Nanotechnology for Gene Delivery to the Eye

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Abstract

The relatively immune-privileged status of the eye makes it an interesting target for gene delivery. Gene delivery to the eye using viral vectors via subretinal and intravitreal injections has been extensively investigated. Recently, the safety of recombinant adeno-associated virus vector expressing RPE65 complementary DNA (cDNA) in a limited clinical trial of three patients has also been reported. Nanotechnology-based non-viral vectors offer the advantages of safety and flexibility in terms of loading capacity and delivery system design compared with viral vectors. An ideal non-viral vector should be non-toxic, efficiently taken up into the target cells and conducive to gene expression, and should protect the gene against enzymatic degradation. Multiple kinds of nanotechnology-based non-viral vectors have been investigated for potential applications for gene delivery to the eye, namely nanoplexes, dendrimers, micelles, nanoparticles and liposomes. This article summarises and discusses key advances in the application of nanotechnology for gene delivery to the eye.

Keywords

Nanotechnology, gene delivery, eye, nanoparticles, liposomes

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Gene therapy is a promising therapeutic approach for the treatment of a wide array of inherited and acquired disorders. The eye is a particularly interesting target for gene delivery owing to the relatively immune-privileged status of the tissue. In the eye, gene delivery has potential applications in treating various disorders including neovascular and non-neovascular retinal degenerative disorders, glaucoma and corneal graft rejection, among others. Indeed, a number of viral vectors have been shown to successfully result in intraocular gene expression.[1]

Recently, Bainbridge et al.[2] published the results of a three-patient clinical trial conducted to assess the safety of subretinally delivered recombinant adeno-associated virus vector expressing RPE65 complementary DNA (cDNA). The study concluded that the vectors were largely safe. The safety and efficacy of viral vectors must be further established in larger clinical trials to enable their clinical acceptance. In the study by Bainbridge et al., although none of the patients showed significant improvement in visual acuity, one patient showed improvement in visual function on microperimetry and dark-adapted perimetry and in a subjective test of mobility. Thus, clinical use of gene therapy is challenged by effective gene delivery *in vivo*. In addition, viral vectors have the drawback of limited gene-carrying capacity, immunogenicity and toxicity.[3]

For these reasons, our group and several other investigators are investigating the use of non-viral vectors as an alternative to viral vectors. An ideal non-viral gene delivery vector should be non-toxic, efficiently taken up by target cells and conducive to gene expression, and should protect the gene against enzymatic degradation. In this article, potential routes of administration for gene delivery to the eye and examples of nanotechnology-based nano-size non-viral vectors for ocular gene delivery are discussed.

Routes of Administration for Gene Delivery to the Eye

Gene delivery to the eyes using non-viral nanotechnology-based vectors can be achieved by multiple routes of administration.[4] The various routes assessed for this purpose include topical,[5] intracameral,[5] intracorneal,[6] subconjunctival,[7] intravitreal,[8,9] subretinal[10–12] and intravenous[13–16] (see *Figure 1*). The choice of route of administration is based on the target tissue within the eye. For gene expression in the anterior segment, topical, intracameral and intracorneal routes may be employed. For gene expression in the posterior segment of the eye, intravitreal and subretinal injections are most commonly used. Subconjunctival and peri-ocular injections can potentially deliver the vectors or the expressed proteins to the anterior as well as the posterior segment of the eye. Yoon et al.[7] reported transfection of corneal cells after subconjunctival injection of liposomes loaded with a plasmid encoding the extracellular region of brain-specific angiogenesis inhibitor 1 (BAI1-ECR) in rabbits. Plasmid expression was observed in corneal stroma for seven days. The intravenous route of administration for ocular delivery is usually not preferred because of the presence of blood ocular barriers; however, the intravenous route is now being increasingly investigated for gene delivery purposes.

Figure 1: Routes of Administration for Gene Delivery to the Eye

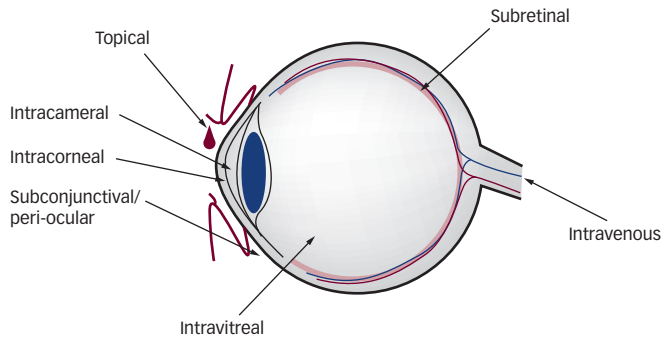
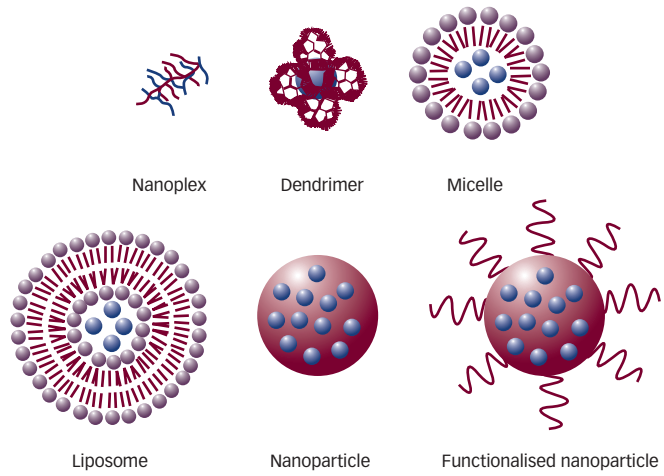


Figure 2: Various Nanotechnology-based Delivery Systems for Gene Delivery to the Eye



Some possible localisations for nucleic acid therapeutics are depicted in purple in the delivery systems.

The gene expression pattern in the eye after intravenous administration of a gene delivery system depends on the delivery system design. Pegylated liposomes resulted in the expression of β-galactosidase in multiple tissues – namely the ciliary body, iris, sebaceous gland, corneal epithelium and outer retina – at 48 hours after intravenous administration.[14] Recent studies from our group indicated that transferrin (Tf) and/or arginine–glycine–aspartic acid (RGD) peptide functionalised poly(lactide-co-glycolide) (PLGA) nanoparticles result in effective gene delivery to the back of the eye after intravenous injection.[13,17] Thus, the route of administration plays a critical role in the gene expression pattern within the eye.

Nanosystems for Gene Delivery to the Eye

Evidence to date indicates the suitability of nanosystems in enhancing the delivery of nucleic acids including oligonucleotides and genes to the tissues of the eye. The nanosystems that have been investigated for delivering genes to the eye include nanoplexes, dendrimers, micelles, nanoparticles and liposomes (see *Figure 2*). Key advances in the use of nanotechnology for gene delivery to the eye are summarised in *Table 1*.

Nanoplexes in Ocular Gene Delivery

Nanoplexes are complexes of the therapeutic macromolecule with one or more carrier materials.[18] Cationic polymers such as polyethyleneimine (PEI) are commonly used to complex and condense DNA.[19] The positively charged groups on the polymer interact electrostatically with the negatively charged phosphate groups in the backbone of the DNA molecule, allowing compaction of DNA and, hence, better transfection.

Nanoplexes have been investigated for improving the outcomes of glaucoma filtration surgery, which is complicated by excessive scarring during wound healing. The scarring can eventually lead to obliteration of surgically created subconjunctival filtration space or bleb for the passage of aqueous humour. Inhibition of transforming growth factor-β2 (TGF-β2) by administration of nano-sized complexes of PEI and anti-TGF-β2 oligonucleotide significantly improves the outcome of glaucoma surgery.[20] The nanocomplexes (220±40nm) administered following encapsulation in porous particles sustained the release of oligonucleotide for 15 days and enhanced the intracellular penetration of the oligonucleotide upon subconjunctival administration of particle suspension in pigmented Fauve de Bourgogne female rabbits. The clinical evaluation of these rabbits was based on the overall inflammatory state of the eye and the time to filtering bleb failure.

Nanoplexes have also been applied to rescue degenerating photoreceptors.[21] Human basic fibroblast growth factor-2 (hFGF2) plasmid was condensed with two synthetic oligopeptides, K8 and JTS-1. Subretinal injection of the complexed DNA into three-week-old dystrophic Royal College of Surgeons (RCS) rats resulted in delayed photoreceptor cell degeneration 60 days after injection. The average analysed field points with delayed cell dystrophy represent 14–17% of the retinal surface compared with 2.6 and 4% in vector- and vehicle-injected eyes, respectively.

Dendrimers for Ocular Gene Delivery

Dendrimers – from the Greek *dendron* (tree) and *meros* (part) – consist of a central core molecule from which a number of highly branched tree-like arms originate in an ordered and symmetrical fashion.[22]

> *While some of the nanosystems have been shown to be efficacious in inhibiting ocular diseases, others need to be tested for their therapeutic applicability.*

Dendrimers have several unique physiochemical properties that make them attractive for biomedical applications. Dendrimers are made by stepwise attachment of layers of a second branched molecule on a central core. The addition of one layer on top of the core molecule results in generation 0 (G0) of a dendrimer; the addition of subsequent layers results in G1, G2, G3, etc. of dendrimers.

Intravitreal delivery of anti-vascular endothelial growth factor (VEGF) oligonucleotide using lipophilic amino acid dendrimers inhibited laser-induced choroidal neovascularisation (CNV).[8] Inhibition of the development of CNV was observed for four to six months with dendrimers. On the other hand, eyes injected with oligonucleotide alone did not show any significant difference compared with the vehicle-treated group. Furthermore, it was observed that the oligonucleotide was delivered to a wide area of the retina and penetrated all of the

Table 1: Key Advances in the Application of Nanotechnology to Ocular Gene Delivery

Nanosystem Type	Route of Administration	Animal Model	Observation	Reference
Nanoplexes	Intravitreal	Rats	Complex of fibronectin antisense oligonucleotide with polyoxyethylene–polyspermine block co-polymer persisted in retinal vascular cells until 6 days and significantly reduced fibronectic mRNA and protein levels.	23
	Intravitreal	Rats	Anti-TGFβ-2- oligonucleotide/polyethyleneimine complexes are preferentially localised in retinal muller cells at 72 hours after injection.	20
	Subretinal	Rats	Basic fibroblast growth factor complexed with two oligopeptides (K8 and JTS-1) efficiently transfected retinal and choroidal cells. At 60 days after injection, a delay in photoreceptor degeneration was observed.	21
	Intravitreal Subretinal	Mice	Intravitreal injection resulted in gene expression in the inner plexiform layer and retinal ganglion cells. Subretinal injection resulted in gene expression in the neural retina, retinal pigment epithelium, choroid and sclera.	10
Dendrimer	Intravitreal	Rats	Anti-VEGF oligonucleotide–amino acid dendrimer significantly inhibited the development of choroidal neovascularisation for 4–6 months. CNV was induced in rats using a Krypton laser (647.1nm). 6–9 burns were applied to each eye (100µm, 0.1 seconds, 150mW).	8
Micelles	Topical	Mice and rabbits	The micelles were prepared using poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) block co-polymers. β-galactosidase expression was observed in the sclera, conjunctiva, iris and tendon of the lateral rectus muscles. Low-level gene expression was also observed in the anterior chamber, cornea, retinal pigment epithelium and vitreous body.	24
Nanoparticles	Intravitreal	Mice	Human serum albumin nanoparticles protected the loaded Cu/Zn superoxide dismutase plasmid against nuclease degradation, sustained plasmid release for up to 6 days, transfected ARPE-19 cells with 80% transfection efficiency and expressed detectable levels of protein in 48 hours *in vivo*.	9
	Intravitreal	Rats	Poly-lactide nanoparticles injected into the eyes led to preferential expression of red nuclear fluorescent protein in the retinal pigment epithelial layer.	29
	Intravenous	Rats	Administration of anti-VEGF intraceptor-loaded poly-(lactide-co-glycolode) nanoparticles targeted gene expression to the neovascular eye and the inhibited laser induced choroidal neovascularisation.	17
Liposomes	Topical	Rats	β-galactosidase expression in the cornea, conjunctival epithelial cells and retinal ganglion cells for up to 1 month.	32
	Intracameral	Rats and rhesus monkeys	HVJ fusogenic liposomes express β-galactosidase in the trabecular meshwork.	38
	Intravitreal	Rabbits	Liposomes increased stability of oligonucleotide in the vitreous. Significant levels of oligonucleotide (37%) were detected in the vitreous at 15 days after injection.	39
	Intravitreal	Rabbits	Cationic liposomes transfected the cornea, aqueous humour, iris-ciliary body, lens, vitreous and retina. Gene expression peaked at 3 days after injection and lasted for at least 7 days.	40
	Intravenous	Mice	Pegylated immunoliposomes resulted in widespread expression of β-galactosidase gene loaded in the ciliary body, iris, sebaceous glands of tarsal plate and retina.	14

CNV = choroidal neovasculrisation; HVJ = haemagglutinating virus of Japan; mRNA = messenger RNA; TGF-β2 = transforming growth factor-β2; VEGF = vascular endothelial growth factor.

retinal cell layers. Immunohistochemistry revealed that the dendrimers were well tolerated and showed no observable signs of inflammation.

Micelles for Ocular Gene Delivery

Micelles are colloidal aggregates of amphiphilic molecules. Micellisation is a self-assembling property exhibited by amphiphilic molecules in aqueous solution above a specific concentration, referred to as critical micelle concentration (CMC). Although any surfactant molecule is suitable for forming a micelle, micelles based on block co-polymers made up of repeating hydrophilic and lipophilic blocks are being extensively investigated as therapeutic carriers for drug and gene delivery. Micelles can be spherical, rod-shaped or lamellar depending on the nature of the monomer units. An example of such a block co-polymer is Pluronic®, which consists of ethylene oxide (EO) 7 and propylene oxide (PO) blocks arranged in the following order: EO_x–PO_y–EO_x.[23]

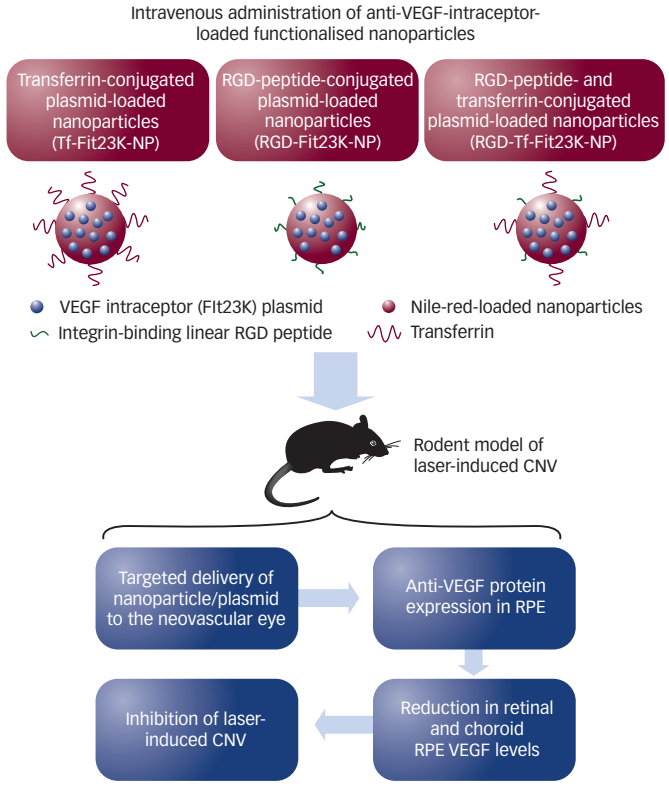
An interesting report by Liaw et al.[24] showed the delivery of reporter *lacZ* gene after topical dosing in mice and rabbits. The micelles were formed using poly(ethylene oxide)–poly(propylene oxide)–poly(ethyleneoxide) triblock co-polymers. The micelles had an average size of 160nm and a zeta potential of -4.4mV. After two days of topical instillation three times a day, the most intense gene expression was observed on days two to three in the iris, sclera, conjunctiva and lateral rectus muscle in rabbits. In mice, gene expression was also observed in intraocular tissues such as the anterior chamber and the retinal pigment epithelium.

Nanoparticles for Ocular Gene Delivery

Nanoparticles are spherical particles with diameters in the nanometer size range. They can be prepared with lipids, proteins, polysaccharides or polymers,[25] and can be broadly classified as nanospheres or nanocapsules: nanospheres are nanoparticles with drug or gene molecules dispersed in a carrier matrix, while nanocapsules are composed of a reservoir of drug solution or solid coated by a rate-limiting layer.[26] Thus, nanocapsules have a distinct core containing drug and an outer shell made up of a membrane.

Our research has made significant contributions in proving therapeutic efficacy and gene delivery to the eye using nanoparticles. We are investigating the use of albumin (natural) and biodegradable-polymer (synthetic) -based nanoparticles for facilitating gene delivery to the eye. With the US Food and Drug Administration (FDA) approval of albumin nanoparticles (130nm) loaded with paclitaxel (Abraxane®) in 2005, albumin serves as an acceptable carrier for preparing nanoparticles. Albumin-based nanoparticles as a gene delivery

Figure 3: Results of Investigation of Anti-vascular Endothelial Growth Factor Intraceptor Gene Delivery Using Functionalised Nanoparticles to Inhibit Laser-induced Choroidal Neovascularisation



CNV = choroidal neovascularisation; RGD = arginine–glycine–aspartic acid; RPE = retinal pigment epithelium; VEGF = vascular endothelial growth factor.
Source: Singh et al., 2009.[17]

system offer the advantages of an established safety profile, biodegradability and potential clinical viability.[27] Biodegradable synthetic polymers such as PLGA and poly(lactide) (PLA) offer an additional platform for preparing nanoparticles for nucleic acid delivery. Several pharmaceutical products containing these polymers are approved for parenteral administration. Currently, Posurdex®, an intravitreal implant system based on these polymers, is undergoing clinical trials for the delivery of a low-molecular-weight drug.

Albumin nanoparticles loaded with Cu, Zn superoxide dismutase plasmid (SOD1) exhibited transfection efficiency as high as 80% in ARPE-19 cells.[9] We encapsulated SOD1 plasmid in human serum albumin nanoparticles and demonstrated that these particles with a diameter of about 150nm sustain the release of the plasmid, protect it against serum and nuclease degradation and allow *in vitro* transfection efficiencies to a greater extent than lipofectamine, a positively charged commercial reagent. Furthermore, intravitreal injection of the albumin nanoparticles in mice followed by Western blot analysis indicated retinal expression of enhanced yellow fluorescence protein in the retina within two days.

Albumin nanoparticles are also useful in enhancing corneal gene expression. Anti-VEGF intraceptor (Flt23K)-loaded albumin nanoparticles inhibit corneal neovascularisation.[6] Intrastromal injection of Flt23K-loaded nanoparticles sustained corneal gene expression for four weeks. Corneal neovascularisation development following mechanical–alkali injury was reduced by Flt23K-plasmid-loaded nanoparticle administration three weeks prior to injury. Corneal neovascularisation area after injection of intraceptor plasmid-loaded nanoparticles and naked plasmid was 35.0±6.0 and 58.3±8.7%, respectively.

We have recently shown that intravenously administered surface functionalised PLGA nanoparticles (~300–400nm) loaded with anti-VEGF intraceptor plasmid (Flt23K) target the gene delivery to the neovascular eye and significantly inhibit CNV.[17] The nanoparticles were surface-functionalised with Tf, RGD peptide or a combination of Tf and RGD peptide (dual) at individual concentrations that were half those used in the Tf and RGD groups (see *Figure 3*). The nanoparticles (~10mg) containing 100µg of plasmid were injected into the tail vein of Brown Norway rats 14 days after CNV induction in the right eye by laser cauterisation of Bruch's membrane. The left eye was used as normal control for each animal. At 24 hours after nanoparticle administration, the particles were detected in the neovascular eye but not in the normal eye in all animals. Furthermore, on comparison of nanoparticle ocular delivery across various groups in the study, we determined that the functionalised nanoparticles were delivered to the retina to a significantly greater extent than non-functionalised nanoparticles. Two weeks after nanoparticle administration, the CNV area as measured by histopathology and choroidal flatmounts was significantly lower in rats treated with plasmid-loaded functionalised nanoparticles compared with vehicle-treated or naked-plasmid-treated groups.

PLGA nanoparticles are useful in enhancing the cellular uptake and efficacy of oligonucleotides. For instance, we observed that PLGA (50:50) nanoparticles (252±3.4nm) enhanced the delivery and activity of a VEGF antisense oligonucleotide in cultured human retinal pigment epithelial cell line (ARPE-19).[28] The cellular uptake of VEGF antisense oligonucleotide encapsulated in PLGA nanoparticles was enhanced by 4.3-fold compared with unencapsulated oligonucleotide. Nanoparticles containing the oligonucleotide significantly reduced VEGF mRNA levels as well as VEGF secretion from ARPE-19 cells, suggesting their potential usefulness for neovascular disorders of the eye. An increase in VEGF secretion has been at least partly implicated in the retinal neovascularisation seen in proliferative diabetic retinopathy, retinopathy of prematurity and age-related macular degeneration. We have also demonstrated the suitability of PLGA-based nanoparticle systems for retinal gene delivery of plasminogen kringle 5 kringle peptide (unpublished data). Other investigators have shown that PLA and PLGA nanoparticles (643±74nm) loaded with plasmids encoding green fluorescent protein or red nuclear fluorescent protein successfully transfect cultured bovine and human retinal pigment epithelial cells (RPE).[29] The nanoparticles also successfully transfected retinal pigment epithelium *in vivo* upon intravitreal injection in male Lewis rats.[29]

Even inorganic nanoparticles such as those made of iron oxide have potential for enhancing gene transfection. Streptavidin-coated superparamagnetic iron oxide nanoparticles can be conjugated to biotin-labelled DNA fragments for enhancing gene transfection.[30] Using these layered particles of 100nm, gene transfection could be enhanced in an immortalised human hepatoma cell line (Huh-7) and adult retinal endothelial cell lines from dog and human sources. However, the toxicity of iron oxide particles has to be evaluated carefully prior to therapeutic applications.

Liposomes for Ocular Gene Delivery

Liposomes are vesicles consisting of hydrated phospholipid bilayers designed to entrap drug in either the core or the bilayer. Based on

structural parameters such as size and number and position of lamellae, liposomes can be classified into multivesicular vesicles (MVVs: >1µm), multilamellar large vesicles (MLVs: >0.5µm), oligolamellar vesicles (OLVs: 0.1–1µm), unilamellar vesicles (UVs: all sizes), giant unilamellar vesicles (GUVs: >1µm), large unilamellar vesicles (LUVs >100nm) and small unilamellar vesicles (SUVs: 20–100nm).[31]

Although a number of studies have investigated the delivery of reporter genes to the eye using liposomes after administration by various routes (see *Table 1*), the therapeutic efficacy of this system has yet to be investigated. An interesting study using liposomes for gene delivery to the eye reported reporter gene expression in retinal ganglion cells for one month after topical instillation of N-(alpha-trimethylammonioacetyl)-didodecyl-D-glatamate (TMAG) and 3-beta[N-(N',N' dimethyaminoethane)-carbamoyl] cholesterol (DC-cholesterol) liposomes, but not with dimethyldioctadecyl ammonium bromide (DDAB) liposomes.[32] Gene expression was also observed in corneal and conjunctival epithelial cells. The instillation of plasmid alone did not result in any gene expression. Although these findings are interesting, the authors failed to suggest a mechanism of gene delivery to retinal ganglion cells after topical instillation of liposomes. Furthermore, the reason for the failure of DDAB liposomes to deliver genes was not explained or discussed.

Other Delivery Systems

Other non-viral approaches for gene delivery to the eye include iontophoresis,[33–35] electron avalanche transfection[36] and ballistic delivery of naked plasmids.[27,37]

Conclusion

Nanotechnology-based gene delivery systems offer a wide range of possibilities for gene delivery to the eye. In comparison with viral vectors, the non-viral vectors offer greater flexibility in customising the system for the purpose of targeting or enhancing the *in vivo* circulation time. The choice of route of administration should be governed by the desired intraocular target. While some of the nanosystems have been shown to be efficacious in inhibiting ocular diseases, others need to be tested for their therapeutic applicability. ■

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Uday B Kompella is a Professor of Pharmaceutical Sciences and Ophthalmology at the University of Colorado in Denver. He is one of the leading investigators in the field of ophthalmic drug/gene delivery. Apart from ophthalmic drug delivery, his laboratory has actively pursued respiratory drug delivery and targeted drug delivery for lung and prostrate cancers. Professor Kompella is an active member of the American Association of Pharmaceutical Sciences (AAPS) and the Association for Research in Vision and Ophthalmology (ARVO).

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