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Nanomedicines for Back of the Eye Drug Delivery, Gene Delivery, and Imaging

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Abstract

Treatment and management of diseases of the posterior segment of the eye such as diabetic retinopathy, retinoblastoma, retinitis pigmentosa, and choroidal neovascularization is a challenging task due to the anatomy and physiology of ocular barriers. For instance, traditional routes of drug delivery for therapeutic treatment are hindered by poor intraocular penetration and/or rapid ocular elimination. One possible approach to improve ocular therapy is to employ nanotechnology. Nanomedicines, products of nanotechnology, having at least one dimension in the nanoscale include nanoparticles, micelles, nanotubes, and dendrimers, with and without targeting ligands, are making a significant impact in the fields of ocular drug delivery, gene delivery, and imaging, the focus of this review. Key applications of nanotechnology discussed in this review include a) bioadhesive nanomedicines; b) functionalized nanomedicines that enhance target recognition and/or cell entry; c) nanomedicines capable of controlled release of the payload; d) nanomedicines capable of enhancing gene transfection and duration of transfection; f) nanomedicines responsive to stimuli including light, heat, ultrasound, electrical signals, pH, and oxidative stress; g) diversely sized and colored nanoparticles for imaging, and h) nanowires for retinal prostheses. Additionally, nanofabricated delivery systems including implants, films, microparticles, and nanoparticles are described. Although the above nanomedicines may be administered by various routes including topical, intravitreal, intravenous, transscleral, suprachoroidal, and subretinal routes, each nanomedicine should be tailored for the disease, drug, and site of administration. In addition to the nature of materials used in nanomedicine design, depending on the site of nanomedicine administration, clearance and toxicity are expected to differ.

Keywords

Nanotechnology; Drug delivery; Gene delivery; Imaging; Controlled release; Stimuli responsive delivery

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1. Implications of Nanomedicines in Posterior Segment Ocular Drug Delivery

As the proportion of ageing in the population continues to grow, the number of individuals suffering from age related ocular diseases will significantly increase over the next few decades and is expected to increase health costs. Significant strides in our understanding of the molecular pathways that are involved in ocular diseases of the back of the eye have enabled the discovery of new biomarkers and drugs for managing these diseases. However, treatment and management of these diseases continues to be a challenging task due to the diversity in disease presentation amongst patients, lack of surrogate biomarkers that can reliably detect early stages of disease, and anatomical and physiological ocular barriers that limit entry of therapeutic agents. There is no therapeutic or prosthetic that can fully restore vision loss and current diagnostic techniques are unable to detect early stages of disease. Therefore, development of improved therapeutics and diagnostics for the back of the eye diseases is receiving immense recognition and resource allocation from pharmaceutical and biotechnology firms alike. More effective therapeutics and diagnostics will not only improve the treatment and management of diseases of the posterior segment of the eye, but are expected to reduce healthcare costs for a greater public benefit.

Nanotechnology is a current hot-topic issue because of its potential to significantly impact several fields related to chemistry, engineering, biology and medicine. It has received significant research attention over the past decade. Nanoscience refers to the study of material characteristics (physical, chemical and biological) at the nanometer scale. Nanotechnology encompasses the design, fabrication, or application of the material with at least one dimension in the nanometer scale (Raghava et al., 2008). Some materials at the nanoscale exhibit unique electrical, mechanical, magnetic, optical, and chemical properties. These properties may be put to use in improving the physical, physicochemical or biological aspects of drugs as well as drug delivery systems.

The use of nanotechnology is being investigated for several different ophthalmic applications for back of the eye diseases. These applications include improved drug and gene delivery to the target tissue for the treatment of posterior segment disorders (e.g., choroid and retina), improving diagnostics, and retinal prosthesis. Various drug delivery devices in the nanoscale including nanoparticles, nanotubes, micelles, liposomes, dendrimers, and nanoconjugates, collectively referred to as nanomedicines or nanoparticles in this article, have been investigated for enhanced drug delivery (Amrite and Kompella, 2006; Raghava et al., 2008). A summary of the types of nanoparticles (bioadhesive, sustained release, stealth, targeted, and stimuli responsive) that may be used for different routes of administration is shown in Figure 1 along with future nanosystems for back of the eye diseases that can be envisaged.

Current treatments for posterior segment diseases suffer from significant disadvantages including frequent administration and potential for injection related adverse events, which threaten patient quality of life. Nanomedicines can be designed to significantly improve patient compliance and ultimately, therapeutic outcome. Nanoparticles are increasingly being used in diagnostics where they offer enhanced properties like increased sensitivity and selectivity as compared to conventional diagnostic agents. Certain nanoparticles can gain access to the intracellular environment due to the process of endocytosis and are being pursued extensively as a safer alternative to viral gene-delivery. Nanodevices are also being pursued in the development of retinal and cortical prostheses and retinal implants. Restoring vision represents a unique challenge since the device or implant must not only be biocompatible and stable with the surrounding tissue, but it must interact electrically with remaining nerve cells to communicate with the brain for presenting images.

First, this review focuses on the fundamentals of nanoparticle behavior in the eye (Section 2) before considering the types of nanoparticles that are under investigation (Section 3), and routes of administration that are used to deliver nanoparticles to the back of the eye (Section 4). Specific examples of applications of nanotechnology for treatment and management of back of the eye diseases are described in Section 5. A case study is discussed in Section 6 for the periocular route to demonstrate the parameters that influence delivery to the choroid and retina, the target tissues for back of the eye diseases. When designing therapeutics and diagnostics it is important to consider their potential toxic effects and therefore, Section 7 describes the preliminary in vitro and in vivo studies that have investigated nanoparticle toxicity. After describing the behavior of nanomedicines in the eye, the use of micro- and nano-electromechanical systems (M/NEMS) in the fabrication of films, implants, and microneedles are briefly discussed (Section 8). The last section (Section 9) reflects on the future of nanoparticles for treatment and management of back of the eye diseases and recognizes alternative approaches including implants, films, and other devices.

2. Fundamentals of Nanosystems

2.1 Enhanced cell uptake and gene delivery

A key attribute of nanotechnology is the design of delivery systems with at least one of the dimensions in the range of 1 to 1000 nm. In nature, a range of solutes and organelles exist in this size range and beyond. Biological molecules range in size from less than a nanometer to tens of nanometers. For instance, pathogens such as viruses are in the range of ten to a few hundred nanometers and bacteria are in the range of a few hundred nanometers to micrometers. Mammalian cells typically have a diameter of a few microns and their organelles are within the nanometer range. A nanometer is a dimension that is one billionth of a micron, which is much smaller than the dimension of a cell. Thus, it is intuitive that cells and organelles can be better targeted with nanoparticles as opposed to micron size delivery systems in general, if the goal is enhanced cellular uptake of the payload. Nanoparticles have some unique advantages over larger size particulate systems. These advantages include enhanced cellular uptake of nanoparticles as compared to larger particles. This uptake process is most likely by endocytosis (Sahay et al., 2010) and has been utilized for gene delivery (Oyewumi and Rice, 2006). Aukunuru and Kompella (Aukunuru and Kompella, 2002) investigated the uptake of different sized nanoparticles (20, 100, 500, 1000, and 2000 nm) into retinal pigment epithelial (RPE) cells. There was a clear size dependency in uptake with the smaller particles having more uptake as compared to larger particles (Figure 2). Aukunuru and colleagues utilized biodegradable poly(lactide-co-glycolide) (PLGA) nanoparticles to deliver an anti-sense VEGF oligonucleotide to RPE cells (Aukunuru et al., 2003).

The nanoparticle formulation delivered significantly higher levels of the oligonucleotide within the cells as compared to a solution formulation and was also more effective in reducing VEGF secretion and VEGF mRNA expression in these cell types. Mo and colleagues developed nanoparticles for delivering Superoxide dismutase (SOD1) gene to RPE cells as well as to retinal tissue in vivo (Mo et al., 2007). The nanoparticle formulation had greater transfection efficiency compared to the commercially available transfecting agent lipofectamine. In addition, the nanoparticles could transfect retinal cells in vivo after intravitreal injection in mice as demonstrated by detectable expression levels of the fusion protein (GFP-SOD) in the retina.

2.2 Reduced settling velocity

All other factors (density of the particles, viscosity of the medium) being equal, the settling velocity of particles in a medium increases with increase in particle size. In terms of drug

products, this parameter is of importance on at least two counts. One, when a nanosuspension as opposed to microsuspension is prepared as a drug product (e.g., eye drop), the nanosuspension will stay suspended uniformly for a much longer period than a microsuspension, ensuring uniformity of dose delivered long after resuspension. Second, if the suspension is injected into a relatively static fluid compartment (e.g., vitreous humor or cerebrospinal fluid) compared to the blood stream, particles will stay suspended for a prolonged period before coming in contact with the surrounding tissue (e.g., retina in case of the vitreous humor). With such ability to remain suspended in these compartments, it can be anticipated that nanoparticles will have limited contact with cells capable of engulfing and clearing the particles, in the absence of cell migration towards nanoparticles, allowing sustained nanoparticle retention. Indeed, at least one report indicates that small nanoparticles have a more prolonged vitreal half-life compared to microparticles (Figure 3) (Sakurai et al., 2001).

2.3. Interactions with large area of cell surface

When nanoparticles are proximal to a cell surface, Brownian motion may contribute to their superior efficacy. Brownian motion is the extent of random walk or movement experienced by a solute or particle in response to small thermal fluctuations in the fluid compartments. The extent of Brownian motion decreases with an increase in particle size. With a greater ability to randomly move around in extracellular fluid compartments, once in the vicinity of tissues, it can be anticipated that nanoparticles may collide more frequently with any adjacent cellular membrane compared to larger microparticles. We speculate that this may be an advantage for receptor targeted nanoparticles, which require more frequent collisions with the receptor for enhanced activity and/or delivery. Although settling due to gravity is expected to allow more rapid contact of larger particles with tissue, Brownian motion and their amplified surface area for a given dose might allow small nanoparticles come in contact with a larger tissue surface, resulting in interactions with a greater number of cell surface receptors.

2.4. Larger drug particles sustain release for longer periods

Drug amount on the particle surface is a critical determinant of drug release. If a pure drug particle is made without any polymeric or carrier material that influences its release, the extent of drug present on the particle surface controls the rate of drug dissolution and hence, release. For instance, a drug molecule with a diameter of 1 nm and unit density, when made into a 1 nm particle, the entire drug content in the particle is exposed to biological media upon administration. Such a particle has a high opportunity to go into solution compared to a particle of 100 nm size of the same material, which has an estimated 0.03% of the drug on the particle surface. As a consequence, the 100 nm particle slowly releases the drug compared to smaller nanoparticles of the drug. Thus, if the goal is to provide sustained release of the drug in the biological compartment, a larger particle is more appropriate. If cell entry is readily achieved by the solubilized drug, a large micron size particle may be most appropriate for very prolonged delivery, in treating chronic diseases such as diabetic retinopathy and macular degeneration. Indeed, we determined that diclofenac in a sodium salt solution, a soluble form of the drug, disappears from the rabbit vitreous humor with a half-life of 2.9 hours, as opposed to a micron-sized (5 μ m) suspension of diclofenac, which sustained diclofenac levels with an apparent half-life of 24 days (Durairaj et al., 2009). The suspension (containing 5 μ m particles) requires more time for dissolution, and hence, it releases the drug for prolonged periods, which results in greater apparent half-life for the drug in the vitreous humor. Thus, larger particles of pure drugs with lower surface to volume ratio sustain drug release for more prolonged periods than smaller particles.

2.5. Enhanced permeation and retention effect increases nanoparticle tissue accumulation

Although not universal to all human tumors, enhanced permeation and retention effect is now well established for materials of a nano- size. Immunoglobulins, drug polymer conjugates, micelles, liposomes, poly(lactide-co-glycolide) nanoparticles, lipid nanoparticles, and DNA-polymer complexes, all undergo EPR effect (Fang et al., 2011). As per this principle, leaky blood vessels in tumors allow accumulation of macromolecules. Further, tumor clearance of macromolecules is reduced due to impaired lymphatic clearance (Maeda and Matsumura, 1989). Since blood vessels can be leaky in inflammatory and angiogenic diseases including choroidal neovascularization and proliferative diabetic retinopathy, it can be envisioned that an effect similar to EPR may be present in the eye. In a laser-induced rat choroidal neovascularization model, we observed extravasation of intravenously administered nanoparticles in the injured eye, but not normal eye, suggesting enhanced permeation of nanoparticles across leaky vasculature in the eye (Singh et al., 2009).

2.6. Multifunctional nanomaterials

Nanomedicines may offer a multitude of functions as opposed to being passive carriers. For instance, nanomaterials can potentially allow drug delivery as well as diagnosis (theragnostics). One such example is microbubbles carrying a therapeutic agent. The bubbles can serve as contrast agents in ultrasound based diagnosis, in addition to serving as therapeutic delivery systems. Another example is the use of magnetic nanoparticles loaded with a drug payload. These systems can serve as magnetic resonance imaging contrast agents as well as drug delivery systems. In addition to the above multi-functionality for therapy and diagnosis based on imaging, other pharmaceutically relevant multi-functionalities are feasible with nanomaterials. For instance, we determined that a g6 dendritic polyguanidylated translocator (DPT; with six guanidine groups) increases solubility and cell uptake of gatifloxacin, while sustaining its delivery in the eye (Durairaj et al., 2010). g6 DPT dendrimer forms complexes with gatifloxacin via ionic, hydrogen bond, and hydrophobic interactions and increases drug solubility in a dose-dependent manner. Being positively charged, the dendrimer is able to electrostatically bind the negatively charged surface of corneal epithelial cells for enhanced cell entry. Further, the drug-dendrimer complex exhibited sustained drug delivery to eye tissues for up to 24 hours after a single dose. This is an example of a single nanomedicine that has three functions: to improve drug solubility, cell uptake and sustain drug release.

3 Types of Nanoparticles Employed for Back of the Eye Diseases

This section provides an introduction into the types of nanoparticles that have been investigated for posterior segment diseases (Figure 4) and describes key features of each. More information regarding ophthalmic nanoparticle delivery systems have been published by Diebold and Calonge (Diebold and Calonge, 2010). Further, fabrication methods for ophthalmic delivery systems are published in the book entitled Nanoparticle Technology for Drug Delivery by Gupta and Kompella (Gupta and Kompella, 2006).

3.1 Polymeric nanoparticles

Polymeric nanoparticles are being studied extensively as targeted and sustained drug delivery systems for effective drug delivery to the target with minimum side effects. Poly (DL-lactic-co-glycolic acid) (PLGA) is of particular interest for ophthalmic use since it is present in Food and Drug Administration (FDA) approved drug products and because it is biodegradable and biocompatible. In addition, some FDA approved ophthalmic products including Ozurdex®, a dexamethasone biodegradable implant, contain PLGA as an excipient. Other polymeric compounds that are currently being used in drug products

include polyvinyl alcohol, poly (ethylene-co-vinyl acetate), polyimide, and poly (methyl methacrylate).

3.2 Polymeric nanogels and hydrogels

Nanogels are hydrogels composed of a nanoscale network of hydrophilic polymers, directly loaded with drug by equilibration or swelling in water, followed by reduction in solvent volume and gel collapse, leading to the formation of compactly packed nanoparticles. Nanogels can be used for controlled release of both hydrophilic and hydrophobic drugs by varying the composition and conformation of polymers, and the degree of crosslinking. The kinetics of drug release from the nanogels can also be controlled by varying the degradation rate of the crosslinks and through external stimuli, especially in stimuli-responsive hydrogels, whose degradation may be controlled by external properties such as pH and temperature. Cationic nanogels have been studied especially for gene delivery following spontaneous encapsulation of negatively charged genes and antisense DNA (McAllister et al., 2002; Vinogradov et al., 2004).

3.3 Liposomes

Liposomes are biocompatible and biodegradable lipid assemblies comprising of an aqueous volume surrounded by a phospholipid bilayer, with drugs entrapped in the core or bilayer. Liposomes can vary in size from nanometers to micrometers. They can be uni- or multilamellar, and can vary considerably in their size, surface charge, lipid composition, fluidity of the bilayers, and method of preparation. Liposomes are versatile in drug loading properties. For instance, they can encapsulate hydrophilic drugs in the core and lipophilic drugs in the bilayer. Ionic drug loading can be further facilitated by using cationic or anionic lipids. The encapsulated drug is usually released by passive diffusion, vesicle fusion, or vesicle disruption. Currently, verteporfin is a clinically used, systemically administered, liposomal photodynamic therapeutic for the treatment of subfoveal choroidal neovascularization (Ruiz-Moreno et al., 2006). Further, intravitreally delivered liposomal formulations have also been found to have minimal tissue toxicity and enhanced drug vitreal half-life by reducing drug elimination from the vitreous cavity (Alghadyan et al., 1988; Peyman et al., 1989).

3.4 Micelles

Micelles are self-assemblies of amphiphilic molecules, similar to liposomes, but exhibiting a higher ordered structure and composed of monolayers, unlike liposomes which are made up of lipid bilayers. Standard micelles are formed at concentrations greater than the critical micellar concentration, wherein the amphiphiles assemble in the aqueous medium forming a hydrophobic core and a hydrophilic shell. Similarly, based on the ambient solvent and the type of amphiphilic molecule used, different structures of micelles predominate in the formulation, such as standard, reverse and unimolecular micelles (Trivedi and Kompella, 2010). In this review we address only nanosized micelles prepared from amphiphilic polymers, with the potential to target posterior segment ocular disorders. Polymeric micelles, typically contain polyethyleneglycol, due to its ability to prevent aggregation, and prolong circulation time since they are not recognized by liver macrophages and hence, are not phagocytosed (Klibanov et al., 1990). Polyethyleneglycol, the component that forms the micellar shell, is usually chosen such that its molecular weight is higher than that of the hydrophobic core forming molecule (Kwon, 2003). This results in micelles having critical micellar concentrations in the micromolar range, and generally smaller than 100 nm (Ashok et al., 2004; Torchilin, 2002). These micellar systems are thus thermodynamically stable upon dilution, and possess reduced toxicity, compared to surfactant-based traditional micelles, with very high critical micellar concentrations.

3.5 Dendrimers

Dendrimers are tree-like branched polymers that are typically water soluble. Dendrimers can have neutral, negative, or positive functional groups at the terminus of their branches. Drugs can be either entrapped in the dendrimer network through hydrogen bonds, hydrophobic interactions, and ionic interactions or they can be conjugated through covalent bonds. Polyamidoamine dendrimers are the most investigated dendrimers for drug and gene delivery. Administration of PAMAM dendrimer encapsulating carboplatin in the subconjunctival space reduced retinoblastoma tumor growth to a much greater extent than drug in solution on day 22 after dosing in a mouse model (Kang et al., 2009). Although PAMAM dendrimer is small in size and expected to be of a size <10 nm, we observed that dendrimer nanoparticles of PAMAM dendrimer form approximately 260 nm particles, as measured by dynamic light scattering. Since 20 nm particles disappear rapidly from the periocular space, while those 200 nm are retained for prolonged periods (Amrite and Kompella, 2005), we speculated that PAMAM loaded with carboplatin persisted for prolonged periods in the subconjunctival space and sustained drug delivery to the tumor.

3.6 Chitosan nanoparticles

A polysaccharide derived from the natural compound chitin, found in the exoskeleton of crustaceans, chitosan has the unique property of mucoadhesion due to surface positive charge, which allows it to reside on the ocular surface for prolonged time after topical administration (Enriquez de Salamanca et al., 2006). The hydroxyl groups in the polysaccharide structure of chitosan compete with water on the surface of tissues and hence, providing additional means of bioadhesion. Section 5.1 discusses nanoparticles capable of bioadhesion and rapid internalization in greater detail.

The use of chitosan nanoparticles has been primarily limited to improving drug and gene delivery for treatment of anterior segment diseases (De Campos et al., 2001), with limited focus on application in posterior segment diseases. Hybrid PLGA-chitosan nanoparticles loaded with a plasmid encoding plasminogen kringle 5, a fragment of plasminogen with antiangiogenic activity, were able to efficiently deliver the plasmid to the retina after intravitreal injection as plasminogen kringle 5 expression was found in the retina up to 2 weeks after injection (Jin et al., 2011). Further, the hybrid PLGA-chitosan nanoparticles loaded with the plasmid exhibited RPE uptake and inhibition of inflammatory cytokines was observed as early as 72 hours after treatment.

3.7 Protein nanoparticles

Albumin, a protein primarily located in blood plasma, offers unique advantages over other types of materials for nanoparticles since it is an endogenous protein. Indeed, an albumin nanoparticle formulation of paclitaxel (Abraxane®) was approved by the FDA for breast cancer treatment. Human serum albumin has several properties that make it an attractive material for drug delivery. For instance, human serum albumin increases drug solubility, decreases drug toxicity, protects drug against oxidation, and increases drug half-life (Kragh-Hansen et al., 2002). Cellular uptake of human serum albumin is mediated by the glycoprotein 60 (albondin) receptor (Schnitzer and Oh, 1994; Vogel et al., 2001). In the eye, serum albumin filtrates through the blood-aqueous barrier and is the major component (50% of total proteins) of aqueous humor and provides important metabolites for the growth of rapidly dividing lens epithelial cells (Tripathi and Tripathi, 1989). Therefore, the desirable drug delivery properties of albumin in combination with its endogenous nature make it a potential candidate for drug delivery, gene delivery, and diagnostic imaging of the back of the eye.

Albumin nanoparticles loaded with a plasmid encoding superoxide dismutase exhibited an 84% transfection efficiency, 5-fold higher than lipofectamine (Mo et al., 2007), which is likely due to the ability for albumin to undergo receptor mediated cell uptake. These in vitro results translated into detectable expression levels of superoxide dismutase in the mouse eye following intravitreal injection for 48 h whereas plasmid solution injection had no protein detected.

4. Routes of Drug Delivery to the Back of the Eye

The key routes for delivering drugs to the posterior segment of the eye include topical, systemic, periocular, and intraocular (e.g., suprachoroidal and intravitreal) routes. Figure 1 provides a summary of the routes of administration and drug delivery strategies employed in the delivery of nanomedicines.

The retina is located in the posterior segment of the eye and drug delivery to the retina is at best miniscule from the topical route of delivery due to a multitude of anatomical and physiological barriers (Kompella et al., 2010). Systemic routes such as intravenous and oral routes can deliver drugs to the retina, but delivery is limited by the presence of inner and outer blood-retinal-barriers, blood dilution of the drug, and additionally, gastrointestinal barriers in the case of oral route. This necessitates administration of large systemic doses to deliver effective concentrations of the drug to the retina. Such large doses may cause systemic side effects. Intravitreal injection, an intraocular delivery mode, provides the highest bioavailability of the drug to the retina because of the proximity of the vitreous to the retina. However, intravitreal injections are associated with complications such as retinal hemorrhage, retinal detachment, endophthalmitis, and cataracts (Jonas et al., 2008).

Periocular routes of delivery include subconjunctival, sub-Tenon, retrobulbar, peribulbar, and posterior juxtасcleral routes, which involve injecting outside the globe of the eye and in the proximity of the sclera to exploit high scleral permeability for retinal drug delivery. This route of administration may therefore reduce the risk of injection related complications associated with intravitreal administration. The delivery of the drug to the retina is most likely achieved by the diffusion of the drug through the sclera, choroid, and the retinal pigment epithelium. This mode of delivery to the retina is aptly known as the transscleral route of delivery of drugs to the eye (Raghava et al., 2004).

Suprachoroidal delivery of pharmacologic agents is a newer method of drug delivery under investigation, with a potential to minimize systemic adverse effects while localizing higher levels of the drug to the posterior ocular tissues. It has been directed at primarily treating choroidal, retinal and optic nerve diseases such as choroidal neovascularization, age-related macular degeneration, and diabetic retinopathy. Also, intraocular pressure (IOP) reduction in glaucoma treatment is a potential application for this approach.

The route of injection for a given therapeutic or diagnostic use will depend upon the nature of the disease and therapeutic/diagnostic. For example, treatment of choroidal neovascularization may be best achieved by delivering the drug via the suprachoroidal route, which provides drug in close proximity to the choroid. However, if the goal is to design a non-invasive treatment for a short-duration, the topical route may be the best approach. For delivering drug for longer periods of time via the topical route, bioadhesive nanomedicines may be employed to reduce precorneal clearance and increase corneal surface time. The sections below describe each of these different routes of administration as well as the advantageous and disadvantageous of each route.

4.1 Topical

Several earlier attempts aiming at eye drop delivery for back of the eye drug delivery met with little clinical success due to the presence of formidable membrane barriers and elimination pathways on the eye surface (Maurice, 2002). Further, the normal intraocular fluid flow gradient is from vitreous to aqueous, and therefore, topical application of compounds are delivered successfully to the anterior segment, but are practically non-transferable to the retina. Although nanoparticle size is generally perceived to be an advantage in delivery, even a dimension of 20 nm may be inadequate for high dose delivery to the cornea (Kompella et al., 2006). Following topical eye drop administration to ex vivo bovine eye, about 2% of the 20 nm particles could be delivered to the cornea in 5 min. In human cornea, which occupies a much smaller area, even lower dose delivery is anticipated. However, after removal of corneal epithelium, the delivery of nanoparticles to cornea was about 20%, suggesting the barrier nature of corneal epithelium for small nanoparticles. Compared to cornea, nanoparticle entry as well as transscleral transport is superior in the conjunctiva, which might be beneficial for transscleral delivery to the back of the eye (Kompella et al., 2006).

Corneal and conjunctival routes are the two local pathways for drug entry into posterior ocular tissues and blood vessels, from a topical eye drop. Corneal pathway involves entry into the cornea followed by interior tissues including iris, aqueous humor, lens, and iris ciliary body. Conjunctival pathway, on the other hand, involves drug permeability across conjunctiva followed by entry into the sclera, choroid, retinal pigment epithelium, and retina. Drug delivery to the back of the eye from an eye drop primarily occurs via the conjunctival pathway. A detailed analysis of entry pathways for a drug applied as an eye drop to reach the posterior segment has been provided by Maurice (Maurice, 2002). Furrer and colleagues demonstrated that a TNF- α inhibitory single-chain antibody fragment (scFv), was delivered to the vitreous, neural retina, and choroid-RPE of New Zealand White rabbits, after repeated topical administration of eye drops every hour up to 10 hours (Boddu et al., 2010). The peak drug level (C_{max}) achieved in the vitreous humor was about 25-fold higher than the aqueous humor. In addition, the peak drug level in the vitreous humor after topical administration was 4.7-fold higher than after intravenous administration. The conjunctival pathway was identified as the key pathway for the back of the eye delivery of the TNF- α antibody fragment (Mitra et al., 2011). Conjunctival entry and transport can be further enhanced by nanoparticles functionalized with peptide and protein ligands such as deslorelin and transferrin that recognize cell surface receptors and allow nanoparticle internalization (Kompella et al., 2006; Trivedi et al., 2012). Indeed a recent study found that 1 week after topical dosing in a rat model, drug levels in the rat choroid-RPE were significantly higher for transferrin functionalized nanoparticles loaded with diclofenac, when compared to diclofenac in solution (Kompella and Upadhyay, 2012).

4.2 Intravenous

Intravenous administration is routinely used in treating cancers with nanomedicines such as liposomes and albumin nanoparticles. Matsumura and Maeda identified enhanced permeability and retention effect (EPR) associated with polymer conjugates of small size that evade rapid renal elimination (Matsumura and Maeda, 1986). According to this principle, the drug conjugates escape leaky vasculature in tumors (enhanced permeation) and remain in the tumors due to reduced clearance (enhanced retention). The prolonged circulation of drug conjugates further assists drug distribution to the tumor tissue. Likewise, small molecule-, peptide-, and plasmid-loaded nanoparticles can be administered systemically to target specific sites via the bloodstream. Singh and colleagues investigated the application of functionalized nanoparticles in the intravenous delivery of an anti-VEGF intraceptor plasmid Flt23K, for the treatment of choroidal neovascularization (Singh et al.,

2009). The investigators engineered transferrin-, RGD-peptide-, or dual-functionalized PLGA nanoparticles to specifically target anti-VEGF intraceptor plasmid Flt23K to CNV lesions. The plasmid-loaded transferrin-, RGD-peptide, and dual-functionalized nanoparticles transfected RPE cells and endothelial cells in vivo, significantly reducing the CNV area by 54, 64, and 51%, respectively, in comparison to the plasmid-loaded non-functionalized nanoparticles; and by 64, 71, and 61%, respectively, in comparison to the naked-plasmid treated group. In addition to the above, confocal microscopy of the posterior ocular segment indicated confinement of nanoparticles to the laser-treated CNV-induced eye but not the contralateral eye, suggesting that surface functionalization targeted and highly localized drug delivery to the disease site. Thus, efficient drug targeting to the posterior ocular segment can be achieved by intravenous administration, using nanoparticles that target the disease site, in order to enhance biodistribution to the target and reduce high-dose requirements. Further, unlike intraocular drug delivery, intravenous administration is likely safer for the eye.

4.3 Periocular

The periocular route of delivery has received attention as a potentially safe alternative to the intravitreal injection, for delivering drugs to the retina. A comprehensive review of the periocular route of administration has been presented by Singh and Kompella (Raghava et al., 2004). Periocular route is also a potential route for gene delivery since intravitreal injections may result in undesired transfection of cells important for vision (Mac Gabhann et al., 2007).

Although it is certain that the retinal bioavailability from the periocular route is lower than the intravitreal route (Raghava et al., 2004), potent drugs (such as carboplatin) administered by the periocular route can most likely reach therapeutic drug levels in the retina. Pre-clinical studies have demonstrated that subconjunctivally administered carboplatin is effective in inhibiting tumor progression in a dose-dependent manner in a rabbit model of retinoblastoma (Murray et al., 1997). Van Quill and colleagues also demonstrated that the subconjunctival route is an effective means of delivering carboplatin for the treatment of retinoblastoma (Van Quill et al., 2005). In addition, it has been demonstrated in murine retinoblastoma models that subconjunctival anecortaveacetate is effective in reducing tumor burden when given alone, and increases the effectiveness of carboplatin when given as an adjuvant to subconjunctival carboplatin therapy (Jockovich et al., 2006).

Clinical effectiveness of the periocular mode of administration for the treatment of choroid-retinal disorders has also come to light in the past decade. A clinical study investigated the therapeutic effectiveness of posterior juxtascleral infusion of triamcinolone acetonide in 18 patients (22 eyes) with refractory diabetic macular edema (Veritti et al., 2009). The study found that there is visual acuity improvement by one line in 64% of the treated patients and visual acuity improvement by 3 lines in 27% of the treated patients post one-year. In a large clinical study in patients with uveal melanoma (N= 163), following periocular injection of triamcinolone (40 mg at 0, 4 and 8 months) as an adjuvant to plaque radiotherapy, significantly lower occurrence of OCT evident macular edema was observed as compared to controls (no triamcinolone and plaque radiotherapy only) (Hazard ratio: 0.45; 95% Confidence Interval: 0.19–0.70, $P < 0.001$) (Horgan et al., 2009). In addition, moderate and severe vision loss occurred less frequently in the triamcinolone treated group as compared to controls.

The disposition of particles administered by the subconjunctival route of administration highly depends upon particle size. For instance, Amrite and Kompella found that negatively charged carboxylated fluorescent polystyrene particles in the 20–2000 nm size range exhibited negligent penetration into the ocular tissues after subconjunctival administration

(Amrite and Kompella, 2005). Nearly the entire dose of 200 nm and 2 μ m particles persisted in the subconjunctival space for 60 days whereas 20 nm particles were cleared rapidly via circulation, with only 15 and 8% of the administered dose remaining after 1 and 7 days, respectively. Therefore, larger particles are retained for much longer periods in the subconjunctival space compared to smaller particles and hence, larger particles are more appropriate for sustained delivery by the subconjunctival route.

4.4 Intravitreal

Intravitreal drug delivery has been one of the most sought after routes for ocular therapeutics intended for the posterior eye due to its proximity to the retina, choroid, and RPE. Apart from comfort and biocompatibility, intravitreal delivery systems should possess attributes such as capability of achieving high drug loads, safety from rupture and retinal detachment, and ease of administration. Intravitreal delivery of nanomedicines is expected to enhance localization of the drug to the target tissue and sustain drug release for a prolonged period of time, thereby alleviating repeated injections which otherwise exacerbate the clinical condition. However, frequent intravitreal injections have been associated with injection related adverse events such as retinal detachment and endophthalmitis (Ladas et al., 2009). Further, an increase in intraocular pressure has also been associated with intravitreal administration (Young et al., 2001). Therefore, although intravitreal administration delivers drug in close proximity to the target diseased tissues, the potential disadvantages of this route should be considered.

Several pre-clinical studies have been conducted in vivo, for small molecule-, peptide- and plasmid-loaded nanoparticles in treating neovascularization and retinal degeneration. Efficacy of intravitreally injected controlled release bevacizumab-loaded PEG (b-PEG) and PLGA (b-PLGA) nanoparticles was assessed in laser-induced CNV rat model (Pan et al., 2011). In addition to successful retention of bevacizumab anti-angiogenic activity, the investigators also noticed that a single dose of b-PEG sustained bevacizumab release for as long as eight weeks, decreasing CNV area by 40.9% at the end of 4 weeks and 36.3% at the end of 8 weeks. Likewise, a single intravitreal dose of b-PLGA reduced CNV area by 34.8% and 16.5% at the end of 4 and 8 weeks, respectively. In all groups, CNV lesions were induced 2 weeks prior to harvesting. In another study conducted on a laser-induced CNV rat model (Jin et al., 2011), PLGA nanoparticles loaded with plasminogen kringle-5 (K5-NP) were injected intravitreally. A single dose of K5-NP resulted in significant expression of K5 in the retina for up to 2 weeks, leading to reduction of VEGF levels, suppression of angiogenesis and retinal vascular leakage, and a 40% decrease in CNV area compared to the untreated group. Similarly, a single dose of intravitreally injected K5-NP in streptozotocin-induced diabetic rat model (Park et al., 2009) has been shown to inhibit growth of endothelial cells and attenuate VEGF and intracellular adhesion molecule-1 overexpression, in addition to significantly inhibiting leukostasis and ischemia-induced retinal neovascularization, for at least four weeks. Besides the successful use of PLGA for encapsulation of peptides and plasmids, endogenous proteins like human serum albumin (HSA) have also been evaluated in preparing nanoparticles (Mo et al., 2007). Cu, Zn superoxide dismutase (SOD1) gene-loaded HSA nanoparticles prepared by desolvation crosslinking method, presented high encapsulation efficiency (84%) and sustain the expression of the fusion protein in vivo for up to 48 hour following a single intravitreal dose of 130 ng. In addition to being a safer biodegradable option, nanoparticles prepared with an endogenous protein such as HSA may be less prone to degradation in the vitreous humor. In conclusion, the intravitreal route has received considerable attention due to its ability to deliver drug in close proximity to the retina and choroid and has shown promise in sustaining drug and gene delivery for effective treatment.

4.5 Suprachoroidal

Recently, the suprachoroidal space is being explored as a potential site of drug delivery for the treatment of posterior segment ocular disorders (Einmahl et al., 2002; Gilger et al., 2006; Gilger et al., 2010; Jiang et al., 2007; Jiang et al., 2009; Olsen et al., 2006; Olsen et al., 2011; Patel et al., 2011; Peden et al., 2011; Touchard et al., 2012). Theoretically, the suprachoroidal space is a preferred site for drug delivery to the posterior tissues such as choroid, RPE and macula, due to its non-interference with the optical pathways and improved diffusional access to the choroid.

Drug diffusion from the suprachoroidal space obviates conventional restrictive kinetic barriers such as the internal limiting membrane and the sclera, otherwise seen in intravitreal and transscleral delivery, respectively. Several studies have pointed that the diffusional kinetics from the suprachoroidal space could be optimized using sustained release formulations such as nano- and micro-systems (Gilger et al., 2006; Olsen et al., 2006). It is noteworthy that macromolecules such as bevacizumab are rapidly removed from the suprachoroidal space (Olsen et al., 2011) and hence optimally formulated particulate matrix would perhaps sustain release and activity for longer duration. The investigators also found significant differences in the immune responses elicited by the intravitreal and suprachoroidal routes of administration. Intravitreally administered bevacizumab showed a granulomatous reaction (vitritis and vasculitis) in contrast to no inflammation when delivered by the suprachoroidal route. Olsen and colleagues used microcannulation injections to successfully deliver bevacizumab in vivo into the suprachoroidal space of porcine eyes (Olsen et al., 2011). Patel and colleagues used hollow microneedles to deliver nanoparticle and microparticle suspensions in the suprachoroidal space of rabbit, porcine, and human ex-vivo eyes (Patel et al., 2011). In the latter study, hollow microneedles as long as several hundred micrometers were demonstrated to be a minimally invasive strategy for suprachoroidal delivery, capable of localizing the drug to the adjacent choroid and sclera. Suprachoroidal delivery of 20 and 1000 nm FluoSpheres into excised porcine eyes resulted in the spread of 1000 nm particles within only the suprachoroidal space, whereas 20 nm particles moved through the sclera to a much greater extent. In addition to the influence of particle size on the success of suprachoroidal delivery, elevated infusion pressure and increased microneedle length increase the rate of success of suprachoroidal delivery (Patel et al., 2011) (Figure 5). The rationale for this result is that elevated intraocular pressure reduces tissue surface deflection during microneedle injection and thereby increases the depth of penetration into sclera.

Suprachoroidal delivery is thus anticipated to offer unique advantages in addition to being safe and efficacious in localizing drug levels for the treatment of choroidal, retinal and macular diseases such as choroidal neovascularization, diabetic retinopathy, and retinal degenerations.

5. Applications of Nanotechnology for Back of the Eye Diseases

5.1 Nanoparticles capable of bioadhesion and/or rapid internalization

Topical route of administration, although most commonly employed for ophthalmic drug product use, suffers from rapid precorneal clearance, leading to little or no drug delivery to the back of the eye. This route of administration can primarily benefit from nanomedicines that rapidly adhere to and/or internalize in the eye surface tissues including corneal and conjunctival epithelia. A number of approaches have been assessed to improve ocular surface adhesion including the use of polyethylene glycol, poly(acrylic acid), and other mucoadhesive polymers to enhance corneal or conjunctival adhesion and hence retention (Ceulemans and Ludwig, 2002; Durrani et al., 1995; Hornof et al., 2003; Lehr et al., 1994). However, these approaches allowed limited prolongation in precorneal residence. Positively

charged polymeric materials on the other hand, may allow more prolonged retention on the eye surface. Using DPT dendrimers, we determined that dendrimers gained rapid entry into human corneal epithelial cells and sustained gatifloxacin levels in eye tissues for at least 24 hours (Durairaj et al., 2010). We prepared PAMAM G3.0-PEG-acrylate hydrogel crosslinked with UV light as a positively charged dendrimer hydrogel capable of adhering to mucin and enhancing human corneal epithelial cell uptake of brimonidine and timolol (Holden et al., 2012). Our additional studies in rabbits indicated that when timolol and brimonidine loaded nanoparticles are formulated with the dendrimer hydrogel, single topical application sustained eye surface retention of nanoparticles and drug delivery in various eye tissues for up to 7 days, without inducing any gross toxicity to the cornea or conjunctiva (Yang et al., 2012). Further, dendrimer hydrogel increased the uptake of nanoparticles in cultured human corneal epithelial cells, resulting in about 25% uptake within 5 minutes.

Another approach to enhance the bioadhesive properties of nanoparticles is to associate them with chitosan or its derivatives. Chitosan is a polysaccharide widely assessed for pharmaceutical applications (Thanou et al., 2001b, c). It has also been demonstrated that chitosan adheres to mucus layers in vivo, via electrostatic interactions between the positively charged amino groups within chitosan, and the negatively charged sialic groups of mucin (Hassan and Gallo, 1990; Lehr et al., 1992; Meng et al., 2011; Thanou et al., 2001d), in addition to intermolecular hydrogen bonds. Furthermore, chitosan may enhance the paracellular absorption of hydrophilic molecules by promoting a structural reorganization of the tight junction-associated proteins (Thanou et al., 2001a; van der Merwe et al., 2004a.; van der Merwe et al., 2004b). Cyclosporin A was used as a model drug to demonstrate enhanced drug delivery to the eye surface, in the treatment of dry eye syndrome. De Campos and colleagues studied the ocular disposition of cyclosporine A-loaded chitosan nanoparticles, following topical instillation in rabbits (De Campos et al., 2001). All groups maintained drug levels in cornea, conjunctiva, aqueous humor, and blood at 24 h time point, with the drug levels being significantly higher in cornea and conjunctiva with the nanosuspension of cyclosporine A in chitosan, when compared to the levels achieved by an aqueous solution of cyclosporin A or an admixture of cyclosporine A with chitosan. While drug levels in aqueous humor were equal for all three groups at 24 h, no drug was detectable in this tissue at 48 h for any of the treatment groups. Thus, chitosan nanoparticles can increase ocular surface tissue drug delivery, possibly through mucoadhesion and/or enhanced paracellular delivery.

5.2 Nanomedicines with one or more surface modifications that enhance target recognition and/or cell entry

While plasma membranes of cellular barriers allow passive diffusion of small molecule drugs, they are more restrictive for the entry of hydrophilic small molecules as well as macromolecules, which are typically hydrophilic. For the entry of such poorly permeable molecules, specialized mechanisms of cell entry may exist. For instance, solute transporters may facilitate the entry of small molecules while proteins and large peptides may gain entry into cells via receptor mediated endocytosis. Utilization of receptor mediated endocytosis is an attractive approach to enhance cellular uptake of nanoparticles. It can be envisioned that a few protein or peptide ligands coated on the surface of a nanoparticle might enable receptor interaction followed by internalization of the nanoparticle. Nanoparticles modified on their surface with unique features for enhanced delivery are referred to as functionalized nanoparticles. Such an approach can permit cellular entry of a larger amount of drug encapsulated in the nanoparticle, which may otherwise not enter the cells. While selecting a receptor that is suitable for cell entry of a nanoparticle, it is critical to choose a receptor that undergoes rapid internalization.

Transferrin receptor is well known to undergo internalization and recycling (Ciechanover et al., 1983). Further, our earlier investigations indicated that respiratory epithelial cells internalize deslorelin, a peptide agonist of luteinizing hormone releasing hormone (LHRH) receptor (Koushik et al., 2004). To enhance nanoparticle cell affinity and uptake in ocular tissues, we investigated the potential value of conjugating transferrin or deslorelin on nanoparticle surface (nanoparticle functionalization). Nanoparticles (20 nm, polystyrene) functionalized with transferrin or deslorelin showed rapid (within 5 min) entry into bovine cornea and conjunctiva, and exhibited enhanced uptake as well as transscleral transport in these tissues, when compared to the non-functionalized nanoparticles (Kompella et al., 2006). Such enhanced uptake and transport in conjunctiva, is likely to facilitate transscleral drug delivery to the back of the eye from nanoparticles.

Singh and colleagues compared tissue localization and gene delivery for RGD, transferrin, and dual functionalized nanoparticles with non-functionalized nanoparticles (Singh et al., 2009). Interestingly, the retinal delivery of nanoparticles following intravenous administration was higher for functionalized nanoparticles when compared to non-functionalized nanoparticles (Figure 6).

5.3 Nanomedicines capable of sustained release

Several diseases afflicting the posterior segment of the eye including diabetic retinopathy and age related macular degeneration require chronic treatment for periods of several years. The two main routes that can potentially deliver effective drug levels to the retina include intravitreal and periocular routes of delivery. Frequent injections by either route can lead to serious complications, raising safety concerns and decreased patient compliance. To obviate or reduce injection frequency, sustained drug delivery systems can be employed to deliver drug to the retina for prolonged periods. Several nano- and micro- technology based drug delivery systems have been investigated for transscleral sustained delivery of drugs to the retina. These systems include particulate systems like nano- and micro- particles (Amrite et al., 2006; Amrite and Kompella, 2006; Ayalasomayajula and Kompella, 2005; Carrasquillo et al., 2003; Kompella et al., 2003; Saishin et al., 2003), episcleral implants (Kato et al., 2004; Kim et al., 2005; Kim et al., 2004; Okabe et al., 2007; Pontes de Carvalho et al., 2006), scleral buckles (Lloyd et al., 2001), and fibrin sealants (Martin et al., 2008).

When selecting a slow release drug delivery system, the rationale of selecting nanoparticles as opposed to microparticles is debatable. Although polymeric nanoparticles are effective in sustaining delivery compared to drug in solution, they are generally not as effective as microparticles in sustaining delivery due to their high surface to volume ratio (see Section 2.4). For instance, polylactide nanoparticles (345 nm) loaded with budesonide exhibited a high initial burst release followed by very slow rate of release unlike microparticles (3.6 μ m), which exhibited low burst release followed by greater release rates and more continuous release in vitro (Kompella et al., 2003). Consistent with this, in vivo retinal drug levels were higher with microparticles when compared to nanoparticles on days 7 and 14 after periocular administration. In another in vivo study, periocularly administered microparticles of triamcinolone acetonide maintained drug levels in the retina for up to 2 months, whereas triamcinolone acetonide levels were undetectable for both nanoparticles and drug in suspension (Kadam et al., 2012). These studies demonstrate that microparticles better sustain drug levels in vitro and in vivo compared to nanoparticles or drug in solution. When embedded in a hydrogel, burst release of drug from nanoparticles and microparticles may be reduced and the overall drug release duration may be prolonged. Duvvuri and colleagues demonstrated that ganciclovir-loaded PLGA microspheres dispersed in a PLGA-poly(ethylene glycol)-PLGA aqueous gel sustained drug release in the vitreous for 14 days compared to only 54 hours for ganciclovir solution after intravitreal administration in rabbits (Duvvuri et al., 2007). Although the above hydrogels have demonstrated potential in

sustaining drug delivery, the intravitreal route is an invasive route with potential injection related adverse events such as vitreal hemorrhage and retinal and choroidal detachment. Thus, topically applied hydrogel formulations that enhance back of the eye drug delivery would be ideal.

Hydrogels administered by the subconjunctival route have also demonstrated sustained release and offer a safe alternative to intravitreal injections for targeting the posterior segment. For instance, insulin loaded biodegradable, subconjunctivally implantable hydrogels can effectively sustain drug release and treat neurodegenerative disorders such as diabetic retinopathy (Misra et al., 2009). The team employed poly(*N*-isopropylacrylamide-co-dextran macromer containing multiple hydrolytically degradable oligolactate-(2-hydroxyethyl methacrylate) hydrogel discs as small as 2 mm in diameter, accommodating insulin with a loading efficiency of 98%. In vitro and in vivo studies indicated that these thermoresponsive hydrogels were non-toxic to the R28 retinal neuronal cells or the retina and sustained insulin release for at least 7 days when administered subconjunctivally. Hydrogels of a larger diameter (4.5 mm) showed less burst and slower release compared to hydrogels of a smaller diameter (2 mm). In order to further minimize the invasiveness of hydrogel implantation, subconjunctivally delivered in-situ forming hydrogels may be employed for the treatment of retinal degenerations. Another alternative is the design of nanogels based on similar polymers for administration using narrow gauge needles. Other nanomedicines including liposomes (Fujisawa et al., 2012; M et al., 2012), micelles (Chopra et al., 2012), and dendrimers (Durairaj et al., 2010; Iezzi et al., 2012) have also demonstrated potential in sustaining drug release.

5.4 Nanomedicines capable of enhancing gene transfection and duration of transfection

Cellular entry is critical for delivering macromolecules that act on intracellular targets. This is particularly relevant for RNA therapies such as siRNAs and miRNAs and plasmid delivery for protein expression. Nanoparticles can offer several advantages for the delivery of the above nucleic acids. First, the nucleic acid can be protected from degradation by the ubiquitous nucleases in the body. Our earlier studies indicated that poly(lactide-co-glycolide) nanoparticles protect STAT1 siRNAs (Scheinman et al., 2011) as well as an anti-VEGF intraceptor (Sundaram et al., 2009) from degradation by nucleases. Second, encapsulation in nanoparticles can potentially reduce the interaction of siRNAs with toll like receptors on cell surface, thereby minimizing off target effects of siRNAs (Kleinman et al., 2008). Third, nanoparticles can enhance cell entry of nucleic acids that are otherwise impermeable or have poor cell entry. Several studies have demonstrated improvement in gene delivery with the use of nanoparticles compared to naked gene in addition to biocompatibility (Cho et al., 2012; Jin et al., 2011; Mayo et al., 2010; Qazi et al., 2012). Fourth, nanoparticles, depending on the makeup, can sustain intracellular delivery and hence, effects of the nucleic acid.

It is therefore desirable to design nanoparticles that accomplish all of the advantages described above to ultimately enhance gene transfection and duration of gene transfection. It can be envisioned that based on the case studies provided in Section 5.2, functionalized nanoparticles may be more effective than non-functionalized nanoparticles in gene therapy. Ligands such as transferrin, deslorelin, and RGD peptide are expected to enhance cell entry of nanoparticles and the associated payload into target cells. Functionalized nanoparticles are therefore expected to enhance gene delivery to the cell and hence, therapeutic effect compared to non-functionalized nanoparticles. Singh and colleagues found that after a single intravenous injection of an anti-VEGF plasmid loaded RGD, transferrin or RGD and transferrin functionalized nanoparticles, RGD functionalized nanoparticles exhibited the most gene expression in the RPE compared to all other groups (Singh et al., 2009). Efficacy

results of the anti-VEGF plasmid loaded RGD, transferrin and RGD and transferrin functionalized nanoparticles correlated with delivery studies wherein the RGD functionalized nanoparticles were more effective in inhibiting laser induced choroidal neovascularization compared to transferrin and dual RGD and transferrin functionalized nanoparticles in a rat model. These RGD, transferrin, and dual RGD and transferrin functionalized nanoparticles were effective until day 14 after a single dose, suggesting their ability to sustain drug effects. Thus, functionalized nanoparticles are capable of sustaining gene delivery and efficacy in vivo in back of the eye tissues to a much greater degree than non-functionalized nanoparticles. A comprehensive review on nanoparticle-based gene therapy for retinal diseases by Conley and Naash is an excellent source for more information (Conley and Naash, 2010).

5.5 Nanomedicines responsive to stimuli including light, heat, ultrasound, electrical signals, pH, and oxidative stress

If triggered release of a high dose is desired in a localized manner, stimuli responsive nanomedicines would be useful. Triggers for drug release can include light, heat, ultrasound, electrical signals, pH, and oxidative stress among others. In addition, induction of a phase change such as a transition from a gel to a solution state or solid to a gel state can be used to trigger release due to enhanced diffusion in the transformed phase.

Eye, being a receptacle for light, is particularly suited for light responsive nanomedicines, which was previously reviewed in literature (Christie and Kompella, 2008). Light can induce changes in liposomal membranes such as an elevation in temperature, leading to disruption of the lipid bilayer followed by drug release. Additionally light-sensitive polymeric systems can be envisioned for back of the eye applications. Photodynamic therapy, approved for treating subfoveal choroidal neovascularization, employs liposome encapsulated photosensitizer for localization in the neovascular lesion following intravenous administration. Following accumulation, a nonthermal laser is used to activate the photosensitizer, subsequently leading to occlusion of the neovasculature. pH sensitive liposomes can be triggered to release their content in endosomal compartments that are acidic. Iontophoresis can potentially be employed for triggered release of solutes from nanosystems, by driving suitable ions into the vicinity of the drug delivery system in the eye. Microbubbles, currently employed for diagnostic purposes in cardiovascular medicine, are responsive to ultrasound. The bubbles can be disrupted by ultrasound, resulting in triggered release of any cargo within the microbubble or in the membrane.

Stimuli responsive materials are also under investigation for their potential use in gene transfection as an alternative to viral systems, which are difficult to manufacture and may incur toxicity (Howard et al., 2008). Magnetolipofection, a method utilizing magnetic beads and magnetic forces, was developed as an alternative to viral vectors without compromising high transfection efficiency. Fujii and colleagues prepared magnetite and green fluorescent protein plasmid loaded cationic liposomes and demonstrated the ability of magnetic forces to enhance the cellular expression of green fluorescent protein (GFP) when compared to lipofectamine (Fujii et al., 2010). RPE cells were cultured in flasks placed horizontally or vertically and the cells were treated with either magnetite cationic liposomes loaded with plasmid or lipofectamine with plasmid. A magnet was placed under the culture plate at the center of the well (for horizontal cells) or on the side of the flask (for vertical cells) to induce transfection. Magnetolipofection was applied for different time points (5 or 30 min) and different concentrations of liposomes (2.5, 5, 12.5, 25, and 50 pg/cell). Interestingly, no GFP-positive cells were found in cells transfected with lipofectamine in the horizontal direction whereas cells treated with magnetolipofection had GFP-positive cells for all liposomal concentrations (2.5 pg/cell to 50 pg/cell). In addition, the number of GFP positive cells correlated directly with magnetolipofection dose and duration of magnetolipofection.

However, in the vertical direction, lipofectamine performed as well as 25 pg/cell liposomal dose. Similar to the horizontal direction, the number of GFP-positive cells correlated directly with duration of magnetolipofection. Although lipofectamine performed as well as magnetolipofection in inducing GFP expression, lipofectamine was more toxic than magnetolipofection as indicated by a cytotoxicity assay.

An alternative to triggered release is formation of delivery systems including controlled release systems in situ. It can be envisioned that some of the above triggers can be used to form delivery systems in situ. For instance, UV light sensitive polymers (Holden et al., 2012) can potentially be coadministered with a drug to the eye, followed by UV crosslinking to form controlled release systems in situ.

Some of the nanoparticles described above for drug and gene delivery are summarized in Table 1.

5.6 Diagnostics and imaging of the eye

A number of diagnostic imaging applications for the eye are anticipated based on advances in nanotechnology and nanomedicine. Light signals from the nanoparticles and changes in emission properties (e.g., fluorescence lifetime) as a function of the environment are useful measures that can be used to non-invasively assess biomarkers of disease (Wilson et al., 2005)

One imaging based application is polychromatic angiography. Current diagnostic imaging for detection of retinal angiography is conducted by intravenously administering a fluorescent molecule, fluorescein, which permeates across the blood retinal barrier in diseased eye, but not in a normal healthy eye. This diagnostic technique cannot differentiate early from late stage and is an “all or none” diagnostic of retinal angiography. Therefore, a method that can distinguish early from late stage retinal angiography would allow for a better assessment of disease progression, improve ability to choose appropriate treatment and dose, and allow for monitoring effects of treatment and dose. Tari and colleagues propose a polychromatic angiography diagnostic that will be able to distinguish early from moderate and late stages of disease and hence, offers potentially several advantages in the treatment and management of retinal angiography (Tari et al., 2009). The idea is that small particles extravasate through leaky vasculature or compromised blood barriers in early stage disease, but not larger particles. However, in late stage disease, large and small particles alike will leak through vasculature or permeate across blood barriers and colocalize in the tissue. If the small particles contain a different colored dye than large particles, then the color can be tracked to monitor the stage of disease. The detection of both large and small particles indicates breakdown or dysfunction of blood barriers, whereas the detection of only small particles indicates that lesser breakdown or dysfunction exists.

Polychromatic angiography can also be applied to detect vasculature blockage; for instance, both large and small particles will travel through normal vessels, but when blockage occurs, only the small particles or no particles will travel across the blockage. Therefore, immediately after the blockage either no fluorescence or only fluorescence from small particles will be detected after the blockage and fluorescence of large and small particles will be detected before the blockage. In addition, the polychromatic diagnostic described may also potentially serve as a therapeutic wherein drugs are loaded into the particles and are delivered to the damaged tissue. Different therapeutics can be loaded into the particles such that small particles will contain a different therapeutic than larger particles in order to specialize therapy for each stage of disease and hence, provide more appropriate and effective treatment for the stage of disease.

5.7 Nanotechnology for retinal prosthesis

Ocular diseases such as diabetic retinopathy and age related macular degeneration permanently damage photoreceptor cells, which impairs vision. There are no therapies or approaches to restoring vision in these patients and therefore, there is a need to develop methods that are capable of restoring vision. Several implants and devices are currently being investigated for their potential to stimulate the retina to produce visual percepts. Materials on the nanoscale are being used to improve interaction with the surrounding tissue (including photoreceptor cells) and hence, improve biocompatibility.

One such device, a nanophotonic device, has been designed by Khraiche and colleagues for optimal product properties such as reduced foot print, power consumption and computational needs of the current retinal prosthesis, while reproducing high resolution vision (Khraiche et al., 2011). Nanotopography, the deviation of a surface within a spatial wavelength of around 0.2 to 20 nm, is an important parameter when designing silicon wafers. In other words, a surface with a high wavelength and low frequency is considered true surface nanotopography). This surface type is used to bridge the gap between rough and flat surfaces. Indeed, the unique structure of nanotopography has been shown to improve tissue integration of prosthetic devices and recovery from injury (Friedburg et al., 2001). Nanotopography is expected to reduce the amount of current needed to stimulate neural tissue, which would minimize power consumption of the device and hence, reduce potential tissue damage. The nanophotonics device designed by Khraiche and colleagues has inherent nanotopography, which would interface with ganglion cells. In addition, having nanowires (200 nm diameter) on the surface of the devices is expected to improve tissue integration of prosthetic devices. However, the utility of this device in vivo as a retinal prosthetic implant needs to be assessed.

Several other devices that employ nanotechnology are being developed for retinal prosthesis and the review article by Scholz provides an excellent summary (Scholz, 2010).

6. Dynamics of drug targeting – a case study based on the periocular route of delivery

Achievement of therapeutic doses and high drug activity in conjunction with spatiotemporal control of drug levels is critical for therapeutic effects in the confined anatomical space of the eye. It is noteworthy that there are multidimensional considerations in ocular drug delivery. In the emerging field of nanomedicines, the optimal physiochemical spaces for drugs and delivery systems are gradually being unveiled, molecular size, lipophilicity-hydrophilicity balance, and charge of the drug nanomedicine remain key players. Further, slow dissolution of the drug is another sought after physiochemical feature that alleviates rapid clearance from intraocular space, thus sustaining the delivery.

6.1 Influence of location of injection

The periocular route of drug delivery can be divided into subconjunctival, subtenon, peribulbar, and retrobulbar routes depending on the anatomical location of the injection. Retinal drug delivery can be significantly affected by the location of the periocular injection.

In a human study, patients with rhegmatogenous retinal detachment were administered dexamethasone sodium phosphate by the peribulbar (N= 50, dose = 5 mg), subconjunctival (N= 49, dose = 2.5 mg) or oral (N= 49, dose = 7.5 mg) route. The mean maximum concentration of dexamethasone in the subconjunctival fluid was 8.7 fold higher with the subconjunctival injection compared to peribulbar injection. Both periocular routes (peribulbar and subconjunctival) produced significantly higher maximal dexamethasone

concentrations in the subretinal fluid compared to the oral route. The dose-normalized mean maximal dexamethasone concentrations in the sub-retinal fluid were 120 and 13 fold higher for the subconjunctival and peribulbar routes of injection, respectively, compared to the oral route (Weijtens et al., 2000). In a similar study, it was observed that subconjunctival dexamethasone (N= 50, dose 2.5 mg) (Weijtens et al., 1999) produced higher peak concentrations of dexamethasone in the vitreous compared to peribulbar administration of dexamethasone (N= 61, dose 5 mg) (Weijtens et al., 1997). The dose normalized peak dexamethasone concentrations in the vitreous following the subconjunctival administration were 11 fold higher than after peribulbar administration. Comparison of these levels to the oral dexamethasone administration study (N= 54 dose 7.5 mg) (Weijtens et al., 1998), reveals that both peribulbar as well as subconjunctival administration produced higher dexamethasone vitreal concentrations compared to the oral route. Vitreous dexamethasone concentrations after peribulbar administration were 4 fold higher than vitreous levels obtained after oral administration. These clinical studies demonstrate that the route of administration (subconjunctival, peribulbar, or oral) influence drug levels in different eye tissues (subconjunctival fluid, subretinal fluid, and vitreous).

Preclinical studies also have demonstrated the influence of route of administration on ocular drug tissue levels. In a rabbit model, mean peak choroid-retinal concentrations of sodium fluorescein were higher following retrobulbar injection as compared to intravenous injection (Ghate et al., 2007). Peak choroid-retinal concentrations following retrobulbar injection were also higher compared to posterior subtenon or subconjunctival injection with the order of peak choroid-retinal concentrations being: retrobulbar>posterior subtenon>subconjunctival. Following periocular injection, sodium fluorescein was also detected in the anterior segment with the order of anterior segment concentrations being subconjunctival>posterior subtenon> retrobulbar. Following intravenous injection, there was a rapid rise and decline in the choroid-retina sodium fluorescein concentrations whereas the levels were sustained for a much longer period for all three periocular modes of administration.

Okabe and colleagues placed non-biodegradable implants releasing betamethasone at three different locations the anterior episclera (anterior subconjunctival), posterior episclera (posterior subconjunctival) and anterior intrasclera in rabbits (Okabe et al., 2007). They observed the highest concentration of betamethasone in the retina-choroid 4-weeks post administration with the intra-scleral implant. The authors also determined concentrations of betamethasone at three different sites in the retina-choroid, which were around the site of implantation, opposite to the site of implantation, and at the posterior pole. There was a spatial gradient in the betamethasone concentrations with the implants, with the concentration being highest around the site of implantation in all the three locations. With the anterior episcleral placement and anterior intra-scleral placement the concentration at the opposite site (anterior episcleral at the other side of the eye) was higher than the concentration at the posterior pole. With the posterior episcleral placement, the concentration was higher at the posterior pole rather than the opposite site. Thus, there was a spatial distribution of betamethasone concentrations from the site of implantation (Okabe et al., 2007).

6.2 Influence of drug molecular weight

The sclera is mostly an acellular tissue made up of collagenous matrix with fluid filled channels. Previous studies have shown that the sclera is permeable to a wide range of solutes differing in molecular weights. Bill demonstrated that large proteins like albumin and large macromolecule like dextran when injected in the supra-choroidal space of the rabbit eye were able to diffuse across the sclera and could be detected in the extraocular tissues (Bill, 1965). Even molecules like 70 kDa dextran (Kim et al., 2002) and a 150 kDa IgG antibody

(Ambati et al., 2000) have been shown to be permeable across the sclera. Drug diffusion in general is dependent of the molecular size. It is intuitive to believe that drug diffusion increases with decrease in molecular size and because molecular size and molecular weight are significantly correlated, drug diffusion generally decreases with an increase in drug molecular weight. Several mechanistic studies have demonstrated that scleral permeability is dependent on the permeant molecular weight (Edelhauser and Maren, 1988); (Maurice and Polgar, 1977); (Ambati et al., 2000); (Amaral et al., 2005). Ambati et al. (Ambati et al., 2000) in their in vitro transport studies with rabbit sclera have demonstrated that the molecular size is a better predictor of transscleral permeability as compared to molecular weight.

The choroid-RPE offers more resistance to the transport of molecules particularly macromolecules. Pitkanen and colleagues demonstrated that bovine choroid-RPE is 10–100 times less permeable to hydrophilic and macromolecular compounds compared to the sclera (Pitkanen et al., 2005). Using FITC-dextran molecules of molecular weight ranging from 4 to 80 kDa, they demonstrated that permeability decreased significantly with increasing molecular size of the probe.

6.3 Influence of drug lipophilicity

The hydrophilic-lipophilic properties of molecules play an important role in drug diffusion and drug transport across various biological tissues. The permeability of molecules across a biological interface is dependent on the physicochemical nature of the permeating molecule as well as on the properties of the biological tissue including the hydrophilicity/lipophilicity of the tissue.

In trans-scleral drug delivery, the drug has to pass through tissues with different hydrophilicities. The sclera is a relatively hydrophilic tissue whereas the choroid and the RPE are relatively lipophilic. It has been demonstrated using in-vitro culture systems that the permeability across the RPE in general increases with an increase in drug lipophilicity until a plateau is reached (Pitkanen et al., 2005). Cheruvu and Kompella investigated the effect of lipophilicity on solute transport across the sclera and the sclera-choroid combination (Cheruvu and Kompella, 2006). Drug permeability across the sclera for molecules of similar molecular radii and molecular weights was found to inversely relate to the drug lipophilicity. This was observed across both bovine as well as porcine scleral tissue. The permeability coefficients across bovine sclera ranged from 3.33×10^{-6} cm/s for the most hydrophilic solute (sodium fluorescein, Log D: -0.68) to 0.91×10^{-6} cm/s for the most lipophilic solute (rhodamine 6G, Log D: 4.02), whereas they ranged from 3.33×10^{-6} cm/s to 0.91×10^{-6} across porcine sclera. An alternative explanation for the increased permeability of sodium fluorescein (a negatively charged molecule) and rhodamine 6G (a positively charged molecule) is the molecular charge. The sclera structure is made up of collagen fibrils and glycosaminoglycans, which are negatively charged and can bind to the positively charged rhodamine, thereby decreasing its effective permeability. These observations have been made by others including Maurice and Polgar who observed that permeability across sclera was greater with anionic dyes as compared to cationic dyes (Maurice and Polgar, 1977). In agreement with the previous study, Cruysberg and colleagues demonstrated that the permeability of sodium fluorescein across human sclera in an in vitro transport/diffusion experimental design is higher than that of rhodamine 6G (Cruysberg et al., 2002).

Cheruvu and Kompella also investigated the effects of the underlying tissues (choroid-Bruch's membrane) on the transscleral permeability of solutes (Cheruvu and Kompella, 2006). The trend of decreasing permeability with increasing lipophilicity is steeper for sclera-choroid combination as compared to sclera alone, indicating that the choroid-Bruch's

membrane is a more effective barrier for lipophilic drugs as compared to hydrophilic ones. Similar results were observed when solute molecule transport was studied across sclera-choroid-RPE, wherein the transport was the least for highly lipophilic molecules compared to hydrophilic molecules (Figure 8) (Kadam et al., 2011).

In addition to the influence of drug lipophilicity on permeability, species differences in tissue thickness also influence drug permeability (Kadam et al., 2011). The transport of eight beta-blockers (ranging from hydrophilic to lipophilic) in different species was found to be dependent on sclera thickness. Cumulative transport across sclera followed the rank order: rabbit > porcine > bovine and inversely correlated with scleral thickness. When the permeability was normalized to thickness, sclera of all species exhibited similar drug permeability. However, when the transport was assessed across the sclera-choroid-RPE, the differences in transport could no longer be explained solely based on tissue thickness differences. Differences in melanin pigment content in choroid also contributed to the differences in permeability across sclera-choroid-RPE.

When designing a nanomedicine for the treatment of posterior segment eye diseases, drug properties as well as biological tissue properties that influence drug diffusion and transport must be considered.

6.4 Influence of eye pigmentation

Sclera is not highly pigmented, whereas pigmentation is present in the choroid as well as the RPE (Cheruvu et al., 2008). Melanin is a class of pigment molecules wherein eumelanin is black or brown in color and pheomelanin is red or yellow in color. Within the eye, pigmentation is found in the choroid and the RPE. Melanin molecules are polyanionic with several carboxyl groups and semiquinones with a tendency to bind lipophilic and cationic drugs through hydrophobic and electrostatic interactions (Schoenwald et al., 1998). Binding of several drugs to melanin has been reported in the literature and lipophilic drugs with $pK_a > 7$ have a strong tendency to bind to melanin a class of pigment molecules wherein eumelanin is black or brown in color and pheomelanin is red or yellow in color. Within the eye, pigmentation is melanin molecules are with several carboxyl groups and semiquinones (Leblanc et al., 1998). Binding of drugs to pheomelanin and eumelanin may differ significantly depending upon the drug. For instance, chloroquine binding to pheomelanin is higher (92%) when compared to eumelanin (27%), following incubation of 2.5 μ M chloroquine with 5 mg of pheomelanin or eumelanin (Mars and Larsson, 1999).

Due to the melanin content in the choroid and RPE, there is a potential for drugs to bind the choroid and RPE when administered periocularly. Indeed Cheruvu and Kompella speculated that melanin content was contributing to the results they observed in their study that demonstrated reduced transport of lipophilic molecules compared to hydrophilic molecules across the sclera-choroid (Cheruvu and Kompella, 2006). The investigators then designed an in vivo study to better understand the effect of pigmentation on transscleral drug delivery to the retina. Cheruvu and colleagues administered celecoxib suspension periocularly to weight and age matched Sprague Dawley (SD-non pigmented) and Brown Norway (BN-pigmented) rats (Cheruvu et al., 2008). The celecoxib retinal and vitreal exposure ($AUC_{0-\infty}$) was 1.4 ($p = 0.001$) and 1.6 ($p = 0.001$) fold higher at day 8 in non-pigmented rats compared to pigmented rats, respectively. This study also demonstrated that melanin binding of celecoxib significantly reduced transport across the choroid and RPE. Further, this study demonstrated several fold greater delivery in SD rats when compared to BN rates for a slow release delivery system of celecoxib (Figure 9). Thus, drug binding to the pigment reduces retinal drug delivery, with the differences being more when non-saturating levels of drug are present. A similar study by Kadam and colleagues demonstrated that melanin pigment in the choroid and RPE reduces transport of beta-blockers in rabbits (Figures 7 and 8, albino vs.

pigmented rabbit) (Kadam et al., 2011). In addition, species differences in tissue thickness and melanin content influenced drug delivery (Figures 7 and 8). Thus, pigmentation differences can have a profound impact on drug delivery to the retina following periocular administration, especially for drugs with a strong tendency to bind to melanin.

The binding of drugs to melanin and subsequent reduction in the target tissue availability and efficacy has been well documented in the literature (Salazar-Bookaman et al., 1994). Using ^{14}C -brimonidine, Acheampong and colleagues demonstrated that higher accumulation of drug in pigmented tissues compared to non-pigmented tissues following topical or systemic administration in rats, monkeys, and rabbits (Acheampong et al., 2002a) (Acheampong et al., 2002b). The binding of drugs to pigmented ocular tissues can raise efficacy as well as safety concerns, although the direct association between melanin binding and ocular toxicity is a matter of active debate (Leblanc et al., 1998); (Steiner et al., 1990); (Bernstein, 1967).

6.5 Influence of disease state

The delivery of drug to the target tissue is influenced by several factors, which can be grouped into three broad categories: 1) factors related to the physicochemical parameters of the drug, 2) route of drug administration and 3) factors influencing the physiological mechanisms involved in drug disposition. For drug delivery to the retina by the periocular route, the drug has to cross the sclera, choroid, and the RPE. Changes to these tissues as a result of physiological changes (such as aging) or pathophysiological alterations can significantly impact the delivery of drugs to the retina. It has been demonstrated by Cheruvu and colleagues that the total retinal exposure of celecoxib (AUC) is 1.5–2.0 fold higher (depending on the rat strain) when given to diabetic rats by the periocular mode of administration as compared to normal non-diabetic rats (Figure 10) (Cheruvu et al., 2009). In addition, the peak exposure (C_{max}) of celecoxib was between 1.3–2.2 fold higher in diabetic rats as compared to the non-diabetic controls. The authors hypothesized that disease progression of diabetes leads to a significant breakdown in the blood – retinal barrier. Drug leakage into the retina increased 3.5 folds in BN rats compared to only 2.4 fold in SD rats with diabetes for 2 months as compared to non-diabetic controls. Previously it was demonstrated by the same group that the contribution from local delivery pathways to the retinal concentration of celecoxib is significantly higher (>90%) as compared to the systemic route (Ayalasomayajula and Kompella, 2003). The technique used by the authors cannot differentiate between the breakdown of the outer and the inner blood–retinal barrier and hence, relative contribution from each of those mechanisms could not be estimated.

The breakdown of the blood-retinal barrier can be estimated using several different techniques. The widely used techniques for these assessments include leakage of human serum albumin (HSA) into the retina, leakage of FITC-dextran of different molecular weights ranging from 4 to 150 kD, and Evans-blue dye leakage into the retina. For all of these techniques, the marker is injected systemically and then measured in the plasma and the retina and the ratio of the marker in the retinal tissue to the marker in the plasma is estimated. Any changes to this ratio reflect changes to the barrier properties of the blood-retinal barrier. It has been demonstrated in several animal studies that diabetes leads to an increased leakage of the marker into the retina and thus breakdown of the blood retinal barrier can be inferred (El-Remessy et al., 2003); (Do Carmo et al., 1998); (Cunha-Vaz, 1998). This breakdown also suggests that greater drug delivery to the retina from the systemic mode is also likely as diabetes progresses due to compromised barrier function.

The compromise in the integrity of the barrier functions can be effectively utilized in drug and gene delivery to the retina. Singh and colleagues utilized the concept of leaky vasculature in the choroid-retina which occurs as a result of choroidal neovascularization (a

condition developed in progressive wet age related macular degeneration) to deliver genes to the choroid-retina by the systemic route (Singh et al., 2009). The investigators demonstrated that nanoparticles functionalized with certain cell surface receptor markers can effectively deliver an anti-VEGF intraceptor plasmid to the choroidal neovascular lesions leading to a decrease in CNV area. The authors also demonstrated that the nanoparticles can passively localize in the areas of CNV lesions, but under normal physiology are not delivered to the retina to any significant extent. In another study, periocular administration of polymeric microparticles of triamcinolone acetonide resulted in enhanced delivery to various eye tissues in CNV rats when compared to normal rats (Kadam et al., 2012). In conclusion, alterations in the physiology of the ocular tissues including break down of the blood retinal barrier can significantly alter drug delivery and these changes should be considered when designing nanomedicines or diagnostics for back of the eye diseases.

6.6 Influence of particle size on disposition

In addition to studying the influences of route of administration, drug properties, and physiological and pathophysiological properties (pigmentation and membrane barriers) on drug delivery, particulate size also contributes significantly to drug disposition. Over the past few years, there has been an increased interest in the delivery of drugs as well as genes to the ocular tissues using particulate systems.

Amrite and Kompella investigated the effect of particle size on the clearance of particulate systems after periocular administration using non-biodegradable particles of 20, 200, and 2000 nm size (Amrite and Kompella, 2005). The authors utilized the non-biodegradable particles in order to study the influence of particle size without the confounding factor of polymer biodegradation which can alter the size of the particles in vivo. Their studies suggest that following periocular administration particles larger than 200 nm are completely retained at the site-of administration. This retention behavior is not affected by the dose of the particles administered or the surface properties (charge and hydrophilicity). The smallest size investigated was 20 nm and these particles were rapidly lost from the site of administration after periocular administration, which was further confirmed in a later follow-on study (Amrite et al., 2008b). For the smaller particles (20 nm) the investigators found some possible dependency on the surface properties with hydrophobic particles having a tendency to be retained better as compared to the hydrophilic particles.

None of the particles investigated (20–2000 nm) gained any significant access to the retinal tissue following periocular administration. There were quantifiable levels of the 20 nm particles in the sclera-choroid only. This has also been observed with in vitro transport studies across bovine sclera and sclera-choroid-RPE combination where the investigators found some quantifiable transport of 20 nm nanoparticles across sclera, but not across sclera-choroid-RPE (Amrite et al., 2008b). The cumulative % transported across sclera, however, was only 0.46% at the end of 24 hours. Based on their study on effect of particle size on periocular retention of nano- and micro- particles, the authors conclude that particulate systems at or above 200 nm are likely to be retained at the periocular site of administration for prolonged periods and can be used as sustained retinal drug delivery devices utilizing the transscleral mode of delivery (Amrite and Kompella, 2005). If the delivery of intact particles to the retina is a goal (e.g., for gene delivery) then the periocular route of administration is not the preferred route as there is negligible delivery to the retina with this route with any particle size in the 20–2000 nm range.

6.7 Mechanisms of particle clearance

Smaller particles (20 nm or smaller) are cleared from the site of administration after periocular administration. Amrite and colleagues have investigated the mechanisms of the

clearance of these particles following periocular administration (Amrite et al., 2008b). Their studies suggest that the clearance of particles is largely dependent on the blood and lymphatic circulation in the periocular tissue. The investigators injected 20 nm particles by periocular administration to live (circulation intact) and dead (devoid of circulation) rats in order to evaluate the effect of circulation on particle clearance. At the end of 6 hours post-particle dosing, the mean retention at the periocular site of administration was only 45% of the amount injected in live animals, whereas it was 77% in the dead animals. This percent retention in the dead animals is close to the retention observed immediately after dosing (about 80% particles quantified) (Amrite and Kompella, 2005). Also the tissue levels of the particles in the sclera-choroid were 19 folds higher in the dead animals as compared to the live animals. In addition, the particles could be detected in the intraocular tissues including the retina and vitreous of the dead animals but not in the live animals, indicating a possible breakdown of some of the transport barriers in the dead animals (Amrite et al., 2008b). The authors also demonstrated using confocal microscopy that the particles (20 nm) administered periocularly are cleared by both the blood and lymphatic clearance systems as the particles were observed in the organs of the reticulo-endothelial system (RES) (liver and spleen) as well as in the cervical, mesenteric and axillary lymph nodes (Amrite et al., 2008b).

The role of RES in clearance of particulate systems administered by intravenous route has been demonstrated in several in vivo studies (Geze et al., 2007; Moghimi et al., 1991; Schipper et al., 2009; Simon et al., 1995). In addition, the lymphatic system is known to be involved in clearance of particulate systems. It has been observed that the conjunctival follicle associated epithelium in the rabbit is able to bind to and translocate particles in studies with fluorescent latex particles (size 200 nm) after instillation into the conjunctival cul-de-sac (Liu et al., 2005). The particles, after being translocated from the conjunctival epithelium, were visualized in the cervical lymph nodes indicating the role of the lymphatic circulation in clearance of these particles. Similar observations have been made with clearance of *Maackiaamurensis* leucoagglutinin (a relatively large macromolecule with size in the nanometer scale) in guinea pigs where the antigen was translocated by the M cells present in the conjunctiva (Meagher et al., 2005). Amrite and Kompella also observed presence of inflammatory cells in the histological sections of the periocular tissue 60-days following administration of the 200 and 2000 nm particles which were retained at the site of administration for that period (Amrite and Kompella, 2005). These inflammatory cells could also be playing a role in particle clearance after periocular administration. The role of inflammatory cells in particle clearance has been demonstrated in several studies after pulmonary administration of particles (Zhang et al., 2000).

Thus after periocular administration, particles of a smaller size are likely cleared by the systemic and lymphatic circulation. Larger particles are retained at the site of administration and probably would degrade at the site (if biodegradable). Larger particles are better systems for sustained delivery of drugs to the retina after periocular administration.

6.8 Predictions of retinal drug delivery

Mathematical modeling is an effective tool, which when utilized correctly can be helpful in better understanding physiological systems as well as drug delivery kinetics and dynamics. Simulations can be performed using well validated mathematical models, which can provide a quantitative understanding of the probable drug exposure. Mathematical modeling has been used in the ocular field for many years, though the concept has been recently applied to better understand the periocular mode of delivery. The modeling approaches for posterior segment drug delivery using the periocular route have been summarized by Pekka-Ranta and Urtti (Ranta and Urtti, 2006). Classical pharmacokinetic models can be used to describe drug behavior in the retina following periocular administration.

Amrite and colleagues developed a mathematical model to describe the corneal and retinal pharmacokinetics of lipophilic small molecules like celecoxib following periocular administration (Amrite et al., 2008a). This model was developed based on the in vivo data from Cheruvu and colleagues (Cheruvu et al., 2008) and validated using the celecoxib retinal pharmacokinetic data following periocular administration in SD rats (Ayalasomayajula and Kompella, 2004) and corneal prednisolone in vivo data in rabbits (Tsuji et al., 1988). The models fitted the observed data well (correlation coefficients above 0.95 between predicted and observed data). Based on the modeling studies, the authors concluded that the leak back from the site of injection after periocular administration likely contributes to the observed drug levels in the cornea. The models also demonstrate that drug clearance is significantly influenced by the periocular circulation and lymphatics and this is the major clearance pathway for drugs administered by periocular injection. In addition, the models suggest that corneal pharmacokinetics of small lipophilic molecules following periocular administration can be explained by using similar mathematical models in both rats and rabbits. The model was further fine-tuned to adapt it for use with sustained drug delivery systems like nanoparticles and microparticles, where the clearance of the system itself was not negligible (Amrite and Kompella, 2005). This refined model is presented in the work of Amrite and colleagues (Amrite et al., 2008b). Using this refined mathematical model, simulations were performed to evaluate the retinal levels of celecoxib following periocular administration with delivery systems that differed in terms of their drug release rates and in terms of the elimination of the formulation following periocular administration. It was observed that the 200 nm particle delivery system (not eliminated after periocular administration) better sustained retinal celecoxib drug levels compared to 20 nm particles (which are eliminated after periocular administration) with different hypothetical first order release rate constants of the drug from the delivery system. The drug concentration profiles were simulated using the estimated half-lives of 5.5 hours and 180 days for 20 nm and 200 nm, respectively, previously determined by Amrite and Kompella (Amrite and Kompella, 2005). It was also demonstrated that the differences in the retinal celecoxib levels between the 20 nm and 200 nm particle systems become more apparent with slower drug release rates (Figure 11).

A similar approach to using mathematical modeling for predicting vitreal pharmacokinetics has been used by Lee and Robinson (Lee and Robinson, 2004). Their model was simplified by removing the aqueous chamber and systemic circulation compartments, which are not likely to contribute significantly to the vitreous level after subconjunctival injection (periocular route of administration). The best model was selected and validated by comparing the predicted pharmacokinetic values to experimental values for prednisolone administered at different doses subconjunctivally. The most accurate model incorporated a distribution compartment where the drug diffuses into and out of from the vitreous chamber ($r^2 = 0.99$). The authors found that the rate constant for drug to move from the subconjunctival space to the vitreous ($8.51 \times 10^{-5} \text{ min}^{-1}$) was much slower than elimination immediately from the subconjunctival space ($1.28 \times 10^{-2} \text{ min}^{-1}$) and vitreous ($1.14 \times 10^{-2} \text{ min}^{-1}$). This is in agreement with the study by Amrite and colleagues that demonstrated periocular and lymphatic clearance is high for drugs like prednisolone (Amrite et al., 2008b).

7. Nanosystem and Dose-dependent Toxicities

Nanoparticles that are biocompatible and biodegradable are the most sought after emerging therapeutics in ocular and other biomedical applications. Carrier selection for designing nanomedicines should ideally be based on biocompatible, generally recognized as safe (GRAS), as indicated by the FDA, ingredients or those ingredients that are likely to be safe. Ideally, the carrier materials should be metabolized to safe products that can be readily

cleared from the system. Alternatively, the material itself should be amenable to clearance from the body. If the material is intended to remain in the body, it should ideally be inert, biocompatible, with no adverse effects after long term exposure. A variety of materials including polymers, lipids, carbohydrates, and proteins have been used to prepare nanomedicines. More recently, inorganic materials including gold and porous silica have been made inroads for ophthalmic applications. In contrast to organic compounds, inorganic metals persist without breaking down and this may be of toxicological concern. The composition, size, shape, surface charge, lipophilicity, and surface modifications, are the key physicochemical properties influencing the biocompatibility of nanoparticles (McNeil, 2005). In addition, the route of administration, dose, dose frequency, and patient related factors should be considered when minimizing toxicity.

The potential risks posed by different nanosystems, perhaps more specific to the route of delivery, target tissue, and the nanoparticle composition, include inflammation, immune-stimulation (Dobrovolskaia and McNeil, 2007; Zolnik et al., 2010), immunosuppression, aggregation, accumulation in non-target tissues, membrane disruption (Panessa-Warren et al., 2009), hemolysis, oxidative stress generation (Medina et al., 2007), and adsorption of plasma proteins onto the surface. These responses could further lead to vitreal haze, epithelial damage, membrane opacity, hemorrhage, fibrosis, and retinal damages such as traction, thickening, tear, fold and degeneration in the back of the eye (Prow et al., 2008). A brief summary of the various adverse effects reported with different nanoparticles administered intravenously and subretinally is shown in Figure 12. However, there is a dearth of reports that have observed significant toxicities associated with nanomedicine delivery to the back of the eye.

There are reports that have investigated the in vivo toxicity of nanoparticles administered by routes including intravitreal and topical. One particular study investigated the toxicity of 50 nm and 4 μ m iron oxide nanoparticles coated with dextran and labeled with Alexa 488 dye after intravitreal injection in Sprague Dawley rats (Raju et al., 2011). There was no significant difference between rats injected with PBS and particles (50 nm or 4 μ m) in intraocular pressure and electroretinogram measurements up to 5 months after a single injection. However, the corneal endothelial cell count was significantly lower for eyes injected with 4 μ m particles compared to 50 nm particle or PBS injected eyes at 5 months; whereas the corneal endothelial cell count was similar amongst 50 nm, 4 μ m, and PBS for other time points (1 hour, 1 week and 1 month). In addition, slightly more iron deposition was observed in the choroid for only 4 μ m particles at 5 months compared to eyes injected with PBS. Since iron deposition in the choroid was detected in control eyes injected with PBS only, the authors suggested that iron deposition is representative of normal aging. This study concluded that the changes in corneal endothelial cell count and slight increase in iron deposition are minor and therefore, iron oxide particles are generally considered non-toxic in the eye. However, this study did not assess the toxicity of particles after frequent injections of nanoparticles. It is expected that toxicity will depend upon dose and dose frequency of nanoparticles administered and more appropriate studies should be designed to characterize these parameters.

In vitro investigations of nanotechnology toxicity are typically conducted by studying the effects of nanoparticles over certain time periods (e.g., 24–48 hours) on cell cultures. Effects measured may include cell survival, cell proliferation, inflammatory cytokine levels, membrane permeability, and ATP production among others. For example, Mo and colleagues investigated the cytotoxicity of albumin nanoparticles loaded with a plasmid were cytotoxic (Mo et al., 2007). Study design included assessment of RPE cell viability after a single treatment for 96 hours at various different concentrations (5, 10, 50, 100, 500, 1000, 5000 μ g/ml). Albumin was not cytotoxic up to 5000 μ g/ml.

Undesired consequences such as aggregation and oxidative stress generation can be minimized by varying the surface charge and composition of the nanoparticle. Membrane disruption is sometimes a sought after drug delivery pathway, especially in gene delivery (Akagi et al., 2010; Kiang et al., 2004), which may cause significant toxicity due to leakage of ions and disruption of cellular composition. A less investigated major issue related nanomedicines that is under-investigated is immunotoxicity, and there is a need to standardize this evaluation.

8 Emerging Micro- and Nano- Electromechanical Systems (M/NEMS)-Based Fabrication

For posterior ocular segment diseases, M/NEMS-based fabrication methods are under investigation for their ability to fabricate microneedles (described below), implants (Lo et al., 2009), and micro- and nano-particles (Yang and Zahn, 2004) (Gong et al., 2009). Over the last decade, submicron engineered M/NEMS have been widely implemented in the design of sensors and devices for biomedical applications including diagnosis and treatment (Grayson et al., 2004). Manufacturing of N/MEMS devices is similar to the techniques for fabricating integrated circuits meaning they have an electrical component in addition to a physical component. The electrical component can be used to conduct work such as monitoring fluid flow into the device. The physical component of the device can allow the device to do the actual physical work such as a valve that opens and closes for channeling or routing fluid flow through the device. Typically, the devices are made with silicon, polymers (such as polyester, acrylate, polymethyldisiloxane, and parylene), metals (such as gold, copper, titanium, platinum, and silver), or ceramics (such as silicon carbide and nitrides of silicon, aluminum, and titanium). General fabrication methods include photolithography and micromolding, which are shown in Figure x.

8.1 Example of M/NEMS-based fabrication: microneedles

Microneedles are essentially micron sized syringe needles and are fabricated with M/NEMS techniques and have the potential to be used as pain-free or reduced pain injections for the delivery of both small and large molecular weight drug molecules (Kaushik et al., 2001). Polymeric microneedles are of particular interest due to their cost effectiveness, biodegradability, biocompatibility, strong, and tough (Aoyagi et al., 2007) (Lee et al., 2008). When microneedles are used to puncture the globe in intraocular drug delivery, they by-pass ocular diffusional barriers and create transient transport pathways of micron dimensions, thus enhancing intraocular permeability and providing the drug within close proximity to the target tissue. The micropores created, are larger than the therapeutic molecules, which permit the diffusion of hydrophilic macromolecules, microparticles, and supramolecular complexes (Prausnitz, 2004).

While silicon has traditionally been used in microneedle fabrication (Hashmi et al., 1995) (Henry et al., 1998), glass (Wang et al., 2000), maltose (Miyano et al., 2005) (Kolli and Banga, 2008), dextrin (Ito et al., 2006), ceramic (Ovsianikov et al., 2007), stainless steel (Verbaan et al., 2008), galactose (Donnelly et al., 2009), and various polymers (McAllister et al., 2003) (Park et al., 2005, 2006) (Pérennès et al., 2006) may also be used. Microneedles can be manufactured by M/NEMS-based microfabrication methods including reactive ion etching, surface/bulk micromachining, laser drilling, chemical isotropic etching, polysilicon micromolding, lithography electroforming replication, and injection molding (Yang and Zahn, 2004) (Davis et al., 2005) (Moon et al., 2005) (Park et al., 2005) (Stoeber and Liepmann, 2005).

As described in Section 5.5, Patel and colleagues have fabricated hollow microneedles for precisely delivering drug to the suprachoroidal space (Patel et al., 2011). Briefly, fire-polished borosilicate glass with desired dimensions was pulled using a micropipette puller, detached into two, and beveled at the tip using a glass grinder. These microneedles were fabricated to overcome diffusional barriers of the eye and deliver drug to the sclera and beyond, into the suprachoroidal space for delivering drug to the choroid and the retina, the posterior segment of the eye. The investigators used the syringe coupled hollow microneedle device to deliver sulforhodamine B solution, Nile red encapsulated nanoparticle suspension, and fluorescein-labeled microparticles to ex-vivo eyes. The authors observed that a minimum pressure of 250 kPa and a minimum microneedle length of 800 μm was required to penetrate the sclera and successfully deliver particles of sizes 20 to 100 nm into the suprachoroidal space. Similarly, in order to deliver particles of sizes 500 to 1000 μm , a minimum pressure of 250 to 300 kPa and a minimum microneedle length of 1000 μm , was required. Hollow microneedle assisted puncturing of the sclera is thus a potential drug delivery strategy to overcome the scleral diffusional barrier and localize the drug to posterior ocular tissues, facilitating treatment of choroidal and retinal degeneration related diseases. Clearside Biomedical, Inc. is currently initiating clinical investigation of microneedles to assess their utility and safety in delivering drug solutions and micro- and nano-particle suspensions to the suprachoroidal space.

9. Future Prospects

Nanosystems have been evaluated for various applications in preclinical studies, including controlled drug and gene delivery systems, nano-electromechanical devices for imaging and diagnosis, and implantable nanomaterials for prolonged, local therapy. Although preclinical nanomedicine research for ocular use has witnessed technological advances, few have reached market approval for human clinical use. However, there are several ocular nanotechnology based US patents, some of which are briefly described below. US Patent publication no. 2007/0020336 A1 describes the use of cyclodextrin nanomedicine for drug delivery to the posterior segment of the eye (Fujii et al., 2010). This ophthalmic nanosuspension, proposed to be administered as eye drops, contains a drug, cyclodextrin, and water, in which approximately 0.1% to 90% (w/v) of the drug in the solubilized form and the rest as a nanosuspension with cyclodextrin, with a particle size of 10 nm and above. Another patent describes a drug-filled scleral implant capable of releasing the drug into the sclera and claims that at least one portion of the device includes a nanotechnology formulation (US Patent publication no. 2005/0181018 A1 (Karlsson et al., 2012)). US Patent publication no. 2008/0152694 describes a MEMS-based device with a drug delivery pump and a catheter which can be used to administer one or more drugs such as nanoparticles and nanosuspensions, locally into the ocular tissues (Mars and Larsson, 1999). US Patent publication no. 2010/0255061 discusses an intraocular implant that can deliver corticosteroids or anti-VEGF therapeutics into the vitreous for at least one month, and proposes the use of binding agents and/or stabilizers that can facilitate prolonged release of the therapeutic from the drug-filled compartment (Eugene de Juan et al., 2010). This device has been further claimed to be used for the delivery of nanosized therapeutics. In conclusion, the patent landscape for ocular nano-based therapeutics includes many novel nanotechnologies that use nanosystems either solely as a treatment or as a component of an implantable device. Several clinical trials are also underway to translate nanomedicines for effective clinical use in the treatment of posterior segment ocular diseases. A Phase 2 clinical trial for albumin-stabilized nanoparticle formulation of paclitaxel ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00738361) Identifier No. NCT00738361), with both cytotoxic and cytostatic effects, is under investigation in patients with metastatic melanoma of the eye. Another ongoing Phase 2 and 3 clinical trial is investigating the safety and efficacy of cyclodextrin nanoparticle eye drops of dexamethasone for treating diabetic macular edema ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00738361) Identifier No. NCT00738361).

NCT01523314). Further, the clinicaltrials.gov website states that pre-clinical studies with this formulation have demonstrated increased drug absorption into the eye and decreased systemic distribution of the drug, thereby reducing side effects.

As discussed in this article, nanomedicines can be prepared primarily using the pure drug or in association with carrier materials such as polymers, lipids, proteins, carbohydrates, and metals. They can be administered by various routes including intravenous, topical, periocular, intravitreal, and suprachoroidal. The selection of the route of administration as well as the nanosystem depends on a variety of factors including drug properties, disease state, target tissue/cells, and nanomaterial properties such as drug loading, encapsulation efficiency, and safety. Various nanocarriers ranging from the more established systems like liposomes, polymeric nanoparticles, solid-lipid nanoparticles and micelles, to the more novel systems like nanosuspensions, nanoemulsions, nanocrystals, hydrogels, and dendrimers, have been studied for the delivery of ocular therapeutics. This article summarized the nanomedicine advances in treating and managing back of the eye disorders. However, prior to translation of nanomedicines and nanosystems for treatment and management of ocular diseases, a number of obstacles need to be overcome. We envision that the following aspects need to be addressed in future.

1. Reproducible manufacturing of nanoparticles is a key barrier for translation of nanomedicines. While reproducible manufacturing of liposomes and nanoemulsions is well established for FDA approval, large scale GMP manufacturing of a variety of other nanoparticles will be established in future.
2. Systematic studies that compare different types of nanoparticles (polymer, liposomal, dendrimeric, etc.) should be conducted to better select the appropriate nanoparticle type that offers maximal desired effects while minimizing toxic effects.
3. A key requirement of pharmaceutical products is low quantities of impurities. Currently, while several nanoparticles are being manufactured for proof of concept purposes, more directed effort in quantifying and reducing impurities is required. Further, when impurities are identified, their batch-to-batch reproducibility, and safety has to be established.
4. It is routine for several investigators to report a presumptive particle size for various nanoparticles based on their molecular size or based on electron microscopy measurements. While these measures sometimes tend to be of a very small dimension less than 100 nm, in our own experience, even such small materials tend to form loose aggregates in aqueous media, with measured dimensions larger than 100 nm. Thus, the aqueous environment dramatically influences the size of particles. Further, nanoparticles can grow in size during storage. Future studies will understand the fundamentals of nanoparticle aggregation and devise approaches to minimize such aggregation in aqueous and biological media as well as the shelf.
5. Once a nanoparticle is administered into a tissue, the particles can interact with each other, forming larger aggregates. In our experience, nanoparticles administered in the periocular space, when retained over a few hours, tend to aggregate. Such aggregates form implant like structures which can persist for prolonged periods. Future studies will investigate the influence of vitreous humor, aqueous humor, blood, and other tissues on the properties of nanoparticles.
6. To date, largely spherical nanoparticles were investigated for drug and gene delivery to the eye. Future studies will investigate the influence of various shapes of nanoparticles on their biological properties.

7. Toxicity of nanoparticles is incompletely explored in the eye, especially after repeated dosing. In future, more data in this regard is anticipated, establishing safe materials and doses for the eye.
8. Hybrid nanosystems such as those that employ combination of viral and nonviral vectors, dendrimers and nanoparticles, and nanoparticles in conjunction with devices such as microneedles are anticipated.
9. Nanotechnology is not a universal solution for problems in treatment and management of ocular diseases. Other approaches and delivery systems including implants (which are on the micro- or sometimes millimeter scale) continue to be improved with more research and form a part of a repertoire of delivery systems for the treatment of retinal disorders. The choice of the delivery system and the route of administration will be dependent on the drug, disease targeted, the disease stage as well as physician and patient related factors.

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References

- Acheampong AA, Shackleton M, John B, Burke J, Wheeler L, Tang-Liu D. Distribution of brimonidine into anterior and posterior tissues of monkey, rabbit, and rat eyes. *Drug Metab Dispos.* 2002a; 30:421–429. [PubMed: 11901096]
- Acheampong AA, Small D, Baumgarten V, Welty D, Tang-Liu D. Formulation effects on ocular absorption of brimonidine in rabbit eyes. *J Ocul Pharmacol Ther.* 2002b; 18:325–337. [PubMed: 12222763]
- Akagi T, Kim H, Akashi M. pH-dependent disruption of erythrocyte membrane by amphiphilic poly(amino acid) nanoparticles. *J Biomater Sci Polym Ed.* 2010; 21:315–328. [PubMed: 20178688]
- Alghadyan AA, Peyman GA, Khoobehi B, Milner S, Liu KR. Liposome-bound cyclosporine: clearance after intravitreal injection. *Int Ophthalmol.* 1988; 12:109–112. [PubMed: 3229899]
- Amaral J, Fariss RN, Campos MM, Robison WG Jr, Kim H, Lutz R, Becerra SP. Transscleral-RPE permeability of PEDF and ovalbumin proteins: implications for subconjunctival protein delivery. *Invest Ophthalmol Vis Sci.* 2005; 46:4383–4392. [PubMed: 16303924]
- Ambati J, Canakis CS, Miller JW, Gragoudas ES, Edwards A, Weissgold DJ, Kim I, Delori FC, Adamis AP. Diffusion of high molecular weight compounds through sclera. *Invest Ophthalmol Vis Sci.* 2000; 41:1181–1185. [PubMed: 10752958]
- Amrite AC, Ayalasomayajula SP, Cheruvu NP, Kompella UB. Single periocular injection of celecoxib-PLGA microparticles inhibits diabetes-induced elevations in retinal PGE₂, VEGF, and vascular leakage. *Invest Ophthalmol Vis Sci.* 2006; 47:1149–1160. [PubMed: 16505053]
- Amrite AC, Edelhauser HF, Kompella UB. Modeling of corneal and retinal pharmacokinetics after periocular drug administration. *Invest Ophthalmol Vis Sci.* 2008a; 49:320–332. [PubMed: 18172109]
- Amrite AC, Edelhauser HF, Singh SR, Kompella UB. Effect of circulation on the disposition and ocular tissue distribution of 20 nm nanoparticles after periocular administration. *Molecular vision.* 2008b; 14:150–160. [PubMed: 18334929]
- Amrite AC, Kompella UB. Size-dependent disposition of nanoparticles and microparticles following subconjunctival administration. *J Pharm Pharmacol.* 2005; 57:1555–1563. [PubMed: 16354399]
- Amrite, AC.; Kompella, UB. Nanoparticles for ocular drug delivery. In: Gupta, RB.; Kompella, UB., editors. *Nanoparticle Technology for Drug Delivery.* Informa Healthcare; 2006. p. 319-360.
- Aoyagi S, Hayato I, Yuichi I, Mitsuo FHO. Laser fabrication of high aspect ratio thin holes on biodegradable polymer and its application to a microneedle. *Sens Actuators A Physical.* 2007; 139:293–302.

- Ashok B, Arleth L, Hjelm RP, Rubinstein I, Onyukse H. In vitro characterization of PEGylated phospholipid micelles for improved drug solubilization: effects of PEG chain length and PC incorporation. *Journal of pharmaceutical sciences*. 2004; 93:2476–2487. [PubMed: 15349957]
- Aukunuru JV, Ayalasomayajula SP, Kompella UB. Nanoparticle formulation enhances the delivery and activity of a vascular endothelial growth factor antisense oligonucleotide in human retinal pigment epithelial cells. *J Pharm Pharmacol*. 2003; 55:1199–1206. [PubMed: 14604462]
- Aukunuru JV, Kompella UB. In Vitro Delivery of Nano- and Micro-Particles to Human Retinal Pigment Epithelial (ARPE-19) Cells. *Drug Delivery Technology*. 2002; 2:50–57.
- Ayalasomayajula SP, Kompella UB. Celecoxib, a selective cyclooxygenase-2 inhibitor, inhibits retinal vascular endothelial growth factor expression and vascular leakage in a streptozotocin-induced diabetic rat model. *Eur J Pharmacol*. 2003; 458:283–289. [PubMed: 12504784]
- Ayalasomayajula SP, Kompella UB. Retinal delivery of celecoxib is several-fold higher following subconjunctival administration compared to systemic administration. *Pharmaceutical research*. 2004; 21:1797–1804. [PubMed: 15553225]
18. Ayalasomayajula SP, Kompella UB. Subconjunctivally administered celecoxib-PLGA microparticles sustain retinal drug levels and alleviate diabetes-induced oxidative stress in a rat model. *Eur J Pharmacol*. 2005; 511:191–198. [PubMed: 15792788]
- Bernstein HN. Chloroquine ocular toxicity. *Surv Ophthalmol*. 1967; 12:415–447. [PubMed: 4980087]
- Bill A. Movement of Albumin and Dextran through the Sclera. *Arch Ophthalmol*. 1965; 74:248–252. [PubMed: 14318504]
- Boddu SH, Jwala J, Chowdhury MR, Mitra AK. In vitro evaluation of a targeted and sustained release system for retinoblastoma cells using Doxorubicin as a model drug. *Journal of ocular pharmacology and therapeutics : the official journal of the Association for Ocular Pharmacology and Therapeutics*. 2010; 26:459–468. [PubMed: 20874666]
- Carrasquillo KG, Ricker JA, Rigas IK, Miller JW, Gragoudas ES, Adamis AP. Controlled delivery of the anti-VEGF aptamer EYE001 with poly(lactic-co-glycolic)acid microspheres. *Invest Ophthalmol Vis Sci*. 2003; 44:290–299. [PubMed: 12506087]
- Ceulemans J, Ludwig A. Optimisation of carbomer viscous eye drops: an in vitro experimental design approach using rheological techniques. *European journal of pharmaceuticals and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V.* 2002; 54:41–50. [PubMed: 12084501]
- Cheruvu NP, Amrite AC, Kompella UB. Effect of eye pigmentation on transscleral drug delivery. *Invest Ophthalmol Vis Sci*. 2008; 49:333–341. [PubMed: 18172110]
- Cheruvu NP, Amrite AC, Kompella UB. Effect of diabetes on transscleral delivery of celecoxib. *Pharmaceutical research*. 2009; 26:404–414. [PubMed: 18987961]
- Cheruvu NP, Kompella UB. Bovine and porcine transscleral solute transport: influence of lipophilicity and the Choroid-Bruch's layer. *Invest Ophthalmol Vis Sci*. 2006; 47:4513–4522. [PubMed: 17003447]
- Cho YK, Uehara H, Young JR, Tyagi P, Kompella UB, Zhang X, Luo L, Singh N, Archer B, Ambati BK. Flt23k nanoparticles offer additive benefit in graft survival and anti-angiogenic effects when combined with triamcinolone. *Investigative ophthalmology & visual science*. 2012; 53:2328–2336. [PubMed: 22427553]
- Chopra P, Hao J, Li SK. Sustained release micellar carrier systems for iontophoretic transport of dexamethasone across human sclera. *J Control Release*. 2012; 160:96–104. [PubMed: 22306336]
- Christie JG, Kompella UB. Ophthalmic light sensitive nanocarrier systems. *Drug Discov Today*. 2008; 13:124–134. [PubMed: 18275910]
- Ciechanover A, Schwartz AL, Lodish HF. Sorting and recycling of cell surface receptors and endocytosed ligands: the asialoglycoprotein and transferrin receptors. *J Cell Biochem*. 1983; 23:107–130. [PubMed: 6327736]
- Conley SM, Naash MI. Nanoparticles for retinal gene therapy. *Progress in retinal and eye research*. 2010; 29:376–397. [PubMed: 20452457]
- Cruysberg LP, Nuijts RM, Geroski DH, Koole LH, Hendrikse F, Edelhauser HF. In vitro human scleral permeability of fluorescein, dexamethasone-fluorescein, methotrexate-fluorescein and rhodamine 6G and the use of a coated coil as a new drug delivery system. *Journal of ocular*

- pharmacology and therapeutics : the official journal of the Association for Ocular Pharmacology and Therapeutics. 2002; 18:559–569. [PubMed: 12537682]
- Cunha-Vaz J. Diabetic macular edema. *Eur J Ophthalmol*. 1998; 8:127–130. [PubMed: 9793763]
- Davis SP, Martanto W, Allen MG, Prausnitz MR. Hollow metal microneedles for insulin delivery to diabetic rats. *IEEE Trans Biomed Eng*. 2005; 52:909–915. [PubMed: 15887540]
- De Campos AM, Sanchez A, Alonso MJ. Chitosan nanoparticles: a new vehicle for the improvement of the delivery of drugs to the ocular surface. Application to cyclosporin A. *International journal of pharmaceutics*. 2001; 224:159–168. [PubMed: 11472825]
- Diebold Y, Calonge M. Applications of nanoparticles in ophthalmology. *Progress in retinal and eye research*. 2010; 29:596–609. [PubMed: 20826225]
- Do carmo A, Ramos P, Reis A, Proenca R, Cunha-vaz JG. Breakdown of the inner and outer blood retinal barrier in streptozotocin-induced diabetes. *Experimental eye research*. 1998; 67:569–575. [PubMed: 9878219]
- Dobrovolskaia MA, McNeil SE. Immunological properties of engineered nanomaterials. *Nat Nanotechnol*. 2007; 2:469–478. [PubMed: 18654343]
- Donnelly RF, Morrow DI, Singh TR, Migalska K, McCarron PA, O'Mahony C, Woolfson AD. Processing difficulties and instability of carbohydrate microneedle arrays. *Drug Dev Ind Pharm*. 2009; 35:1242–1254. [PubMed: 19555249]
- Durairaj C, Kadam RS, Chandler JW, Hutcherson SL, Kompella UB. Nanosized dendritic polyguanidylated translocators for enhanced solubility, permeability, and delivery of gatifloxacin. *Invest Ophthalmol Vis Sci*. 2010; 51:5804–5816. [PubMed: 20484584]
- Durairaj C, Kim SJ, Edelhauser HF, Shah JC, Kompella UB. Influence of dosage form on the intravitreal pharmacokinetics of diclofenac. *Invest Ophthalmol Vis Sci*. 2009; 50:4887–4897. [PubMed: 19516015]
- Durrani AM, Farr SJ, Kellaway IW. Precorneal clearance of mucoadhesive microspheres from the rabbit eye. *J Pharm Pharmacol*. 1995; 47:581–584. [PubMed: 8568625]
- Duvvuri S, Janoria KG, Pal D, Mitra AK. Controlled delivery of ganciclovir to the retina with drug-loaded Poly(D,L-lactide-co-glycolide) (PLGA) microspheres dispersed in PLGA-PEG-PLGA Gel: a novel intravitreal delivery system for the treatment of cytomegalovirus retinitis. *Journal of ocular pharmacology and therapeutics : the official journal of the Association for Ocular Pharmacology and Therapeutics*. 2007; 23:264–274. [PubMed: 17593010]
- Edelhauser HF, Maren TH. Permeability of human cornea and sclera to sulfonamide carbonic anhydrase inhibitors. *Arch Ophthalmol*. 1988; 106:1110–1115. [PubMed: 3401140]
- Einhahl S, Savoldelli M, D'Hermies F, Tabatabay C, Gurny R, Behar-Cohen F. Evaluation of a novel biomaterial in the suprachoroidal space of the rabbit eye. *Invest Ophthalmol Vis Sci*. 2002; 43:1533–1539. [PubMed: 11980871]
- El-Remessy AB, Behzadian MA, Abou-Mohamed G, Franklin T, Caldwell RW, Caldwell RB. Experimental diabetes causes breakdown of the blood-retina barrier by a mechanism involving tyrosine nitration and increases in expression of vascular endothelial growth factor and urokinase plasminogen activator receptor. *Am J Pathol*. 2003; 162:1995–2004. [PubMed: 12759255]
- Enriquez de Salamanca A, Diebold Y, Calonge M, Garcia-Vazquez C, Callejo S, Vila A, Alonso MJ. Chitosan nanoparticles as a potential drug delivery system for the ocular surface: toxicity, uptake mechanism and in vivo tolerance. *Investigative ophthalmology & visual science*. 2006; 47:1416–1425. [PubMed: 16565375]
- Eugene de Juan, J.; Alster, Y.; Chamow, SM.; Farinas, KC.; Hanson, S.; Gifford, I.; MacFarlane, KA.; Reich, CJ.; Barrett, M.; Campbell, RE.; George, R.; Sutton, D. Posterior segment drug delivery. United States: ForSight Labs, LLC; 2010. A61F 9/00; A61K 39/395; A61P 27/02; A61K 31/5575; A61K 31/58; A61F 2/14; B23P 17/04 ed.
- Fang J, Nakamura H, Maeda H. The EPR effect: Unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect. *Advanced drug delivery reviews*. 2011; 63:136–151. [PubMed: 20441782]
- Friedburg C, Thomas MM, Lamb TD. Time course of the flash response of dark- and light-adapted human rod photoreceptors derived from the electroretinogram. *The Journal of physiology*. 2001; 534:217–242. [PubMed: 11433004]

- Fujii Y, Kachi S, Ito A, Kawasumi T, Honda H, Terasaki H. Transfer of gene to human retinal pigment epithelial cells using magnetite cationic liposomes. *The British journal of ophthalmology*. 2010; 94:1074–1077. [PubMed: 19955204]
- Fujisawa T, Miyai H, Hironaka K, Tsukamoto T, Tahara K, Tozuka Y, Ito M, Takeuchi H. Liposomal diclofenac eye drop formulations targeting the retina: Formulation stability improvement using surface modification of liposomes. *International journal of pharmaceutics*. 2012; 436:564–567. [PubMed: 22828072]
- Geze A, Chau LT, Choisnard L, Mathieu JP, Marti-Batlle D, Riou L, Putaux JL, Wouessidjewe D. Biodistribution of intravenously administered amphiphilic beta-cyclodextrin nanospheres. *International journal of pharmaceutics*. 2007; 344:135–142. [PubMed: 17692481]
- Ghate D, Brooks W, McCarey BE, Edelhauser HF. Pharmacokinetics of intraocular drug delivery by periocular injections using ocular fluorophotometry. *Invest Ophthalmol Vis Sci*. 2007; 48:2230–2237. [PubMed: 17460284]
- Gilger BC, Salmon JH, Wilkie DA, Cruysberg LP, Kim J, Hayat M, Kim H, Kim S, Yuan P, Lee SS, Harrington SM, Murray PR, Edelhauser HF, Csaky KG, Robinson MR. A novel bioerodible deep scleral lamellar cyclosporine implant for uveitis. *Invest Ophthalmol Vis Sci*. 2006; 47:2596–2605. [PubMed: 16723476]
- Gilger BC, Wilkie DA, Clode AB, McMullen RJ Jr, Utter ME, Komaromy AM, Brooks DE, Salmon JH. Long-term outcome after implantation of a suprachoroidal cyclosporine drug delivery device in horses with recurrent uveitis. *Vet Ophthalmol*. 2010; 13:294–300. [PubMed: 20840106]
- Gong X, Peng S, Wen W, Sheng P, Li W. Design and fabrication of magnetically functionalized core/shell microspheres for smart drug delivery. *Adv Funct Mater*. 2009; 19:292–297.
- Grayson ACR, Shawgo RS, Johnson AM, Flynn NT, Yawen L, Cima MJ, Langer R. A BioMEMS review: MEMS technology for physiologically integrated devices. *Proceedings of the IEEE*. 2004; 92:6–21.
- Gupta, RB.; Kompella, UB. *Nanoparticle Technology for Drug Delivery*. New York: Taylor & Francis Group; 2006.
- Hashmi S, Ling P, Hashmi G, Reed M, Gaugler R, Trimmer W. Genetic transformation of nematodes using arrays of micromechanical piercing structures. *Biotechniques*. 1995; 19:766–770. [PubMed: 8588914]
- Hassan EE, Gallo JM. A simple rheological method for the in vitro assessment of mucin-polymer bioadhesive bond strength. *Pharmaceutical research*. 1990; 7:491–495. [PubMed: 1694990]
- Henry S, McAllister DV, Allen MG, Prausnitz MR. Microfabricated microneedles: a novel approach to transdermal drug delivery. *J Pharm Sci*. 1998; 87:922–925. [PubMed: 9687334]
- Holden CA, Tyagi P, Thakur A, Kadam R, Jadhav G, Kompella UB, Yang H. Polyamidoamine dendrimer hydrogel for enhanced delivery of antiglaucoma drugs. *Nanomedicine*. 2012; 8:776–783. [PubMed: 21930109]
- Horgan N, Shields CL, Mashayekhi A, Salazar PF, Materin MA, O'Regan M, Shields JA. Periocular triamcinolone for prevention of macular edema after plaque radiotherapy of uveal melanoma: a randomized controlled trial. *Ophthalmology*. 2009; 116:1383–1390. [PubMed: 19481812]
- Hornof M, Weyenberg W, Ludwig A, Bernkop-Schnurch A. Mucoadhesive ocular insert based on thiolated poly(acrylic acid): development and in vivo evaluation in humans. *J Control Release*. 2003; 89:419–428. [PubMed: 12737844]
- Howard DB, Powers K, Wang Y, Harvey BK. Tropism and toxicity of adeno-associated viral vector serotypes 1, 2, 5, 6, 7, 8, and 9 in rat neurons and glia in vitro. *Virology*. 2008; 372:24–34. [PubMed: 18035387]
- Iezzi R, Guru BR, Glybina IV, Mishra MK, Kennedy A, Kannan RM. Dendrimer-based targeted intravitreal therapy for sustained attenuation of neuroinflammation in retinal degeneration. *Biomaterials*. 2012; 33:979–988. [PubMed: 22048009]
- Ito Y, Hagiwara E, Saeki A, Sugioka N, Takada K. Feasibility of microneedles for percutaneous absorption of insulin. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences*. 2006; 29:82–88. [PubMed: 16828268]

- Jani PD, Singh N, Jenkins C, Raghava S, Mo Y, Amin S, Kompella UB, Ambati BK. Nanoparticles sustain expression of Flt intrareceptors in the cornea and inhibit injury-induced corneal angiogenesis. *Invest Ophthalmol Vis Sci.* 2007; 48:2030–2036. [PubMed: 17460257]
- Jiang J, Gill HS, Ghate D, McCarey BE, Patel SR, Edelhauser HF, Prausnitz MR. Coated microneedles for drug delivery to the eye. *Invest Ophthalmol Vis Sci.* 2007; 48:4038–4043. [PubMed: 17724185]
- Jiang J, Moore JS, Edelhauser HF, Prausnitz MR. Intrasceral drug delivery to the eye using hollow microneedles. *Pharm Res.* 2009; 26:395–403. [PubMed: 18979189]
- Jin J, Zhou KK, Park K, Hu Y, Xu X, Zheng Z, Tyagi P, Kompella UB, Ma JX. Anti-inflammatory and antiangiogenic effects of nanoparticle-mediated delivery of a natural angiogenic inhibitor. *Invest Ophthalmol Vis Sci.* 2011; 52:6230–6237. [PubMed: 21357401]
- Jockovich ME, Murray TG, Escalona-Benz E, Hernandez E, Feuer W. Anecortave acetate as single and adjuvant therapy in the treatment of retinal tumors of LH(BETA)T(AG) mice. *Invest Ophthalmol Vis Sci.* 2006; 47:1264–1268. [PubMed: 16565356]
- Jonas JB, Spandau UH, Schlichtenbrede F. Short-term complications of intravitreal injections of triamcinolone and bevacizumab. *Eye (Lond).* 2008; 22:590–591. [PubMed: 18292795]
- Kadam RS, Cheruvu NP, Edelhauser HF, Kompella UB. Sclera-choroid-RPE transport of eight beta-blockers in human, bovine, porcine, rabbit, and rat models. *Invest Ophthalmol Vis Sci.* 2011; 52:5387–5399. [PubMed: 21282583]
- Kadam RS, Tyagi P, Edelhauser HF, Kompella UB. Influence of choroidal neovascularization and biodegradable polymeric particle size on transscleral sustained delivery of triamcinolone acetate. *International journal of pharmaceutics.* 2012; 434:140–147. [PubMed: 22633904]
- Kang SJ, Durairaj C, Kompella UB, O'Brien JM, Grossniklaus HE. Subconjunctival nanoparticle carboplatin in the treatment of murine retinoblastoma. *Arch Ophthalmol.* 2009; 127:1043–1047. [PubMed: 19667343]
- Karlsson AC, Ekebergh M, Larsson Mauleon A, Almerud Osterberg S. Only a whisper away. A philosophical view of the awake patient's situation during regional anaesthetics and surgery. *Nursing philosophy : an international journal for healthcare professionals.* 2012; 13:257–265. [PubMed: 22950729]
- Kato A, Kimura H, Okabe K, Okabe J, Kunou N, Ogura Y. Feasibility of drug delivery to the posterior pole of the rabbit eye with an episcleral implant. *Invest Ophthalmol Vis Sci.* 2004; 45:238–244. [PubMed: 14691179]
- Kaushik S, Hord AH, Denson DD, McAllister DV, Smitra S, Allen MG, Prausnitz MR. Lack of pain associated with microfabricated microneedles. *Anesth Analg.* 2001; 92:502–504. [PubMed: 11159258]
- Khraiche ML, Lo Y, Wang D, Cauwenberghs G, Freeman W, Silva GA. Ultra-high photosensitivity silicon nanophotonics for retinal prosthesis: electrical characteristics. *Engineering in Medicine and Biology Society.* 2011:2933–2936.
- Kiang T, Bright C, Cheung CY, Stayton PS, Hoffman AS, Leong KW. Formulation of chitosan-DNA nanoparticles with poly(propyl acrylic acid) enhances gene expression. *J Biomater Sci Polym Ed.* 2004; 15:1405–1421. [PubMed: 15648571]
- Kim H, Csaky KG, Gilger BC, Dunn JP, Lee SS, Tremblay M, de Monasterio F, Tansey G, Yuan P, Bungay PM, Lutz RJ, Robinson MR. Preclinical evaluation of a novel episcleral cyclosporine implant for ocular graft-versus-host disease. *Invest Ophthalmol Vis Sci.* 2005; 46:655–662. [PubMed: 15671296]
- Kim H, Robinson MR, Lizak MJ, Tansey G, Lutz RJ, Yuan P, Wang NS, Csaky KG. Controlled drug release from an ocular implant: an evaluation using dynamic three-dimensional magnetic resonance imaging. *Invest Ophthalmol Vis Sci.* 2004; 45:2722–2731. [PubMed: 15277497]
- Kim TW, Lindsey JD, Aihara M, Anthony TL, Weinreb RN. Intraocular distribution of 70-kDa dextran after subconjunctival injection in mice. *Invest Ophthalmol Vis Sci.* 2002; 43:1809–1816. [PubMed: 12036983]
- Kleinman ME, Yamada K, Takeda A, Chandrasekaran V, Nozaki M, Baffi JZ, Albuquerque RJ, Yamasaki S, Itaya M, Pan Y, Appukuttan B, Gibbs D, Yang Z, Kariko K, Ambati BK, Wilgus TA, DiPietro LA, Sakurai E, Zhang K, Smith JR, Taylor EW, Ambati J. Sequence- and target-

independent angiogenesis suppression by siRNA via TLR3. *Nature*. 2008; 452:591–597. [PubMed: 18368052]

- Klibanov AL, Maruyama K, Torchilin VP, Huang L. Amphipathic polyethyleneglycols effectively prolong the circulation time of liposomes. *FEBS Lett*. 1990; 268:235–237. [PubMed: 2384160]
- Kolli CS, Banga AK. Characterization of solid maltose microneedles and their use for transdermal delivery. *Pharm Res*. 2008; 25:104–113. [PubMed: 17597381]
- Kompella UB, Bandi N, Ayalasomayajula SP. Subconjunctival nano- and microparticles sustain retinal delivery of budesonide, a corticosteroid capable of inhibiting VEGF expression. *Invest Ophthalmol Vis Sci*. 2003; 44:1192–1201. [PubMed: 12601049]
- Kompella UB, Kadam RS, Lee VH. Recent advances in ophthalmic drug delivery. *Ther Deliv*. 2010; 1:435–456. [PubMed: 21399724]
- Kompella UB, Sundaram S, Raghava S, Escobar ER. Luteinizing hormone-releasing hormone agonist and transferrin functionalizations enhance nanoparticle delivery in a novel bovine ex vivo eye model. *Molecular vision*. 2006; 12:1185–1198. [PubMed: 17102798]
- Kompella UB.; Upadhyay, A. Trasferrin-functionalized PLGA Nanopartilces Sustain Diclofenac Delivery to Choroid-RPE, The Association for Research in Vision and Ophthalmology. Fort Lauderdale, FL: 2012.
- Koushik K, Bandi N, Sundaram S, Kompella UB. Evidence for LHRH-receptor expression in human airway epithelial (Calu-3) cells and its role in the transport of an LHRH agonist. *Pharmaceutical research*. 2004; 21:1034–1046. [PubMed: 15212170]
- Kragh-Hansen U, Chuang VT, Otagiri M. Practical aspects of the ligand-binding and enzymatic properties of human serum albumin. *Biological & pharmaceutical bulletin*. 2002; 25:695–704. [PubMed: 12081132]
- Kwon GS. Polymeric micelles for delivery of poorly water-soluble compounds. *Crit Rev Ther Drug Carrier Syst*. 2003; 20:357–403. [PubMed: 14959789]
- Ladas ID, Karagiannis DA, Rouvas AA, Kotsolis AI, Liotsou A, Vergados I. Safety of repeat intravitreal injections of bevacizumab versus ranibizumab: our experience after 2,000 injections. *Retina*. 2009; 29:313–318. [PubMed: 19287287]
- Leblanc B, Jezequel S, Davies T, Hanton G, Taradach C. Binding of drugs to eye melanin is not predictive of ocular toxicity. *Regul Toxicol Pharmacol*. 1998; 28:124–132. [PubMed: 9927562]
- Lee JW, Park JH, Prausnitz MR. Dissolving microneedles for transdermal drug delivery. *Biomaterials*. 2008; 29:2113–2124. [PubMed: 18261792]
- Lee TW, Robinson JR. Drug delivery to the posterior segment of the eye II: development and validation of a simple pharmacokinetic model for subconjunctival injection. *Journal of ocular pharmacology and therapeutics : the official journal of the Association for Ocular Pharmacology and Therapeutics*. 2004; 20:43–53. [PubMed: 15006158]
- Lehr CM, Bouwstra JA, Kok W, De Boer AG, Tukker JJ, Verhoef JC, Breimer DD, Junginger HE. Effects of the mucoadhesive polymer polycarbophil on the intestinal absorption of a peptide drug in the rat. *The Journal of pharmacy and pharmacology*. 1992; 44:402–407. [PubMed: 1359054]
- Lehr CM, Lee YH, Lee VH. Improved ocular penetration of gentamicin by mucoadhesive polymer polycarbophil in the pigmented rabbit. *Invest Ophthalmol Vis Sci*. 1994; 35:2809–2814. [PubMed: 8188475]
- Liu H, Meagher CK, Moore CP, Phillips TE. M cells in the follicle-associated epithelium of the rabbit conjunctiva preferentially bind and translocate latex beads. *Invest Ophthalmol Vis Sci*. 2005; 46:4217–4223. [PubMed: 16249501]
- Lloyd AW, Faragher RG, Denyer SP. Ocular biomaterials and implants. *Biomaterials*. 2001; 22:769–785. [PubMed: 11246945]
- Lo R, Li PY, Saati S, Agrawal RN, Humayun MS, Meng E. A passive MEMS drug delivery pump for treatment of ocular diseases. *Biomedical microdevices*. 2009; 11:959–970. [PubMed: 19396548]
- M H, T A, T U, Y A, N O, M T. Suppression of choroidal neovascularization by intravitreal injection of liposomal SU5416. *Archives in Ophthalmology*. 2012; 129:317–321.
- Mac Gabhann F, Demetriades AM, Deering T, Packer JD, Shah SM, Duh E, Campochiaro PA, Popel AS. Protein transport to choroid and retina following periocular injection: theoretical and experimental study. *Ann Biomed Eng*. 2007; 35:615–630. [PubMed: 17277991]

- Maeda H, Matsumura Y. Tumorotropic and lymphotropic principles of macromolecular drugs. *Crit Rev Ther Drug Carrier Syst.* 1989; 6:193–210. [PubMed: 2692843]
- Mars U, Larsson BS. Pheomelanin as a binding site for drugs and chemicals. *Pigment cell research / sponsored by the European Society for Pigment Cell Research and the International Pigment Cell Society.* 1999; 12:266–274. [PubMed: 10454295]
- Martin NE, Kim JW, Abramson DH. Fibrin sealant for retinoblastoma: where are we? *Journal of ocular pharmacology and therapeutics : the official journal of the Association for Ocular Pharmacology and Therapeutics.* 2008; 24:433–438. [PubMed: 18788992]
- Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumorotropic accumulation of proteins and the antitumor agent smancs. *Cancer Res.* 1986; 46:6387–6392. [PubMed: 2946403]
- Maurice DM. Drug delivery to the posterior segment from drops. *Surv Ophthalmol.* 2002; 47(Suppl 1):S41–S52. [PubMed: 12204700]
- Maurice DM, Polgar J. Diffusion across the sclera. *Experimental eye research.* 1977; 25:577–582. [PubMed: 590384]
- Mayo AS, Ambati BK, Kompella UB. Gene delivery nanoparticles fabricated by supercritical fluid extraction of emulsions. *International journal of pharmaceutics.* 2010; 387:278–285. [PubMed: 20025945]
- McAllister DV, Wang PM, Davis SP, Park JH, Canatella PJ, Allen MG, Prausnitz MR. Microfabricated needles for transdermal delivery of macromolecules and nanoparticles: fabrication methods and transport studies. *Proc Natl Acad Sci U S A.* 2003; 100:13755–13760. [PubMed: 14623977]
- McAllister K, Sazani P, Adam M, Cho MJ, Rubinstein M, Samulski RJ, DeSimone JM. Polymeric nanogels produced via inverse microemulsion polymerization as potential gene and antisense delivery agents. *J Am Chem Soc.* 2002; 124:15198–15207. [PubMed: 12487595]
- McNeil SE. Nanotechnology for the biologist. *J Leukoc Biol.* 2005; 78:585–594. [PubMed: 15923216]
- Meagher CK, Liu H, Moore CP, Phillips TE. Conjunctival M cells selectively bind and translocate *Maackia amurensis* leukoagglutinin. *Experimental eye research.* 2005; 80:545–553. [PubMed: 15781282]
- Medina C, Santos-Martinez MJ, Radomski A, Corrigan OI, Radomski MW. Nanoparticles: pharmacological and toxicological significance. *Br J Pharmacol.* 2007; 150:552–558. [PubMed: 17245366]
- Meng J, Sturgis TF, Youan BB. Engineering tenofovir loaded chitosan nanoparticles to maximize microbicide mucoadhesion. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences.* 2011; 44:57–67. [PubMed: 21704704]
- Misra GP, Singh RS, Aleman TS, Jacobson SG, Gardner TW, Lowe TL. Subconjunctivally implantable hydrogels with degradable and thermoresponsive properties for sustained release of insulin to the retina. *Biomaterials.* 2009; 30:6541–6547. [PubMed: 19709741]
- Mitra M, Misra R, Harilal A, Sahoo SK, Krishnakumar S. Enhanced in vitro antiproliferative effects of EpCAM antibody-functionalized paclitaxel-loaded PLGA nanoparticles in retinoblastoma cells. *Molecular vision.* 2011; 17:2724–2737. [PubMed: 22065926]
- Miyano T, Tobinaga Y, Kanno T, Matsuzaki Y, Takeda H, Wakui M, Hanada K. Sugar micro needles as transdermic drug delivery system. *Biomed Microdevices.* 2005; 7:185–188. [PubMed: 16133805]
- Mo Y, Barnett ME, Takemoto D, Davidson H, Kompella UB. Human serum albumin nanoparticles for efficient delivery of Cu, Zn superoxide dismutase gene. *Molecular vision.* 2007; 13:746–757. [PubMed: 17563725]
- Moghimi SM, Porter CJ, Muir IS, Illum L, Davis SS. Non-phagocytic uptake of intravenously injected microspheres in rat spleen: influence of particle size and hydrophilic coating. *Biochemical and biophysical research communications.* 1991; 177:861–866. [PubMed: 2049107]
- Moon SJ, Lee SS, Lee HS, Kwon TH. Fabrication of microneedle array using LIGA and hot embossing process. *Microsyst Technol Micro Nanosyst Informat Storage Proc Syst.* 2005; 11:311–318.

- Murray TG, Cicciarelli N, O'Brien JM, Hernandez E, Mueller RL, Smith BJ, Feuer W. Subconjunctival carboplatin therapy and cryotherapy in the treatment of transgenic murine retinoblastoma. *Arch Ophthalmol*. 1997; 115:1286–1290. [PubMed: 9338675]
- Okabe K, Kimura H, Okabe J, Ogura Y. Ocular tissue distribution of betamethasone after anterior-episcleral, posterior-episcleral, and anterior-intrascleral placement of nonbiodegradable implants. *Retina*. 2007; 27:770–777. [PubMed: 17621189]
- Olsen TW, Feng X, Wabner K, Conston SR, Sierra DH, Folden DV, Smith ME, Cameron JD. Cannulation of the suprachoroidal space: a novel drug delivery methodology to the posterior segment. *Am J Ophthalmol*. 2006; 142:777–787. [PubMed: 16989764]
- Olsen TW, Feng X, Wabner K, Csaky K, Pambuccian S, Cameron JD. Pharmacokinetics of pars plana intravitreal injections versus microcannula suprachoroidal injections of bevacizumab in a porcine model. *Invest Ophthalmol Vis Sci*. 2011; 52:4749–4756. [PubMed: 21447680]
- Ovsianikov A, Schlie S, Ngezahayo A, Haverich A, Chichkov BN. Two-photon polymerization technique for microfabrication of CAD-designed 3D scaffolds from commercially available photosensitive materials. *J Tissue Eng Regen Med*. 2007; 1:443–449. [PubMed: 18265416]
- Oyewumi, MO.; Rice, KG. DNA Nanoparticle GeneDelivery Systems. In: Gupta, RB.; Kompella, UB., editors. *Nanoparticle Technology for Drug Delivery*. New York: Taylor & Francis Group; 2006. p. 361-373.
- Pan CK, Durairaj C, Kompella UB, Agwu O, Oliver SC, Quiroz-Mercado H, Mandava N, Olson JL. Comparison of long-acting bevacizumab formulations in the treatment of choroidal neovascularization in a rat model. *Journal of ocular pharmacology and therapeutics : the official journal of the Association for Ocular Pharmacology and Therapeutics*. 2011; 27:219–224. [PubMed: 21574814]
- Panessa-Warren BJ, Maye MM, Warren JB, Crosson KM. Single walled carbon nanotube reactivity and cytotoxicity following extended aqueous exposure. *Environ Pollut*. 2009; 157:1140–1151. [PubMed: 19201512]
- Park JH, Allen MG, Prausnitz MR. Biodegradable polymer microneedles: fabrication, mechanics and transdermal drug delivery. *J Control Release*. 2005; 104:51–66. [PubMed: 15866334]
- Park JH, Allen MG, Prausnitz MR. Polymer microneedles for controlled-release drug delivery. *Pharm Res*. 2006; 23:1008–1019. [PubMed: 16715391]
- Park K, Chen Y, Hu Y, Mayo AS, Kompella UB, Longeras R, Ma JX. Nanoparticle-mediated expression of an angiogenic inhibitor ameliorates ischemia-induced retinal neovascularization and diabetes-induced retinal vascular leakage. *Diabetes*. 2009; 58:1902–1913. [PubMed: 19491211]
- Patel SR, Lin AS, Edelhauser HF, Prausnitz MR. Suprachoroidal drug delivery to the back of the eye using hollow microneedles. *Pharmaceutical research*. 2011; 28:166–176. [PubMed: 20857178]
- Peden MC, Min J, Meyers C, Lukowski Z, Li Q, Boye SL, Levine M, Hauswirth WW, Ratnakaram R, Dawson W, Smith WC, Sherwood MB. Ab-externo AAV-mediated gene delivery to the suprachoroidal space using a 250 micron flexible microcatheter. *PLoS One*. 2011; 6:e17140. [PubMed: 21347253]
- Pérennès F, Marmioli B, Matteucci M, Tormen M, Vaccari L, Di Fabrizio E. Sharp beveled tip hollow microneedle arrays fabricated by LIGA and 3D soft lithography with polyvinyl alcohol. *Journal of Micromechanics and Microengineering*. 2006; 16:473–479.
- Peyman GA, Schulman JA, Khoobehi B, Alkan HM, Tawakol ME, Mani H. Toxicity and clearance of a combination of liposome-encapsulated ganciclovir and trifluridine. *Retina*. 1989; 9:232–236. [PubMed: 2512600]
- Pitkanen L, Ranta VP, Moilanen H, Urtti A. Permeability of retinal pigment epithelium: effects of permeant molecular weight and lipophilicity. *Invest Ophthalmol Vis Sci*. 2005; 46:641–646. [PubMed: 15671294]
- Pontes de Carvalho RA, Krausse ML, Murphree AL, Schmitt EE, Campochiaro PA, Maumenee IH. Delivery from episcleral explants. *Invest Ophthalmol Vis Sci*. 2006; 47:4532–4539. [PubMed: 17003449]
- Prausnitz MR. Microneedles for transdermal drug delivery. *Adv Drug Deliv Rev*. 2004; 56:581–587. [PubMed: 15019747]

- Prow TW, Bhutto I, Kim SY, Grebe R, Merges C, McLeod DS, Uno K, Mennon M, Rodriguez L, Leong K, Luty GA. Ocular nanoparticle toxicity and transfection of the retina and retinal pigment epithelium. *Nanomedicine*. 2008; 4:340–349. [PubMed: 18640079]
- Qazi Y, Stagg B, Singh N, Singh S, Zhang X, Luo L, Simonis J, Kompella UB, Ambati BK. Nanoparticle-mediated delivery of shRNA.VEGF-a plasmids regresses corneal neovascularization. *Investigative ophthalmology & visual science*. 2012; 53:2837–2844. [PubMed: 22467572]
- Raghava, S.; Goel, G.; Kompella, UB. Ophthalmic Applications of Nanotechnology. In: Tombran-Tink, J.; Barnstable, CJ., editors. *Ocular Transporters in Ophthalmic Diseases and Drug Delivery*. New Jersey: Humana Press; 2008. p. 415-436.
- Raghava S, Hammond M, Kompella UB. Periocular routes for retinal drug delivery. *Expert Opin Drug Deliv*. 2004; 1:99–114. [PubMed: 16296723]
- Raju HB, Hu Y, Vedula A, Dubovy SR, Goldberg JL. Evaluation of magnetic micro- and nanoparticle toxicity to ocular tissues. *PloS one*. 2011; 6:e17452. [PubMed: 21637340]
- Ranta VP, Urtti A. Transscleral drug delivery to the posterior eye: prospects of pharmacokinetic modeling. *Advanced drug delivery reviews*. 2006; 58:1164–1181. [PubMed: 17069929]
- Ruiz-Moreno JM, Montero JA, Arias L, Sanabria MR, Coco R, Silva R, Araiz J, Gomez-Ulla F, Garcia-Layana A. Photodynamic therapy in subfoveal and juxtafoveal idiopathic and postinflammatory choroidal neovascularization. *Acta Ophthalmol Scand*. 2006; 84:743–748. [PubMed: 17083531]
- Sahay G, Alakhova DY, Kabanov AV. Endocytosis of nanomedicines. *J Control Release*. 2010; 145:182–195. [PubMed: 20226220]
- Saishin Y, Silva RL, Saishin Y, Callahan K, Schoch C, Ahlheim M, Lai H, Kane F, Brazzell RK, Bodmer D, Campochiaro PA. Periocular injection of microspheres containing PKC412 inhibits choroidal neovascularization in a porcine model. *Invest Ophthalmol Vis Sci*. 2003; 44:4989–4993. [PubMed: 14578426]
- Sakurai E, Ozeki H, Kunou N, Ogura Y. Effect of particle size of polymeric nanospheres on intravitreal kinetics. *Ophthalmic Res*. 2001; 33:31–36. [PubMed: 11114602]
- Salazar-Bookaman MM, Wainer I, Patil PN. Relevance of drug-melanin interactions to ocular pharmacology and toxicology. *J Ocul Pharmacol*. 1994; 10:217–239. [PubMed: 8207328]
- Scheinman RI, Trivedi R, Vermillion S, Kompella UB. Functionalized STAT1 siRNA nanoparticles regress rheumatoid arthritis in a mouse model. *Nanomedicine (Lond)*. 2011; 6:1669–1682. [PubMed: 22087799]
- Schipper ML, Iyer G, Koh AL, Cheng Z, Ebenstein Y, Aharoni A, Keren S, Bentolila LA, Li J, Rao J, Chen X, Banin U, Wu AM, Sinclair R, Weiss S, Gambhir SS. Particle size, surface coating, and PEGylation influence the biodistribution of quantum dots in living mice. *Small*. 2009; 5:126–134. [PubMed: 19051182]
- Schnitzer JE, Oh P. Albondin-mediated capillary permeability to albumin. Differential role of receptors in endothelial transcytosis and endocytosis of native and modified albumins. *The Journal of biological chemistry*. 1994; 269:6072–6082. [PubMed: 8119952]
- Schoenwald RD, Tandon V, Wurster DE, Barfknecht CF. Significance of melanin binding and metabolism in the activity of 5-acetoxycetylimino-4-methyl-delta2-1,3,4,-thiadiazoline-2-sulfonamide. *European journal of pharmaceuticals and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*. 1998; 46:39–50. [PubMed: 9700021]
- Scholz C. Perspectives on: materials aspects for retinal prostheses. *J Bioact Compact Polym*. 2010; 22:539–568.
- Simon BH, Ando HY, Gupta PK. Circulation time and body distribution of 14C-labeled amino-modified polystyrene nanoparticles in mice. *Journal of pharmaceutical sciences*. 1995; 84:1249–1253. [PubMed: 8801343]
- Singh SR, Grossniklaus HE, Kang SJ, Edelhauser HF, Ambati BK, Kompella UB. Intravenous transferrin, RGD peptide and dual-targeted nanoparticles enhance anti-VEGF intraceptor gene delivery to laser-induced CNV. *Gene Ther*. 2009; 16:645–659. [PubMed: 19194480]

- Steiner K, Buhning KU, Merck E. The melanin binding of bisoprolol and its toxicological relevance. *Lens Eye Toxic Res.* 1990; 7:319–333. [PubMed: 1983104]
- Stoeber B, Liepmann D. Arrays of hollow out-of-plane micro-needles for drug delivery. *J Microelectromech Syst.* 2005; 14:472–479.
- Sundaram S, Roy SK, Ambati BK, Kompella UB. Surface-functionalized nanoparticles for targeted gene delivery across nasal respiratory epithelium. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology.* 2009; 23:3752–3765. [PubMed: 19608628]
- Tari, SR.; Barile, GR.; Kompella, UB. Polychromatic, diversely-sized particles for angiography. Office, USP., editor. United States: The Trustees of Columbia University in the City of New York Board of Regents of the University of Nebraska Varner Hall; 2009. p. 19A61K 51/00; A61K 49/00; A61K 9/127; A61K 9/14; A61K 9/16 ed.
- Thanou M, Nihot MT, Jansen M, Verhoef JC, Junginger HE. Mono-N-carboxymethyl chitosan (MCC), a polyampholytic chitosan derivative, enhances the intestinal absorption of low molecular weight heparin across intestinal epithelia in vitro and in vivo. *Journal of pharmaceutical sciences.* 2001a; 90:38–46. [PubMed: 11064377]
- Thanou M, Verhoef JC, Junginger HE. Chitosan and its derivatives as intestinal absorption enhancers. *Advanced drug delivery reviews.* 2001b; 50(Suppl 1):S91–S101. [PubMed: 11576697]
- Thanou M, Verhoef JC, Junginger HE. Oral drug absorption enhancement by chitosan and its derivatives. *Advanced drug delivery reviews.* 2001c; 52:117–126. [PubMed: 11718935]
- Thanou M, Verhoef JC, Verheijden JH, Junginger HE. Intestinal absorption of octreotide using trimethyl chitosan chloride: studies in pigs. *Pharmaceutical research.* 2001d; 18:823–828. [PubMed: 11474787]
- Torchilin VP. PEG-based micelles as carriers of contrast agents for different imaging modalities. *Advanced drug delivery reviews.* 2002; 54:235–252. [PubMed: 11897148]
- Touchard E, Berdugo M, Bigey P, El Sanharawi M, Savoldelli M, Naud MC, Jeanny JC, Behar-Cohen F. Suprachoroidal Electrotransfer: A Nonviral Gene Delivery Method to Transfect the Choroid and the Retina Without Detaching the Retina. *Mol Ther.* 2012
- Tripathi BJ, Tripathi RC. Hydrogen peroxide damage to human corneal epithelial cells in vitro. Implications for contact lens disinfection systems. *Archives of ophthalmology.* 1989; 107:1516–1519. [PubMed: 2803102]
- Trivedi R, Kompella UB. Nanomicellar formulations for sustained drug delivery: strategies and underlying principles. *Nanomedicine (Lond).* 2010; 5:485–505. [PubMed: 20394539]
- Trivedi, R.; Tyagi, P.; Kompella, UB. Deslorelin and Transferrin Mono- and Dual-functionalized Nanomicelles for Tumor Targeted Drug Delivery. Chicago, IL: American Association of Pharmaceutical Scientists; 2012.
- Tsuji A, Tamai I, Sasaki K. Intraocular penetration kinetics of prednisolone after subconjunctival injection in rabbits. *Ophthalmic Res.* 1988; 20:31–43. [PubMed: 3380524]
- Tyagi, P.; Barros, MD.; Stansbury, JW.; Kompella, UB. Light activated, in situ forming hydrogel for sustained suprachoroidal delivery of a protein drug; 2012 AAPS National Biotechnology Conference, T2014; 2012.
- van der Merwe SM, Verhoef JC, Kotze AF, Junginger HE. N-trimethyl chitosan chloride as absorption enhancer in oral peptide drug delivery. Development and characterization of minitab and granule formulations. *European journal of pharmaceuticals and biopharmaceuticals : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V.* 2004a; 57:85–91. [PubMed: 14729083]
- van der Merwe SM, Verhoef JC, Verheijden JH, Kotze AF, Junginger HE. Trimethylated chitosan as polymeric absorption enhancer for improved peroral delivery of peptide drugs. *European journal of pharmaceuticals and biopharmaceuticals : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V.* 2004b; 58:225–235. [PubMed: 15296951]
- Van Quill KR, Dioguardi PK, Tong CT, Gilbert JA, Aaberg TM Jr, Grossniklaus HE, Edelhauser HF, O'Brien JM. Subconjunctival carboplatin in fibrin sealant in the treatment of transgenic murine retinoblastoma. *Ophthalmology.* 2005; 112:1151–1158. [PubMed: 15885791]

- Verbaan FJ, Bal SM, van den Berg DJ, Dijkman JA, van Hecke M, Verpoorten H, van den Berg A, Luttge R, Bouwstra JA. Improved piercing of microneedle arrays in dermatomed human skin by an impact insertion method. *J Control Release*. 2008; 128:80–88. [PubMed: 18394741]
- Veritti D, Lanzetta P, Perissin L, Bandello F. Posterior juxtascleral infusion of modified triamcinolone acetate formulation for refractory diabetic macular edema: one-year follow-up. *Invest Ophthalmol Vis Sci*. 2009; 50:2391–2397. [PubMed: 19117937]
- Vinogradov SV, Batrakova EV, Kabanov AV. Nanogels for oligonucleotide delivery to the brain. *Bioconjug Chem*. 2004; 15:50–60. [PubMed: 14733583]
- Vogel SM, Minshall RD, Pilipovic M, Tirupathi C, Malik AB. Albumin uptake and transcytosis in endothelial cells in vivo induced by albumin-binding protein. *American journal of physiology. Lung cellular and molecular physiology*. 2001; 281:L1512–L1522. [PubMed: 11704548]
- Wang J, Lu J, Ly SY, Vuki M, Tian B, Adeniyi WK, Armendariz RA. Lab-on-a-Cable for electrochemical monitoring of phenolic contaminants. *Anal Chem*. 2000; 72:2659–2663. [PubMed: 10857651]
- Weijtens O, Feron EJ, Schoemaker RC, Cohen AF, Lentjes EG, Romijn FP, van Meurs JC. High concentration of dexamethasone in aqueous and vitreous after subconjunctival injection. *Am J Ophthalmol*. 1999; 128:192–197. [PubMed: 10458175]
- Weijtens O, Schoemaker RC, Cohen AF, Romijn FP, Lentjes EG, van Rooij J, van Meurs JC. Dexamethasone concentration in vitreous and serum after oral administration. *Am J Ophthalmol*. 1998; 125:673–679. [PubMed: 9625551]
- Weijtens O, Schoemaker RC, Lentjes EG, Romijn FP, Cohen AF, van Meurs JC. Dexamethasone concentration in the subretinal fluid after a subconjunctival injection, a peribulbar injection, or an oral dose. *Ophthalmology*. 2000; 107:1932–1938. [PubMed: 11013202]
- Weijtens O, van der Sluijs FA, Schoemaker RC, Lentjes EG, Cohen AF, Romijn FP, van Meurs JC. Peribulbar corticosteroid injection: vitreal and serum concentrations after dexamethasone disodium phosphate injection. *Am J Ophthalmol*. 1997; 123:358–363. [PubMed: 9063245]
- Wilson DF, Vinogradov SA, Grosul P, Vaccarezza MN, Kuroki A, Bennett J. Oxygen distribution and vascular injury in the mouse eye measured by phosphorescence-lifetime imaging. *Appl Opt*. 2005; 44:5239–5248. [PubMed: 16149347]
- Yang H, Tyagi P, Kadam RS, Holden CA, Kompella UB. A Hybrid Dendrimer Hydrogel/PLGA Nanoparticle Platform Sustains Drug Delivery for One Week and Antiglaucoma Effects for Four Days Following One-Time Topical Administration. *ACS Nano*. 2012
- Yang M, Zahn JD. Microneedle insertion force reduction using vibratory actuation. *Biomed Microdevices*. 2004; 6:177–182. [PubMed: 15377826]
- Young S, Larkin G, Branley M, Lightman S. Safety and efficacy of intravitreal triamcinolone for cystoid macular oedema in uveitis. *Clin Experiment Ophthalmol*. 2001; 29:2–6. [PubMed: 11272779]
- Zhang P, Summer WR, Bagby GJ, Nelson S. Innate immunity and pulmonary host defense. *Immunol Rev*. 2000; 173:39–51. [PubMed: 10719666]
- Zolnik BS, Gonzalez-Fernandez A, Sadrieh N, Dobrovolskaia MA. Nanoparticles and the immune system. *Endocrinology*. 2010; 151:458–465. [PubMed: 20016026]

Figure 1.

Routes of administration (topical, intravitreal, suprachoroidal, periocular and systemic) for delivering different types of nanoparticles (NP) (bioadhesive/rapid uptake, sustained release, stealth, targeted, and stimuli responsive) to the back of the eye. In addition, future applications of nanotechnology include nanofabricated devices, implants, films, and particles.

Figure 2.

Cell uptake decreases with an increase in particle size. Influence of particle concentration on % uptake (mass, number, or surface area) of nano- and micro-particles into ARPE-19 cells after 3 h exposure. Data are expressed as mean $\pm$ S.D. for N=4. (Aukunuru and Kompella 2002) With permission from Drug Delivery Technology.

Key: $\triangle$ 20 nm, $\diamond$ 100 nm, $\square$ 200nm, $\circ$ 500 nm, $\blacksquare$ 1000 nm, $\bullet$ 2000 nm

Figure 3.

Influence of size on vitreal half-life of nanoparticles. Fluorescently labeled polystyrene particles were intravitreally administered to pigmented rabbits. Data are expressed as mean $\pm$ SEM for N=5. Based on the work of Sakurai and colleagues. (Sakurai et al., 2001)

Figure 4.

Some examples of nanoparticles for ophthalmic use. (A) albumin nanoparticles, (B) chitosan nanoparticles, (C) polyamidoamine (PAMAM) dendrimers, (D) poly (lactic-co-glycolic) acid (PLGA) nanoparticles, and (E) polyethylene glycol (PEG)-coated liposomes.(Durazo et al., 2011). Permission requested from Springer.

Figure 5.

Increasing microneedle length and infusion pressure increases success rate of suprachoroidal delivery. The effect of infusion pressure and microneedle length on the success rate of suprachoroidal delivery for (A) 20 nm, (B) 100 nm, (C) 500 nm, and (D) 1,000 nm particles ex vivo into pig eyes. A total of five infusions were attempted at each condition studied. (ANOVA $P < 0.05$). (Patel et al., 2011). Permission requested from Pharmaceutical Research.

Figure 6. Surface functionalization with RGD peptide and transferrin increases retinal delivery of Nile red loaded PLGA nanoparticles following intravenous administration in Brown Norway rats with choroidal neovascularization. At 24 h after nanoparticle administration, the Nile red intensity in the choroidal flatmount of each Brown Norway rat was quantified using image analysis. The average Nile red intensity was compared across various groups in the study. The data are expressed as mean $\pm$ S.D. for N=3. *P < 0.05 as compared to nonfunctionalized Flt23k-plasmid-loaded nanoparticles (Flt23K-NP). (Singh et al., 2009). With permission from Gene Therapy, Nature Publishing Group.

Figure 7. Species differences in beta-blocker transport across sclera were abolished after normalization for scleral thickness. Cumulative percentage of transport at end of 6 hours (A) before and (B) after normalization for tissue thickness. The apparent permeability coefficients (C) before and (D) after normalization for tissue thickness. Results are expressed as the mean $\pm$ S.D. for N=6. (Kadam et al., 2011). With permission from Investigative Ophthalmology & Vision Science.

Figure 8.

Species differences in beta-blocker transport across sclera-choroid-RPE was reduced, but remained after normalization for sclera choroid-RPE thickness and pigment content in choroid-RPE. Cumulative percentage of transport at end of 6 hours (A) before normalization, (B) after normalization for sclera-choroid-RPE thickness, and (C) after normalization for sclera-choroid-RPE thickness and melanin content in the choroid-RPE. Results are expressed as the mean $\pm$ S.D. for N=6. (Kadam et al., 2011). With permission from Investigative Ophthalmology & Vision Science.

Figure 9.

Ratio of ocular tissue AUCs (for celecoxib suspension) or tissue concentrations (for celecoxib-PLA particles) between pigmented (BN) and albino (SD) rats. Data are presented as the ratio of the mean AUCs or concentrations for $n = 4$. In the celecoxib-PLA microparticle group, drug levels were not detected in the contralateral sclera, lens, and cornea in albino SD rats and in the contralateral lens and cornea of BN rats. Therefore, the BN-to-SD rat tissue ratio was not provided for these contralateral tissues. (Cheruvu et al., 2008) Permission requested from Pharmaceutical Research.

Figure 10.

Diabetes breaks down blood-retinal barrier, which increases celecoxib delivery. Relative delivery of celecoxib in diabetic rats compared to control rats following periocular injection of celecoxib. Data are presented as ratio of the mean AUCs or concentrations for N=4. (Cheruvu et al., 2009) With permission from Pharmaceutical Research.

Figure 11.

Simulated retinal delivery of celecoxib from 20 nm and 200 nm particles for 60 days. The compartmental pharmacokinetic model is shown above the simulation data. The panels depict profiles of 20 and 200 nm with a release rate constant of (A) 0.016 min^{-1} , (B) 0.0016 min^{-1} , (C) 0.00016 min^{-1} , and (D) $0.000016 \text{ min}^{-1}$. The insets in each panel are the profiles for the first 24 h of drug release to better show the early differences between the retinal concentrations of celecoxib using 20 and 200 nm particles. (Amrite et al., 2008a). With permission from Molecular Vision.

Figure 12.

Ocular toxicity of three types of nanoparticles after intravitreal or subretinal injection. Gross postmortem evaluations of rabbit eyes 7 days following (A) intravitreal or (B) subretinal injections of gene-encapsulated chitosan, poly(Prow et al.) nanoparticles, and magnetic nanoparticles. Inflammation refers to apparent inflammation anywhere in the eye, whereas haze and membranous opacity suggest inflammatory infiltrates in vitreous. (Prow et al., 2008) Permission requested from Nanomedicine: Nanotechnology, Biology, and Medicine.

Table 1

Some examples of nanomedicine applications for the eye.

Route of Administration	Target Disease	Experimental Model	Nanomedicine Description	Size (nm)	Observation	Mechanism or principle	Reference
Topical	N/A	Ex-vivo bovine eye	Polystyrene NPs were surface conjugated with LHRH-agonist (deslorelin) or transferrin, to facilitate rapid transport across cornea	98.2 ± 0.6 (deslorelin NPs); 84.7 ± 0.8 (Transferrin NPs)	For the deslorelin- and transferrin- NPs, at 5-min following application, corneal epithelial uptake was 3 and 4.5-fold higher than non-functionalized NPs.	Bioadhesion; Rapid receptor-mediated adhesion and entry; Target recognition	(Kompella et al., 2006)
	Bacterial infections of the eye	Human corneal epithelial cells (HCECs); New Zealand White rabbits	An optimized dendritic polyguanidylated translocator (DPT)-gauffloxacin (GFX) formulation	346	Rapid uptake of dendrimers in HCECs within 5-mins; Following topical application, ~13-fold and 2-fold higher areas under the curve, with increased residence times, in rabbit conjunctiva and cornea, respectively	Mucoadhesion and bioadhesion; Rapid internalization; Prolonged residence time in tissues	(Duraiaj et al., 2010)
	Glaucoma	Dutch-belted rabbits	Polyamidoamine dendrimer G3.0 tethered with three PEG-acrylate chains and integrated with PLGA NPs, for co-delivery of brimonidine and timolol maleate	258	Single topical application of the hybrid dendrimer NP formulation maintained an effective IOP reduction for 4 days, and better sustained drug levels for one week compared to controls	Mucoadhesion and bioadhesion; Sustained release; Sustained residence time	(Yang et al., 2012)
	Glaucoma	Human corneal epithelial cells (HCECs)	Dendrimer hydrogel of PAMAM G3.0 tethered with 3 PEG acrylate chains, loaded with brimonidine and timolol maleate	N/A	Mucoadhesion of the dendrimer hydrogel drug formulation was established by a shift towards zero, in the zeta potential of mucin particles; Drug release was sustained in vitro, for as long as 72 hours for brimonidine and 56 hours for timolol maleate	Mucoadhesion; Sustained release	(Holden et al., 2011)
	Corneal neovascularization	BALB/c mice	Human serum albumin NPs encapsulating pCMV.Fit23K	156.2 ± 1.8	Intrareceptor gene expression persisted for at least 5 weeks post injection and reduced corneal angiogenesis by ~40%	Enhanced gene transfection and duration of transfection	(Jani et al., 2007)
Intravenous	Choroidal neovascularization (CNV)	Brown Norway rats with CNV	Transferrin, RGD-peptide or dual-functionalized PLGA NPs loaded with anti-VEGF intrareceptor plasmid Fit23K	270–420	Surface functionalization of the NPs increased delivery to CNV lesions due to receptor mediated targeting	Target recognition and receptor-mediated endocytosis	(Singh et al., 2009)
Posterior subconjunctival	Inflammatory diseases and diseases with VEGF elevation	Sprague-Dawley rats	Budesonide-loaded PLA NPs	345 ± 2	PolymERIC nanoparticles sustained drug delivery better than plain drug.	Sustained release	(Kompella et al., 2003)
	Retinoblastoma	LHβ-Tag transgenic mice	Carboplatin-loaded polyamidoamine dendrimer G3.5 NPs	258 ± 5.7	Significant reduction in mean tumor burden at day 22, following a single injection of the drug containing nanoparticles	Sustained release	(Kang et al., 2009)
Intravitreal	Choroidal neovascularization	Brown Norway rats with CNV	Bevacizumab-loaded PEG and PLGA NPs	819	Persistent effects of bevacizumab with both PEG and PLGA NPs for up to 6 weeks, following laser induction of CNV	Sustained release	(Pan et al., 2011)

Route of Administration	Target Disease	Experimental Model	Nanomedicine Description	Size (nm)	Observation	Mechanism or principle	Reference
	Diabetic retinopathy	Brown Norway rats with diabetic complications	Plasminogenkringle 5 (K5) plasmid-loaded PLGA:chitosan NPs	260 ± 30	Significant attenuation of VEGF and ICAM-1 overexpression and reduction of leukostasis, vascular leakage, and neovascularization for up to 4 weeks	Enhanced and sustained gene transfection	(Park et al., 2009)
	Choroidal neovascularization and retinal inflammation	Brown Norway rats with CNV	Plasminogen kringle 5 plasmid-loaded PLGA:chitosan NPs	N/A	Significant expression of K5 and reduction of CNV area (40%) at the end of 2 weeks following laser induction of CNV	Enhanced and sustained gene transfection	(Jin et al., 2011)
	Retinal degenerative diseases	ARPE-19 cells and SOD1-deficient mice	Cu, Zn superoxide dismutase (SOD1) gene-encapsulated human serum albumin NPs	122 ± 2	80% transfection efficiency in ARPE-19 cells with receptor mediated uptake and rapid endo-lysosomal escape. Significant levels of protein expression was detected in vivo at 48 h post injection	Enhanced and sustained gene transfection	(Mo et al., 2007)
Suprachoroidal	Choroidal neovascularization	Ex-vivo rabbit eye	Bevacizumab entrapped in a hydrogel formed by UV-induced crosslinking of polycaprolactone dimethacrylate and hydroxyethyl methacrylate polymers sustained in vitro drug release in phosphate buffered saline for up to 4 months	N/A	UV (365 nm) exposure for a duration of 10 minutes following suprachoroidal injection of polymers better confined the hydrogel spread in the choroid, the site of injection, when compared to polymers without stimuli or lower durations of light exposure	Stimuli-responsive; Sustained release	(Tyagi et al., 2012)
In vitro studies	Retinoblastoma	Y79-Retinoblastoma cells	Epithelial cell adhesion molecule (EpcAM)-antibody functionalized paclitaxel-loaded PLGA NPs	272 ± 1.6	Cellular uptake was increased by 2-fold due to specific EpcAM mediated endocytosis of the EpcAM-Ab conjugated NPs	Target recognition	(Mitra et al., 2011)
	Retinoblastoma	Y-79 Retinoblastoma cells	Doxorubicin-loaded-PLGA-PEG-folate-micelles suspended in a thermosensitive gel	75.2 ± 2.4 to 101.2 ± 5.63	Formulation sustained drug release for up to 2 weeks and drug uptake was ~4-fold higher in Y-79 cells overexpressing folate receptors; drug delivery occurred upon phase transition of gel at physiologic temperature	Sustained release; Target recognition and cell entry; Stimuli-responsive	(Boddu et al., 2010)

Key: N/A: Not applicable or not available.