticles and the particle degradation rates in vivo, are also key factors. After subconjunctival injection of the drug-loaded particles, the lipophilic steroid distributed to the posterior ocular tissue in order of choroid-RPE > sclera > retina > vitreous based on the drug level. The preferential accumulation of TA in choroid-RPE by the transscleral transport could be due to melanin binding of TA in the melanin-rich choriod-RPE.

Lowe et al. explored subconjunctival administration of degradable hydrogels. These hydrogels were prepared by UV photopolymerization of N-isopropylacrylamide monomer and a dextran macromer containing multiple hydrolytically degradable units. The hydrogel exhibited degradability and thermoresponsive properties for the sustained release of insulin to the retina upon subconjunctival implantation. The hydrogels and their degradation products were not toxic to R28 retinal cells in cell culture (50,000 cells cm^-2) for at least 1 and 7 days, respectively. Hematoxylin and eosin (H and E) staining, immunostaining for microglial cell activation and full field electroretinography (ERG) showed that no adverse effects after implantation of hydrogels in healthy Sprague-Dawley rats. Hydrogel exhibited high loading efficiency (up to 98%) of insulin and released the biologically active insulin in vitro in a PBS solution at 37°C for at least one week. These subconjunctival hydrogels have the potential for sustained delivery of insulin and other drugs to the posterior region.

**Intravitreal delivery systems**

In comparison to periocular routes, intravitreal administration can directly deliver drugs and particles to the vitreous body, enabling further diffusion or penetration to the retina. Behar-Cohen et al. reported that 310 nm poly(lactic acid) (PLA) nanoparticles were preferentially localized in the RPE.
cells after intravitreal administration to Lewis rats, and the nanoparticles were present in the RPE even four months after single intravitreal injection. Kompella et al. showed that the PLA particle retentions at 1 month and 3 month were 60% and 27%, respectively, after the intravitreal injection of 7.6 μm PLA microparticles into healthy New Zealand white rabbit eyes. The drug levels of chemokine (cell surface) receptor CXCR4 antagonist TG-0054 in the vitreous, retina and the RPE at 3 months were similar to levels at 1 month, which were significantly higher than those for the free drug solution at the same time points. Free drugs and large biomolecules can be cleared from the vitreous body by the circulation and vitreous turnover, resulting in short intraocular half-life. Nanoparticle formulations can avoid the quick clearance and can improve retention in the vitreous and the retina for sustained delivery.

Lavik et al. formulated PLGA microspheres and nanospheres containing the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) AG1478 for intravitreal injection in a rat optical nerve crush injury model. Both AG1478 microspheres (~2.6 μm) and nanospheres (~360 nm) promoted optic nerve regeneration at two weeks. However, at 4 weeks, only nanospheres, but not microspheres showed an effect on optic nerve regeneration. The authors suggested that this could be due to the ease of intravitreal administration of nanospheres in comparison to microspheres, which could have led to an increased amount of spheres delivered to the vitreous.

Steroidal and non-steroidal drugs are extensively used for various retinal diseases due to their anti-inflammatory, anti-angiogenic, and neuroprotective properties. Even though they are potent, their administration is often associated with solubility issues leading to either early exclusion or accumulation in ocular tissues causing local toxicity, elevated IOP and optic nerve degeneration. Sustained drug delivery of steriods (e.g. fluocinolone acetonide [FA]) from non-erodible intravitreal implants are undergoing clinical trials. Kannan and Iezzi have developed strategies for delivering FA using PAMAM dendrimers as targeted and sustained drug carriers, for retinitis pigmentosa. PAMAM dendrimer-drug conjugates injected intravitreally into Royal College of Surgeons and s334-ter rat models showed pathology-dependent biodistribution, and selective accumulation in activated microglial cells in the retina. Moreover, the dendrimer-FA conjugate (1 μg dose on a drug basis) showed retinal neuroprotection at least up to 30 days, and was more effective than free drugs at ~20-fold lower concentrations. The activated microglial localization was observed for at least 30 days after a single intravitreal injection. Such treatment strategy with sustained availability of drugs for a prolonged period of time may help reduce the frequency of intravitreal injections and improve drug efficacy through targeted, sustained delivery. Targeting activated microglia to treat neuroinflammation can also be valuable in other conditions, as shown recently in rabbit model of cerebral palsy. The same PAMAM dendrimers used above were found to selectively localize in activated microglia/astrocytes upon systemic administration to newborn rabbit kits with cerebral palsy. One dose of systemically administered dendrimer-N-Acetyl cysteine (D-NAC) conjugate, in the post-natal period, produced a dramatic improvement in the motor function, and improved neuronal injury and myelination in the newborn rabbit kits.

Subretinal and systemic delivery systems

In order to avoid the barrier effect from the vitreous and the penetration through the inner limiting membrane, subretinal injection of gene and drug carriers have been developed. Naash et al. investigated ocular gene therapy using compact DNA nanoparticles through subretinal injection. A block copolymer of 30-mer lysine with a terminal cysteine group conjugated with 10 kDa methoxy-PEG-maleimide (CK30PEG) can condense with plasmid DNA to form rod-shaped nanoparticles with a size of 350 ± 5 nm (length ± width), and a nearly neutral surface charge, and can directly transfect RPE cells. CK30PEG-DNA nanoparticles containing the wild-type retinal degeneration slow (Rds) gene-enabled a partial structural and functional rescue in a retinitis pigmentosa (rds−/−) mouse model by subretinal injection. These nanoparticles were taken up by photoreceptors and the expression of transferred normal mouse peripherin/rds (NMP) co-localized with endogenous retinal degeneration slow protein (RDS), evidenced by confocal fluorescence microscopy. ERG analysis showed that the nanoparticle-mediated gene transfer restored cone function to a near-normal level and improved the rod function, in comparison to the subretinal injection of naked DNA. These
promising in vivo results, combined with a good safety profiles of these nanoparticles upon subretinal injection, offer significant potential for gene therapy to treat different retinal disorders.

Systemic administration has also been explored for delivering drugs to the posterior segments of the eye through the BRB. The inner BRB (iBRB) prevents the penetration of drugs from systemic administration to reach the ocular tissue because it contains tight-junctions forming a selective barrier that can be regulated. Humphries et al. developed a novel approach using RNAi-mediated suppression, that led to the reversible opening of iBRB. The delivery of siRNA to target claudin-5 in the retina caused a transient and size-selective increase in paracellular permeability of microvessels, without the development of retinal edema or changes in retinal function. This reversible opening allowed the delivery of low molecular weight therapeutics to the outer layers of the retina, but excluded molecules larger than 1 kDa. Upon systemic delivery of a siRNA in an IMPDH1-/-mice model with autosomal recessive retinitis pigmentosa, after the reversible barrier opening, the visual function was improved and the rate of photoreceptor cell death was reduced.

PLGA nanoparticles were surface-functionalized with transferrin, a RGD peptide (arginine-glycine-aspartic acid), or both, to deliver anti-VEGF intraceptor plasmid. After systemic administration to laser-induced CNV rat model, these RGD-functionalized PLGA nanoparticles were able to accumulate in the neo-vascular eye but not the control normal eye. Surface functionalized enabled retinal gene delivery to the retinal vascular endothelial cells, photoreceptor outer segments, and RPE cells significantly better, in comparison to non-functionalized nanoparticles. Two weeks after plasmid-loaded nanoparticle administration, significantly smaller CNV area was observed in rats treated with functionalized nanoparticles in comparison to non-functionalized nanoparticles. Naked plasmid did not significantly decrease the CNV. Functionalized nanoparticles did not induce cellular infiltration, inflammation or atrophy of the retina based on H and E staining histological studies.

**SAFETY/TOXICITY OF NANOPARTICLES IN THE EYE**

Intravitreal injection of anti-VEGF reagents is widely used to treat AMD, DR and diabetic macular edema. Intravitreal injection of nanoparticles is a feasible mode of administration, and many of the safety/toxicity studies have focused on intravitreal administration. The toxicity of nanoparticles in ocular tissues can be affected by many factors, including the chemistry, size, dose, time of assessment, and the biodistribution pattern of the particles in the eye. Even though many of the studies have focused on the efficacy of the formulations, recent studies have started to assess the toxicity based on histological evaluation, immunohistochemistry, inflammation and neuronal toxicity. Lutty et al. compared the safety of chitosan, PCEP (poly[(cholesteryl oxocarbonylamido ethyl) methyl bis(ethylene) ammonium iodide] ethyl phosphate), and magnetic nanoparticles which have been investigated as ocular gene delivery vehicles. The toxicity was assessed in New Zealand white rabbits for intravitreal delivery, and Dutch Belted rabbits for subretinal delivery. The evaluation was based on retinal histopathology, retinal degeneration (electroretinography), and inflammation, over a 7-day period. At concentrations required to deliver 1 μg of DNA in the nanoparticle form, intravitreal delivery of chitosan nanoparticles produced an inflammatory response. In comparison, PCEP and magnetic nanoparticles did not show either inflammation, or retinal pathology both upon intravitreal and subretinal administration. Recently, Goldberg et al. reported the effect of magnetic nanoparticle size on ocular toxicity in Sprague-Dawley rats assessed by IOP, ERG, and histopathology. The dextran-coated 50 nm, or uncoated 4 μm magnetic nanoparticles were intravitreally administered at a dose of 1.65 mg/3 μl/eye (10-fold higher level than that used for therapeutic applications). Over a 5 month period, the 50 nm dextran-coated magnetic nanoparticles did not show any signs of toxicity based on the above measures. The 4 μm uncoated magnetic nanoparticles showed no toxicity at short times, but showed some toxicity after 5 months. This was partly attributed to the significant longer ocular residence time of the 4 μm particle, compared to the 50 nm particle.

Differential ocular biodistribution in the presence and absence of disease pathology and targeting can be a significant factor that can have a positive impact on the safety of nanoparticles. Recent studies with PAMAM dendrimers in a rat model suggested that, in healthy eyes, the dendrimers were readily cleared from the retina upon intravitreal administration. In contrast, in the presence of retinal...
degeneration, the dendrimer was retained in the retina, mostly localized to the activated microglial cells even up to 30 days. Such selective localization in cells associated with neuroinflammation may help the toxicity profile of these dendrimers, since little long term accumulation is observed in healthy animals. A lipophilic amino-acid dendrimer bound with an anti-VEGF oligonucleotide (ODN-1) was injected into the eyes of rats by intravitreal administration to inhibit laser-induced choroidal neovascularization. Dendrimer-ODN-1 was well tolerated in vivo and immunohistochemistry showed no observable increase in inflammation-related antigens. PEO-PSP block copolymers self-assembled with oligonucleotide into small particles with neutralized surface charge because of the PEO corona, and histologic studies detected no toxic effect from the PEO-PSP carriers to the ocular tissue after intravitreal injection. The delivery of therapeutic genes or drugs to the eye by intravitreal injection required much low dose in comparison to systemic route, and can help decrease the toxicity of injected nanoparticles to other organs.

Naash et al. have carried out a study on the safety of compacted CK30PEG-DNA nanoparticles in a mouse model by subretinal injection. No infiltration of neutrophils or lymphocytes, or elevation of cytokines was detected in the retinas. Some initial, inflammatory response was observed, but the response returned back to normal after 2 days post-injection. Repeated delivery of these compacted DNA nanoparticles one month after the first injection did not induce toxicity, suggesting that repeated dosing is possible. Through appropriate design of the nanoparticle size, chemistry, and surface modification, it is possible to minimize the toxicity of nanoparticles.

CONCLUDING REMARKS/CLINICAL TRANSLATION

Blindness is a leading health care issue (after cancer) worldwide, with important socio-economical consequences. The critical need to fight blindness is also aided by the large commercial ophthalmic market, which together can be a powerful driving force for clinical translation of novel nanotechnology-based drug delivery approaches. The commercial ophthalmic market in the United States was estimated to be $14 billion in 2009. Blindness and irreversible sight impairment cost an estimated $50 billion each year in the United States. The Ministry of Health in Saudi Arabia, and others in the Middle East have identified ocular diseases as major health issues. Therefore, safe and efficacious nanomedicine approaches may have significant opportunities for translation in the near and intermediate term. The development of drug and gene delivery approaches, appropriately combined with recent discoveries of diseases pathways in AMD and glaucoma, and novel therapeutics, can address many technological road blocks to the efficient treatment of these diseases.

Many intravitreal, polymeric drug delivery implants are already approved by the FDA. These include Retisert (for uveitis) and Ozurdex (for macular edema associated with uveitis and diabetic macular edema). Iluvien, a free floating, non-erodible implant that releases fluocinolone acetonide is undergoing clinical trials in the US and Europe. In fact, the first gene therapy clinical trials (Phase I) for adeno-associated virus (AAV)-based gene therapy is already underway at the King Khaled Eye Specialist Hospital in Riyadh, Saudi Arabia. This is for patients with mutations in MERTK gene leading to retinitis pigmentosa. The MERTK mutation is a leading cause of autosomal recessive form of RP in people of Middle Eastern decent.

A recent study by the Association for Research in Vision and Ophthalmology (ARVO) outlined the important aspects of translating basic research to clinical translation for ocular diseases and identified the five barriers that need to be overcome. These included: (1) development of an effective and safe product; (2) identifying the best mode of delivery and the right delivery system; (3) assessment of the product in appropriate animal models, recognizing the differences in the human equivalence between different small and large animal models; (4) appropriate design of the clinical trials to attain a satisfactory end point; (5) commercialization through spin-offs or finding a commercial partner. The design of the nanomedicine products should involve a ‘quality-by-design’ approach, a stringent evaluation of safety and efficacy by a multi-disciplinary group, including regulatory agencies in the early planning stages for clinical trials. New regulatory guidelines are being developed with close cooperation between US and Europe. The intense interest in nanotechnology also brings with it tremendous public and regulatory scrutiny. Successful translation of nanomedicine
efforts would require a careful risk-benefit analysis, which is often skewed towards risk when it comes to novel nanotherapies. The emerging translational efforts in Saudi Arabia and the Middle East can bring the best of these approaches to have a positive impact on the health of the people, in addition to creating new commercial and research opportunities.

Footnotes

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Figures and Tables
Figure 1

A schematic representation of some nanoparticle platforms used as ocular drug delivery systems. (a) Liposomes (~100-400 nm) are small spherical artificial vesicles typically made with lipid bilayers, (b) Nanoparticles (~20-200 nm) are typically made with biodegradable polymers for sustained drug release, (c) Nanocapsules (~10-1000 nm) can encapsulate relatively large amounts of drugs and nucleic acids such as DNA, microRNA, siRNA and shRNA, (d) Micelles (~10-100 nm) are self-assembled amphiphilic particles that can encapsulate both lipophilic or lipophobic drugs stabilized by surfactants, (e) Dendrimers (~3-20 nm) are monodisperse macromolecules that can be used to encapsulate or covalently conjugate drugs, targeting moieties & imaging agents, (f) Nanoconjugates are polymers to which drug molecules are covalently conjugated.

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