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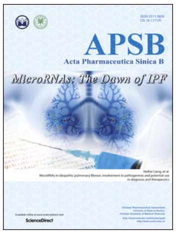

REVIEW

Nanotechnology-based strategies for treatment of ocular disease

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Abstract Ocular diseases include various anterior and posterior segment diseases. Due to the unique anatomy and physiology of the eye, efficient ocular drug delivery is a great challenge to researchers and pharmacologists. Although there are conventional noninvasive and invasive treatments, such as eye drops, injections and implants, the current treatments either suffer from low bioavailability or severe adverse ocular effects. Alternatively, the emerging nanoscience and nanotechnology are playing an important role in the development of novel strategies for ocular disease therapy. Various active molecules have been designed to associate with nanocarriers to overcome ocular barriers and intimately interact with specific ocular tissues. In this review, we highlight the recent attempts of nanotechnology-based systems for imaging and treating ocular diseases, such as corneal d iseases, glaucoma, retina diseases, and choroid diseases. Although additional work remains, the progress described herein may pave the way to new, highly effective and important ocular nanomedicines.

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1. Introduction

Ocular diseases directly affect human vision and quality of life. A survey from 39 countries estimated that 285 million people suffer visual impairment. Of these, 65% are over 50 years old, and 82% of blind patients are over 50[1]. Significant achievements have been made in the discovery of ocular pathological mechanisms and management of ocular disease. However, due to the special physiological barriers and anatomical structures of the human eye, diagnoses and treatments of these disorders can suffer from low efficiency and lack of specificity. The current therapeutic methods seldom can completely restore vision loss or detect severe ocular diseases at an early stage[2]. Therefore, the development of improved diagnostics and therapeutics for ocular diseases is receiving intense attention.

Emerging nanotechnology and nanoscience methods are increasingly being applied to biopharmaceutics. Nanoscience is an interdisciplinary field that combines material science, physics, chemistry and biology, whereas nanotechnology involves the design and fabrication of different materials in nanometer scale at least in one dimension[3–6]. Several nanotechnology-based strategies have been developed and aimed at management of ocular diseases: bioadhesive enhancement, sustainable release, stealth function, specifically targeted delivery, and stimuli responsive release, etc[7–9]. Therefore, many attempts have been focused on fabrication of multi-functional nanosystems for ocular diseases therapy by improving drug (or gene) delivery to both the anterior and posterior segments of the eye.

In this review, we have focused on advances in development of nanotechnology-based systems for ocular diseases therapy and imaging. First, the specific anatomy and the attendant constraints in ocular drug administration are introduced. Some conventional and alternative drug administration routes are summarized and compared as well. Second, for a deeper insight of nanosystems mechanism, several examples of nanosystems for management of ocular disease are highlighted and reviewed. Then, some typical studies are summarized. Finally, we summarize the perspective of nanotechnology and existing challenges in ocular diseases therapy and diagnosis. This review will provide both inspiration and impetus for better design and development of intractable ocular disease managements.

2. Ocular anatomy and constraints to ocular drug delivery

The human eye is a globular structure organ with size of about 24 mm, and consists of two main parts: the anterior and posterior segments[10] (Fig. 1). The both parts have various biological barriers to protect the eye from foreign substances. The anterior portion includes the corneal, iris, lens, and aqueous humor. The posterior portion consists of the vitreous body, retina, choroid, and back of the sclera. The cornea is transparent and contains five layers: epithelium, Bowman's membrane, stroma, Descemet's membrane, and endothelium[11,12]. The human corneal epithelium is the most important part of corneal barrier since it has multilayers of corneal epithelial cells which interconnect by tight junctions. These tight junctions can severely limit ocular penetration of drugs, especially many types of hydrophilic molecules. The corneal stroma is mostly composed of charged and highly organized hydrophilic collagen which hinders passage of hydrophobic molecules[13–15]. In recent studies, various efflux transporters on epithelial cells were proved to be of importance in preventing permeation of anti-viral and anti-glaucoma drugs[16–18].

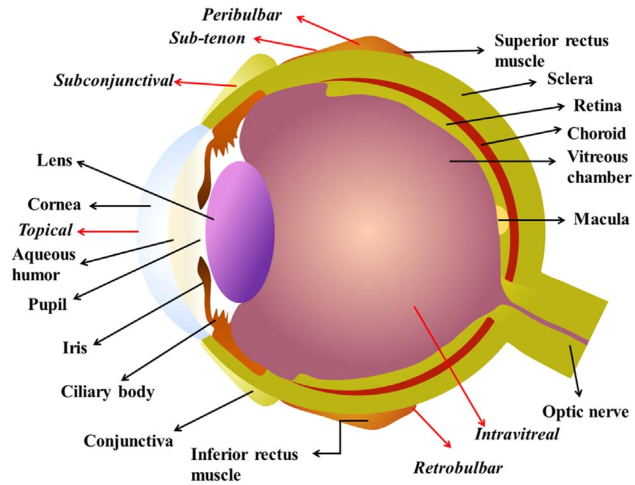

Figure 1 Ocular anatomy and administration routes of both traditional drugs and nanosystems: the black arrows show different eye structures and the red arrows show various administration routes.

The intraocular environment contains two main barriers: blood–aqueous and blood–retina barrier. The blood–aqueous barrier is composed of the nonpigmented epithelium of the ciliary body, which specifically includes the iris epithelium, iris vessel endothelium with tight junction, and Schlemm's canal endothelium. The tight junctions of cells control both active and paracellular transport[14,19,20]. The blood–retinal barrier is divided into inner and outer blood–retinal barriers. The former one is composed of retinal vascular endothelium with tight junctions. The latter includes a monolayer of retinal pigment epithelium (RPE) with tight junctions[19,21]. These two components restrict penetration of molecules into the intraocular chamber, resulting in inefficient therapy on intraocular tissues.

In addition, topical drug administration to the anterior segment of the eye is often limited by clearance mechanisms of the corneal surface and other precorneal factors, including eye blinking, tear film, tear turnover, solution drainage and lacrimation[22]. Human tear film has a rapid restoration time of only 2–3 min. Thus, most topically administered drugs are washed away within a few seconds after instillation. When topical drug solution volume is more than 30 μL (the upper limit volume that can be accommodated in the cul-de-sac), most of the drug is wasted by either nasolacrimal drainage or gravity-induced drainage[23]. Hampered by these factors and ocular barriers, the efficacy of the total administered drugs is less than 5%, suggesting the poor bioavailability of ocular drugs[23,24].

3. Benefits and limitations of ocular delivery routes

3.1. Systemic administrations

Intravenous injection and oral dosing are known systemic administration methods for ocular drug delivery. Since the choroid of the eye has a vascular choroid plexus structure, drugs can easily enter the choroid through blood vessels. However, the outer blood–retinal barrier of RPE cells governs the entry of drugs from the choroid into the retina. The tight junctions of RPE cells hamper most of the drugs and only 1%–2% of administrated drugs can

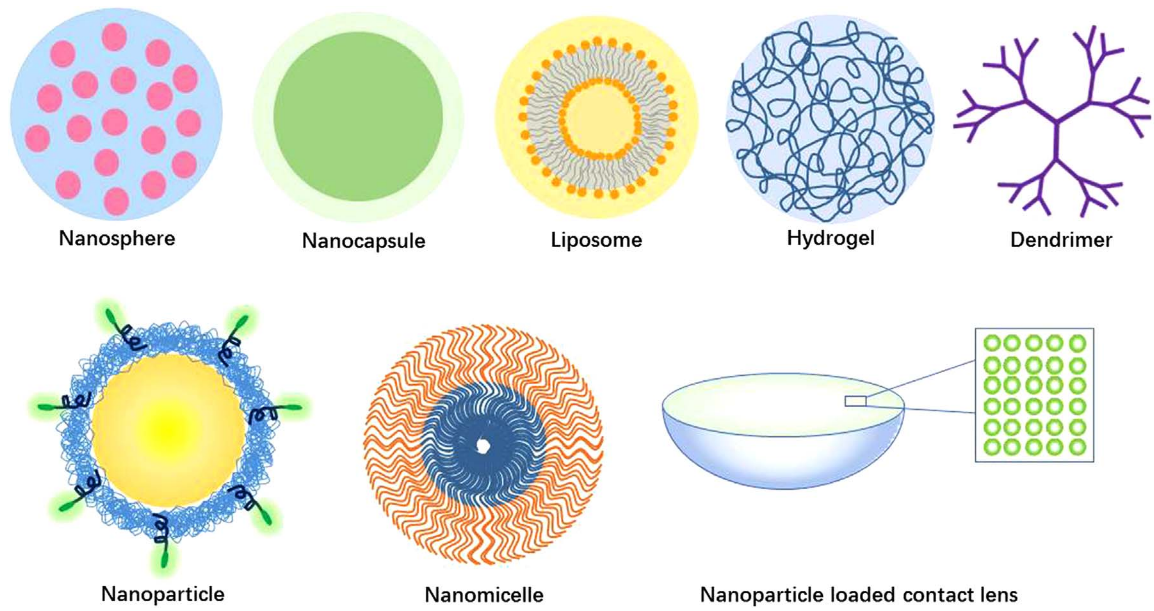

Figure 2 Schematic illustration of different nanotechnology-based ocular delivery systems.

access to the retina and vitreous body[21,25]. Thus, a difficult challenge remains to deliver drugs into the deep inner side of the eye by systemic administration.

3.2. Topical administration

3.2.1. Eye drops

Eye drops are the main form of topical administration due to good patient compliance and economical considerations. Drugs dissolved in eye drops are usually adsorbed by two routes: the corneal route (cornea, aqueous humor, intraocular tissue), and the conjunctiva route (conjunctiva, sclera, choroid, retina, vitreous body). Due to the corneal barrier and pre-corneal factors, less than 5% of totally administered drugs can reach the aqueous humor[26]. As a result, eye drops have to be frequently administered to maintain therapeutic drug concentrations. Eye drops are proven to be efficient in treating corneal diseases, iris diseases and glaucoma. However, they are less efficient in treating posterior eye diseases, including intraocular cancers and retina diseases, even when following frequent dosage regimens[27].

3.2.2. Topical injections

Among various topical injections, intravitreal injection is the most common administration route by injection of drug solution or suspension into the vitreous cavity through a 27- or 30-gauge needle. Usually a 20–100 μL volume solution can be directly injected into the vitreous cavity without discomfort[28]. Intravitreal injections, which result in high local drug concentrations in the vitreous body and retina, can serve as an efficient route of administration for treating posterior eye diseases[29]. However, drug distribution patterns in the vitreous are heterogeneous because of the gel like structure. Molecular distribution is greatly dependent on the drug's molecular weight and the vitreous pathophysiological condition[30–32]. It is reported that small molecules can rapidly spread out in the vitreous, whereas linear molecules with molecular weight more than 40 kDa or globular molecules larger than 70 kDa, have a longer retention time in the vitreous body. In addition, one of the most important compositions in the vitreous body—hyaluronan, is prone to interact with cationic nanoparticles and liposomal gene complexes through electrostatic interaction, leading to nanoparticle aggregation and reduction of the efficiency of gene delivery[33–35]. Furthermore, intravitreal injection is an invasive method which has to penetrate all the layers of the ocular globe and can result in series of side effects such as retinal detachment, iritis, uveitis, cataract, endophthalmitis, and intraocular hemorrhage. Repeated injections increase the incidence of these complications.

Periocular injection includes a series of topical injections which are employed to overcome drawbacks of systemic administration and to increase the drug concentration in intraocular tissues. Periocular deliveries through retrobulbar, peribulbar, sub-tenon and subconjunctival injection are less invasive than intravitreous injection. Drugs administered by periocular delivery routes can reach the posterior segment of the eye by penetration of either corneal choroid or scleral. However, most of these routes suffer from great drawbacks such as inefficiency in prolonging the drug retention time[34,36–38].

4. Types of nanosystems available for treatment and diagnosis of ocular diseases

During the past decades, nanotechnology seems to offer new perspectives in management of ocular diseases by either realizing controlled release, ensuring low eye irritation, improving drug bioavailability or enhancing ocular tissue compatibility[39–42]. Various nanosystems have been designed to deliver their payloads into both anterior and posterior segment of the eye. These nanosystems are mainly made from natural or synthetic polymeric materials. Many colloidal systems such as micelles, liposomes, niosomes, dendrimers, *in situ* hydrogels, and cyclodextrins are of this type. Other forms, including nanoparticles, implants, nanoparticle-contained contact lens, films, as well as other delivery systems, have also been intensely exploited to deliver drug and gene to the inner side of eye *via* appropriate administration routes (Fig. 2). To date, many efforts have been made on both carrier design and

Table 1 Typical nanotechnology-based strategies for ocular anterior diseases application.

Formulation	Material type	Payload	Size (nm)	Functions	Clinical stage	Ref.
Nanowafer	Polymer	Axitinib	500	The drug loaded nanowafer was nontoxic and could treat corneal neovascularization more efficiently compared to the commercial eye drop even at a lower dosage.	Preclinical	44
Nanoparticle	Chitosan	Gene	～200	The nanoparticle showed superior transfection efficiency in anterior segment of the eye.	Preclinical	45
Hydrogel (Virgan)	Polymer	Ganciclovir	–	Topical treatment drug for herpes simplex virus infection in the eye.	Market	46–49
Nanosuspention	Polymer	Diclofenac	105	Enhanced penetration and retention effect in corneal tissues was achieved through topical administration.	Preclinical	50
Nanoparticle	Polymer	Flurbiprofen	200–300	Following topical administration of the formulation, an enhanced anti-inflammation effect was achieved towards to a built animal model.	Preclinical	51
Nanoparticle	Polymer	Dexamethasone sodium phosphate	100–500	The drug loaded nanoparticles could not cause inflammation in the eye and improved the efficacy for prevention of corneal graft rejection.	Preclinical	52
Nanoscale dispersed oilment	Polymer	–	100	The formulation not only retained the advantages of eye ointment, but also showed better efficacy in repairing the tear film and restoring the corneal surface.	Preclinical	53
Hydrogel	Polymer	Diclofenac	–	The micellar supramolecular hydrogel could extend the retention time on corneal surface and improve drug bioavailability in the eye.	Preclinical	54
Nanoparticle	Polymer	Flurbiprofen	100	Nanoparticle formulation showed an inhibition effect of miotic response in a rabbit trauma model with a lower concentration of drugs. More drugs from the nanoparticles penetrated into the aqueous humor compared to commercial eye drops.	Preclinical	55
Nanoparticle	Polymer	Pilocarpine	83	Studies showed that the duration of miotic response had increased by 40% for the nanoparticle formulation.	Preclinical	56
Liposome	Polymer	Coenzyme-Q10	100–200	The liposomes exhibited a markedly anti-cataract effect and could increase the activities of superoxide dismutase and reduced glutathione.	Preclinical	57

–Data not found.

exploring the mechanisms of their biological actions. Meanwhile, much attention is being focused on the fabrication and modification of muti-functional nanocarriers for ocular target therapy.

5. Nanosystems for ocular anterior disease therapy

Eye drops are the most accessible and common formulations for treatment of common ocular anterior diseases, such as corneal injury, dry-eye, keratitis, conjunctivitis and cataract. However, this route of administration suffers from poor bioavailability due to the corneal barrier and pre-corneal factors. Experimental and clinical research has shown that frequent and long-term use of eye drops can result in tear film instability, corneal surface impairment, and cornea and conjunctiva inflammation[43]. Alternatively, considerable effort is being directed towards prolonging drug retention time on the ocular surface and improving drug penetration. Nanosystems are an emerging part of this strategy.

During the past decades, some typical nanosystems have been developed for ocular anterior disease application, as summarized in Table 1[44-57]. For example, flurbiprofen-loaded PLGA nanoparticles with a size distribution around 200 nm have demonstrated a burst release and an ensuing gradual release profile *in vitro*. Therapy with this approach showed an improved anti-inflammatory effect as compared to commercial flurbiprofen eye drops on the rabbit ocular inflammation model[51]. In addition, flurbiprofen-loaded nanoparticles with a uniform size around 100 nm showed an equivalent inhibitory effect on the miotic response in a rabbit surgical trauma model even at a lower dosage than commercial eye drops. This effect was attributed to the increased release of drugs from the nanoparticles and subsequent penetration into the aqueous humor[55]. Such progress indicates the great impact of colloidal nanocarriers on the enhanced bioavailability of ocular drugs such as flurbiprofen[51,55,58,59]. However, some concerns exist regarding the possible rapid clearance of these formulations from the eye surface.

Recently, the *in situ* gel system is becoming a research hotspot, especially stimuli-responsive hydrogel such as pH-, thermo-, and ion-sensitive hydrogels. Moreover, there are commercial products such as Timoptic-XE® and Virgan®, which are ion-activated and pH sensitive hydrogel, respectively. Once the hydrogel is instilled onto the eye surface, the loaded drugs or nanoparticles can escape from the hydrogel upon eye blinking and then release drugs in a sustainable way. Recently, a micellar supramolecular hydrogel was fabricated with methoxy poly (ethylene glycol) block polymer and α-cyclodextrin. *In vivo* distribution results showed that the hydrogel could significantly enhance penetration and retention of the anti-inflammatory drug diclofenac, as compared with the micelle formulation[54]. Similar to hydrogel, nanoparticles loaded contact lens is a kind of polymeric nanodevice encapsulated with drugs.

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Wearers of contact lens can benefit from long drug retention time on the corneal surface[60]. As expected, a nanowafer containing arrays of drugs could withstand eye blinking and remain on the corneal surface for several hours. This formulation not only sustained a controlled drug release for hours to days, but also provided enhanced therapeutic efficacy in treating corneal neovascularization in a murine model[44] (Fig. 3).

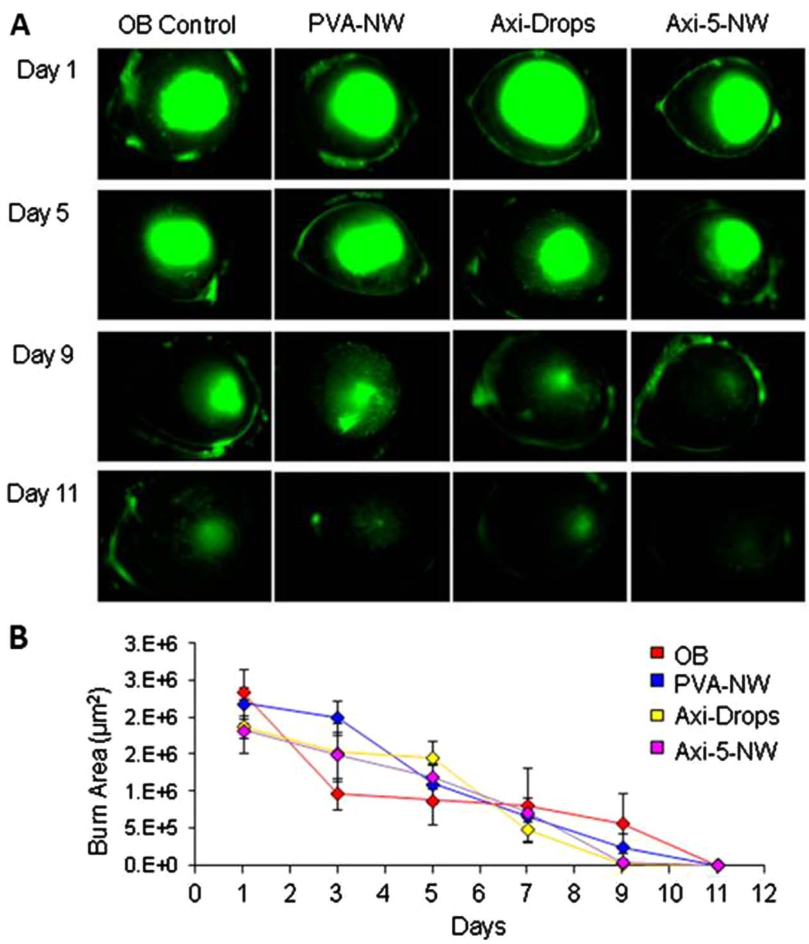

Figure 3 A fabricated nanowafer can improve the corneal wound healing in a mouse cornea burn model[44]. (A) Fluorescence images of mouse corneal surface; (B) Quantitative analysis of corneal surface healing. (Reproduced with permission from ACS artcile (direct link: http://pubs.acs.org/doi/full/10.1021/nn506599f).

Although many studies have applied nanosystems to ocular drug delivery, the mucoadhesive and penetration mechanisms between nanoparticles and corneal barrier deserve more understanding. Corneal epithelium has been shown to be the major barrier for penetration and permeation, which can prevent particles even smaller than 21 ± 1 nm in penetrating into the intraocular space[61]. However, the significance of nanoparticle size and surface chemistry during the penetration process are still controversial. In an earlier study of bovine eyes with removed epithelium, the surface chemistry-dependent penetration characteristics were investigated on two nanoparticles with the same size and different numbers of thiolated groups (SH). Results showed that the interaction between functional groups and collagen of corneal stroma other than the particle size is a major resistance factor during the penetration process. Better penetration into cornea stroma was observed by PEGylation with polyethylene glycol of higher molecular weight (for example 5000 Da) other than the low molecular polyethylene glycol (750 Da)[61] (Fig. 4).

6. Nanosystems for ocular posterior disease therapy

In contrast to diseases of the anterior eye, posterior diseases occur most commonly in the retina and choroid. Examples include age-related macular degeneration (AMD), choroidal neovascularization (CNV), glaucoma, retinoblastoma (Rb) and posterior uveitis. Generally speaking, eye drops present less drug bioavailability in posterior ocular tissues than in the anterior segment, due to the long diffusion distance from corneal surface to the retina or choroid. Moreover, frequent intraocular injections will lead to potential undesired side effects and poor patient compliance[62].

Thus, many efforts during the past decades have been made to improve delivery systems for the treatment of ocular posterior disease. Progress has focused on improving the controlled long-term delivery systems to reduce frequency of injections, including hydrogel, nanoparticles, nanoimplants and nanosized vesicles (Fig. 5). Light-activated solution made from polycaprolactone dimethacrylate (PCM) and hydroxyethyl methacrylate (HEMA) has been successfully fabricated and injected into the suprachoroidal space of rabbit eye

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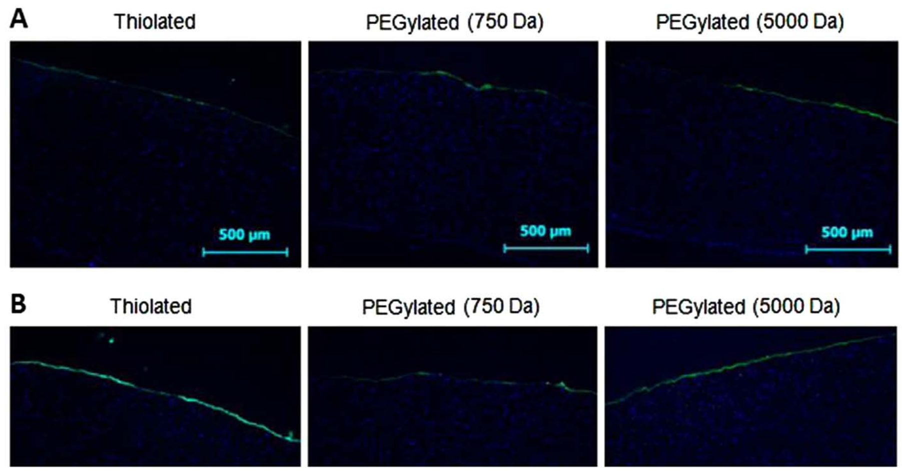

Figure 4 Fluorescence images of bovine cornea with removed epithelium after exposed to silica nanoparticles of 0.5 h (A) and 1 h (B). The nanoparticles had a consistent size distribution and were functioned by thiolated groups and PEGylated 5000 Da, respectively[61]. Reproduced with permission from ACS article. (direct link: http://pubs.acs.org/doi/full/10.1021/mp500332m).

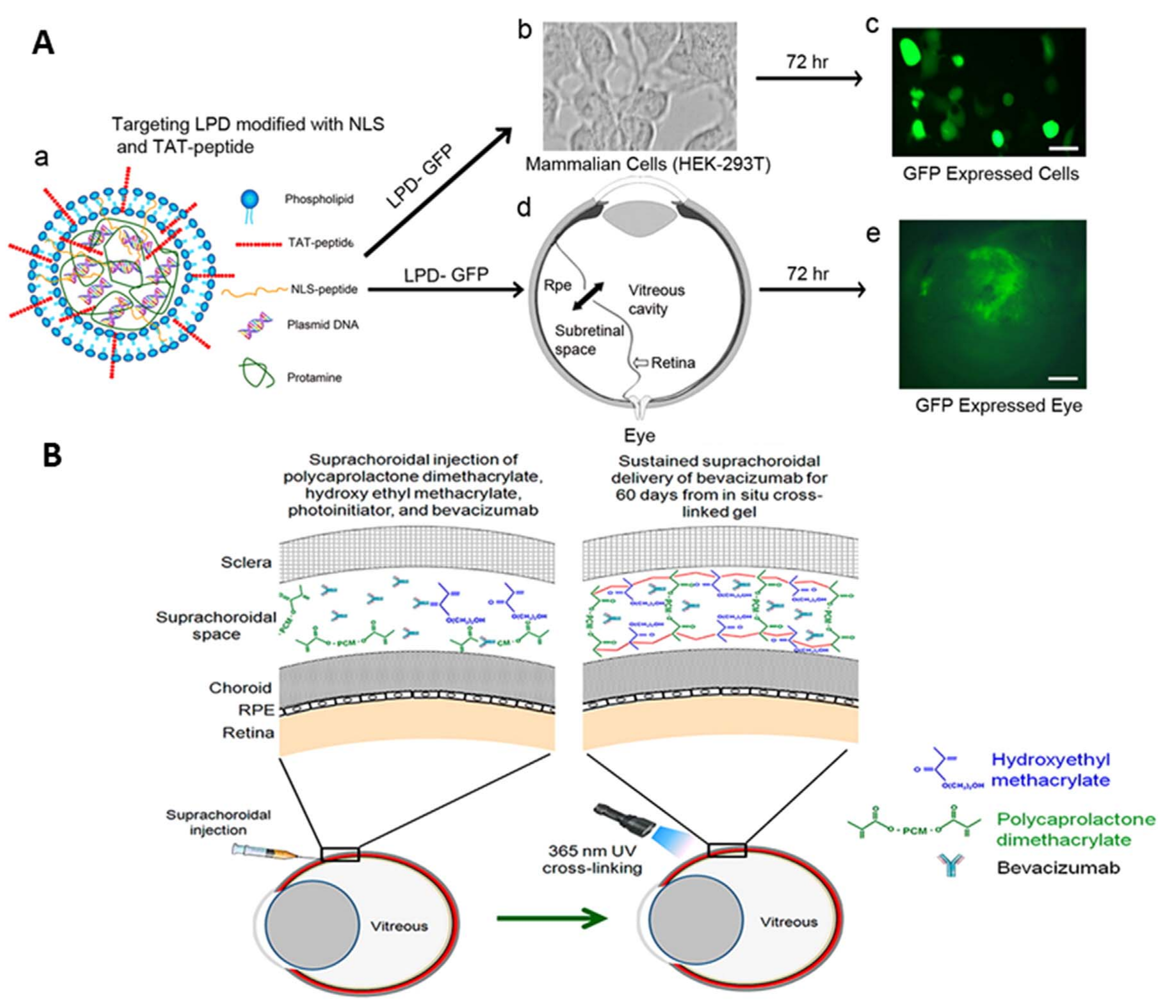

Figure 5 (A) Schematic illustration of a multifunctional nanoparticle modified with a nuclear localization signaling peptide (NLS) and cell permeable peptide (TAT) to deliver gene to the posterior segment of the eye for blinding eye disease treatment63. The strategy includes three functions: (1) A biocompatible lipid molecule was used to pack DNA along with another biocompatible protamine molecule together as a non-viral nanoparticle carrier; (2) The modified peptides have both cell penetrating and nuclei targeting functions thus leading to the gene delivery to eye cells; (3) DNA was used to carry target gene and promote the cell-specific gene expression. (B) A light-activated, *in situ* forming hydrogel system was designed to realize sustainable release of bevacizumab for age-related macular degeneration (CNV) therapy[63]. Reproduced with permission from ACS articles (direct links: http://pubs.acs.org/doi/full/10.1021/nl502275s; http://pubs.acs.org/doi/abs/10.1021/mp300716t).

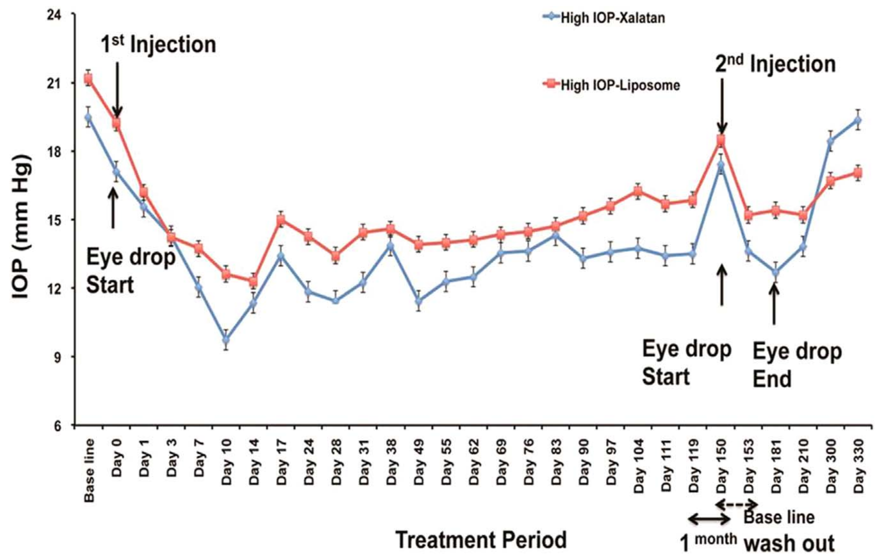

Figure 6 Comparison of the intraocular pressure (IOP) between a commercial eye drop (Xalatan) and latanoprost-loaded liposome in rabbit glaucoma model. The data showed that after a single subconjunctival injection of the liposome, the IOP reduced for up to 120 days and then further reduced over another 180 days after a second injection. The results were comparable to daily eye drop (Xalatan)[64]. Reproduced with permission from ACS article (direct link: http://pubs.acs.org/doi/abs/10.1021/nn4046024).

for CNV therapy. Following the rapid light-activated cross-linking, the solution could form *in situ* hydrogel for a sustained delivery of bevacizumab (an anti-VEGF antibody used to treat CNV) over 60 days[63] (Fig. 5B). However, this system is limited due to the toxicity of the photoinitiator to eyes. Natarajan et al.[64] developed a drug-loaded nano unilamellar vesicle which could obviously reduce the intraocular pressure and realize a sustainable release of drug over 120 days *via* a single subconjunctival injection (Fig. 6). These inspiring results have catalyzed the development of similar systems for glaucoma therapy.

Retina pigment epithelial (RPE) cells are of great importance for vision. They are not only the main forces of blood–retina barrier, but also centrally involved in the pathogenesis of retinal disorders[65,66]. CD44 is overexpressed in the surface of RPE and hence can be used as a key target for a number of drugs and gene-based therapeutics[67,68]. In Martens's work, a nonviral polymeric gene complex with hyaluronic acid (HA) coating was demonstrated and shown to be efficiently taken up by RPE cells *via* the CD44-receptor mediated endocytosis, resulting in a high gene delivery and expression of green fluorescent protein (GFP) in the eye[69].

Analogous approaches to intraocular cancer therapy (such as retinoblastoma (Rb) and uveal melanoma), are more complicated than discussed above. In addition to biological barriers in the posterior segment, the specific microenvironment of intraocular cancers is another therapeutic obstacle. Thus, strategies have been developed to either enable targeted delivery or to improve bioavailability of intraocular cancer drugs. Among numerous moieties that present on intraocular cancer cells, folate receptor has been studied as a delivery target for researchers[70–72]. Based on reports that folate receptors are overexpressed in Rb cells[74], folate-linked PLGA and chitosan nanoparticles have been proposed with sustainable, controllable and targeted delivery of anticancer drug-doxorubicin (DOX) to Rb cells[73,74]. In addition to the single-function nanosystem, multi-functional systems have been drawing great attention to realize diagnosis, treatment, and other functions simultaneously. Mitra et al.[75] prepared polyethyleneimine (PEI) capped gold nanoparticles (AuNPs) which were also conjugated with a novel epithelial cell adhesion molecule (EpCAM) antibody and siRNA molecules. They found these gene delivery systems were significantly internalized by Rb cells resulting in cytotoxicity. Despite great efforts devoted to the intraocular cancer therapy, the current studies are mainly limited in the stage of *in vitro* assessment, due to the lack of mature intraocular cancer animal models.

Photodynamic therapy (PDT) is an emerging therapeutic strategy which has been widely used for numerous disease treatments. PDT consists of three functional modules: a light-activated photosensitizer, an energy laser beam to induce activation, and a surrounding oxygen environment with the ability to produce a toxic compound. One commercial drug Visudyne® used for AMD treatment is a typical PDT product. The active ingredient of Visudyne® is a photoactivated drug-verteporfin. Upon a 689 nm laser depositing with a proper intensity, the drug can generate reactive oxygen species (ROS) and induce neovascular endothelial cell death, resulting in vessel occlusion and ending the growth of choroidal neovascular cells[76,77]. Recently, researchers have designed carbohydrate-targeted mesoporous silica nanoparticles (MSN) encapsulated with both anti-cancer drug camptothecin (CPT) and one-photon or two-photon photosensitizers. Encouraging results were achieved showing that the MSN nanoparticles presented an interesting therapeutic property by killing Rb cells efficiently *in vitro*[78]. Similar results were found in Wang et al.'s work, in which dendrimeric nanocarriers were developed with excellent cellular uptake, significant photoefficiency, and superior phototoxicity in Rb cells[79]. Although PDT showed great promising potential in some cancer treatments, more efforts are required on the development of delivery nanosystems to implement PDT in ocular applications. Some current nanosystems applied in ocular posterior disease treatments are given in Table 2.

7. Nanotechnology in ocular disease diagnostics

There are several approaches employed for clinical ocular disease diagnoses, such as optical coherence tomography (OCT), fundus photography, fluorescein angiography, positron emission

Table 2 Typical nanotechnology-based strategies for ocular posterior disease applications.

Formulation	Material type	Payload	Size (nm)	Function	Clinical stage	Ref.
Hydrogel	Polymer	Bevacizumab	–	The hydrogel showed a sustained release of Bevacizumab in suprachoroidal space of SD rats for 4 months.	Preclinical	63
Nanoparticle	Polymer	Latanoprost acid	80	The nanoparticles provided a sustained drug release by subconjunctival administration.	Preclinical	64
Hydrogel (Timoptic-XE)	Polymer	Timolol maleate	–	Topical treatment drug for glaucoma.	Market	–
Hydrogel	Polymer	Mitomycin C	–	The hydrogel showed good ocular compatibility and realized sustained release in intraocular after glaucoma surgery.	Preclinical	60
Liposome	Polymer	Bevacizumab	100–200	The system could pass through biological barriers by annexin A5 mediated endocytosis after topical administration.	Preclinical	44
Micelle	Polymer	Triamcinolone acetonide	200–350	A micelle combined gel system was well tolerated in rat eyes and had a sustained release for one year after intravitreal injection.	Preclinical	45
Dendrimer	Polymer	Gene	~50	The dendrimer-gene complex exhibited effective gene transfection in RPE cells.	Preclinical	80
Nanoparticle	Peptide/polymer	Gene	~180	The system could rescue the retina degeneration both histological and functional in a mouse model by subretinal injection.	Preclinical	81
Nanoparticle	Polymer	Gene	~250	The nanoparticle-gene complex promoted gene expression of RPE cells in gene deficient mice.	Preclinical	82
Liposome	Polymer	Gene	130–230	The peptide modified liposomes could target RPE cells and had increased the siRNA delivery efficiency 4 times than non-modified liposomes.	Preclinical	83,84

–Data not found.

tomography (PET), magnetic resonance imaging (MRI), ultrasonography and confocal microscopy. They have played a significant role in monitoring disease recovery. For example, MRI is useful for monitoring progress of ocular diseases such as diabetic retinopathy, AMD, and ocular tumor angiogenesis by *in vivo* imaging of neovascularization[85–87]. However, due to poor imaging sensitivity or imaging resolution, each of these approaches has limited advantages for disease diagnosis. For example, PET has high sensitivity but limited spatial resolution, while MRI has good spatial resolution but low sensitivity[88,89]. In order to overcome these drawbacks, nanotechnology seems to provide multiple options. Anderson et al.[90] developed a Gd-perfluorocarbon nanoparticulate emulsion linked with a biotinylated anti-$\alpha_v\beta_3$ monoclonal integrin antibody DM101. The system showed a site-directed contrast enhancement of angiogenic vessels in a rabbit corneal neovasculature model. After administrating the targeted agent for 90 min, the average MRI signal intensity was enhanced by 25% *in vivo*. Gold nanoparticles are particularly attractive contrast agents for OCT. It is reported that the optical resonance wavelengths of gold nanoparticles can be precisely tuned over a broad range because of their easily controlled sizes and shapes[91]. A typical example was shown upon OCT imaging of phantom samples. Gold nanocages (35 nm edge length) showed a cross section absorption about five orders of magnitude larger than conventional indocyanine green in the near-infrared spectral region[92]. Quantum dots have broad excitation spectrum and narrow emission wavelength, which renders them as good choices for tumor imaging[93]. CdSe quantum dots functionalized with targeted peptides could accumulate in tumors by binding tumor blood endothelial cells after intravenous injection[94].

Although nanotechnologies in tumor diagnosis and therapy have been developed and evaluated in recent years, there are only limited studies focusing on ocular disease application. Yet strategies used in other diseases can also guide the treatment and diagnosis in ocular disease. Recently, Hitomi et al.[95] developed a hydrogel nanosystem that combined tumor targeting, triggered drug delivery, and photo-to-heat conversion together to enable multimodal imaging and also controlled release of therapeutic cargo in human tumor xenografts. In this study, peptide targeted phage particles, heat sensitive–based liposome (HSL), mesoporous silica nanoparticles (MSNPs), and photon-to-heat conversion were integrated into a hydrogel system. The HSL and MSNPs could generate heat after NIR laser illumination. The heat induced release of hydrogel contents and meanwhile the loaded drugs were controlled to release at tumor site[95]. Techniques referred in this study offered a nanoplatform that allowing design of different formulations with specific ligands (such as antibodies, peptides and aptamers) and nanocarriers for different types, size and growth rate tumors. Nanoplatforms referred here exhibited great potential for clinical application or diagnostic therapeutic monitoring and targeted delivery to malignant tumors and ocular diseases. Some potential nanotechnology-based strategies in ocular diseases diagnostics are summarized in Table 3.

8. Challenges and perspective

8.1. Challenges

Nanotechnology has been proven to be a powerful and effective tool for treatment and detection of ocular diseases by fabricating nanosystems. In this review, we have focused on advances in design and development of nanosystems for various ocular diseases.

Table 3 Potential nanotechnology-based strategies for ocular disease diagnostics.

Formulation	Material type	Size (nm)	Target	Functions	Clinical stage	Ref.
Nanoparticle	Gd	~260	Corneal neovascularization	The agent showed contrast enhancement of angiogenic vessels in a rabbit corneal neovasculature model.	Preclinical	90
Nanoparticle	Silver	80	Retina	Silver nanoparticles coated with calcium indicator showed minimal damage to retinal cells and could apply for mouse retina imaging.	Preclinical	96
Nanocage	Gold	35	Retina	Gold nanocages exhibited strong optical resonance of 5 orders of magnitude larger than conventional dyes by OCT imaging.	Preclinical	92
Nanoparticle	Quantum dots	3–6	Intraocular cancer	The nanoparticles showed enhanced fluorophores in eye imaging.	Preclinical	94,97
Nanoparticle	Magnetic nanoparticles (Fe_3O_4)	10	Retinal detachment	Magnetically guided diffusion of nanoparticles was found in an *in vitro* model of human vitreous humor.	Preclinical	98

Several nanosystems with different payloads have shown great potential in ocular delivery either *in vitro* or *in vivo*. However, several challenges still remain to be addressed in future studies, including: (1) Among numerous studies of ocular disorder therapy by nanotechnology, many studies are focused on *in vitro* studies, and less *in vivo* studies have been accomplished. In the future, more efforts should be made in this area and animal models especially the ocular cancers model should be established. (2) Although the rabbit is most commonly used animal because of the comparable size of human eye, rabbit eye has a higher surface sensitivity, higher mucus production and lower blinking frequency, lower tear production[99]. These differences would lead to a better result of bioadhesion and retention in the ocular surface thus made the effect of nanosystems unauthentic to human beings. (3) For targeted delivery, the biomarkers are the most common types of target. As a result the ocular disease related biomarkers should be fully understood as well as the cellular and molecular mechanism of their functions. (4) It is reported that nanoparticles seem to grow in size and aggregate inside the tissues after intravitreous injection or other administration route[33–35]. This phenomenon could decrease the delivery efficiency and affect drug distribution. Further studies need to improve our understanding of the fundamentals of nanoparticles and facilitate development of proper delivery routes for application.

8.2. *Perspective*

Considering the above aspects which deserve more efforts, nanotechnology has great application potential in ocular disease therapy and diagnosis. As a unique and relatively closed organ, the eye is always considered to be a perfect research object for gene and drug delivery because the systemic circulation is usually omitted. Data from wiley website revealed that more than 1500 gene therapy clinical trials for ophthalmology are underway[100]. There are various nanomaterials used for nanosystem fabrication. However, their toxicities are not completely understood in the eye, especially for those repeated dosage materials. It seems that colloidal carriers and some FDA approved materials have more potential in application. In addition to delivery systems, future non-invasive delivery routes will be emphasized for ocular diseases in both segments. Finally, all-in-one systems which might combine diagnostic and therapeutic functions may be introduced to enable visual tracking during the ocular disease treatment.

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