R E V I E W

Potential Therapeutic Usage of Nanomedicine for Glaucoma Treatment

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Abstract: Glaucoma is a group of diseases characterized by progressive degeneration of retinal ganglion cells, leading to irreversible blindness. Currently, intraocular pressure reduction is the only established treatment available for glaucoma. With this treatment, the progression of the disease can only be delayed and there is no recovery. In addition, the commercially available eye drops have the disadvantage of low compliance and short therapeutic time, while glaucoma surgery always has the risk of failure due to wound fibrosis. Nanotechnology can overcome the limitations of the current treatment through the encapsulation and conjugation of drugs used for lowering intraocular pressure and antifibrotic agents using biodegradable or biocompatible nanoparticles for the sustained release of the drugs to protect the damaged ocular cells. Furthermore, using nanotechnology, treatment can be administered in various forms, including eye drops, contact lens, and ocular inserts, according to the convenience of the patients. Despite the promising results of delaying the progression of glaucoma, the regeneration of damaged ocular cells, including trabecular meshwork and retinal ganglion cells, is another critical hurdle to overcome. Bone marrow-derived mesenchymal stem cells and Müller glia cells can secrete neurogenic factors that trigger the regeneration of associated cells, including trabecular meshwork and retinal ganglion cells. In conclusion, this review highlights the potential therapeutic applications of nanotechnology- and stem cell-based methods that can be employed for the protection and regeneration of ocular cells.

Keywords: glaucoma, ocular regeneration, protection, stem cell, nanotechnology

Introduction

Glaucoma is the second leading cause of blindness worldwide. According to World Health Organization, 4.5 million or more than 12% of all cases of blindness globally were the result of glaucoma.[1] Glaucoma is a group of optic neuropathies characterized by progressive degeneration of retinal ganglion cells (RGCs) in the inner retina and loss of their axons in the optic nerve.[2] The disease progresses slowly without obvious symptoms until it leads to irreversible visual field loss and optic nerve damage.

There are several reasons for blindness from glaucoma. First, it is often diagnosed late because the patients remain unaware of the gradual contraction of their visual fields until finally their visual acuity begins to fail. Second, the disease is improperly controlled by medication and surgery. Currently, intraocular pressure (IOP) reduction is the only proven treatment for glaucoma.[3] The effectiveness of glaucoma surgery decreases with time because of the fibrosis of the surgical site.[4] In addition, insufficient reduction of IOP by medical treatment is a cause for the

progression of disease. In some patients with glaucoma, the visual field loss can continue to progress even with lowered IOP.[5] Difficulty in detection of IOP fluctuation and non-IOP factors such as neurodegeneration may contribute to the progression.[6] Another reason is the issue of compliance with the treatment. Since glaucoma is a life-long condition, many patients with glaucoma using eye drops experience difficulties in administration of the drops and suffer from ocular discomfort. This difficulty in the use of eye drops can increase the risk of glaucoma progression because of poor compliance.[7,8] Trans-corneal drug penetration through topical eye drops is currently the most commonly used route of drug delivery in ophthalmic medical fields. However, preservative or active ingredients of topical eye drops can have an adverse effect on the ocular surface, leading to dry eye symptom.[9] Thus, the current ophthalmic drug delivery system needs to be improved for better patient compliance and better efficiency.

Among the various fields in which nanotechnology can be applied, biology and medicine have gained increasing attention recently.[10] Nanomedicine is a comprehensive field that combines biology, chemistry, engineering, and medicine to provide more efficient tools for the prevention and treatment of various diseases. It can provide new strategies to clinicians for prevention, diagnosis, and treatment of severe disease such as cancer.[11] Additionally, nanotechnology could be used to introduce remarkable improvements in drug delivery systems,[12] medical imaging and diagnosis platforms,[13] implantable materials,[14] and tissue regeneration strategies.[15,16] Therefore, the use of nanomedicine could result in development of better diagnostic and therapeutic strategies for the prevention of blindness from glaucoma. In this manuscript, the authors have reviewed and discussed promising strategies involving use of nanotechnology for treatment of patients with glaucoma, in an attempt to overcome the limitations of current treatments for glaucoma.

Glaucoma
Definition and Classification of glaucoma
Clinically, glaucoma is defined as a condition with characteristic appearance of optic disc (optic disc cupping) and corresponding visual field loss.[17] The nerve fibers of RGCs pass out of the eye at the optic disc. The neuroretinal rim of the optic disc contains the nerve fibers of RGCs. In glaucoma, the neuroretinal rim of the optic disc becomes progressively thinner, thereby resulting in the enlargement of

the optic disc cup (central depression at optic disc).[18] This is called as optic disc cupping.[19] The loss of the RGC nerve fibers leads to a loss of the connection between the retina and visual pathway of brain, leading to visual field defect.[20]

Elevated IOP is the main risk factor for glaucoma.[21] IOP is determined by the balance between the inflow and outflow of aqueous humor.[22] Aqueous humor is a clear fluid found in the anterior and posterior chambers of the eye, which is required to provide nutrients and remove cellular waste products.[23] In addition, aqueous humor has a role in maintaining the shape of the eyeball and related refractive properties of the eye. Aqueous humor is actively produced by the ciliary body in the posterior chamber and exits the anterior chamber through two distinct routes, including the trabecular pathway and the uveoscleral pathway.[24] Under physiological conditions, the trabecular pathway accounts for about 90% of the total outflow of aqueous humor. In the trabecular pathway, aqueous humor drains through the filter-like region of the trabecular meshwork (TM) and Schlemm's canal (SC), and finally enters systemic circulation through the episcleral veins.[23]

Glaucoma can be clinically classified by several factors. First, based on the appearance of the iridocorneal angle, where TM is located, glaucoma is defined as angle-closure glaucoma or open-angle glaucoma. Angle-closure glaucoma develops when the angle between the peripheral iris and the TM closes due to mechanical contraction, which results in decreased aqueous humor outflow and increased IOP. Conversely, in primary open-angle glaucoma, the IOP is elevated due to increased resistance against the outflow of aqueous humor despite open angle. In primary open-angle glaucoma, the major site of resistance to aqueous outflow is believed to be located in the area between the TM and SC.[25] Both open-angle and angle-closure glaucoma can be primary or secondary, and can be classified into different developmental categories. Primary open-angle glaucoma can occur with or without IOP; the latter is called normal-tension glaucoma. Secondary glaucoma can develop due to trauma, corticosteroids, inflammation, tumor, and conditions such as pigment dispersion or pseudoexfoliation.[17] In secondary glaucoma, the alteration in outflow resistance in the TM and SC is responsible for IOP elevation. Thus, the TM and SC are important therapeutic target tissues for IOP reduction for the treatment of glaucoma.[26]

Pathogenesis of Glaucoma
There are many clinical types of glaucoma; however, optic nerve damage is common to all. The optic nerve is damaged through a mechanism called apoptosis of the RGC.[27] This

Table 1 Limitations of Current Glaucoma Medical Treatments

Mechanism of glaucomatous optic nerve damage	Increased IOP	Stasis of RGC axonal flow →Block NF →apoptotic RGC death
	Local vascular insufficiency	Ischemic damage →Decrease in NFs →RGC death

apoptosis is mediated by two main mechanisms. Mechanical injury, resulting from increased IOP, causes damage to RGCs. Elevated IOP causes stasis of RGC axonal flow at the lamina cribrosa in the optic disc, leading to blockage of neurotrophic proteins (NFPs), which finally results in apoptosis of RGCs.[28] The other mechanism is local vascular insufficiency at the optic nerve head. This ischemic damage can lead to decrease in the levels of neurotrophic factors (NFs) in the optic nerve head, which results in RGC death (Table 1 and Figure 1A and B).[29,30] Besides, mitochondrial

dysfunction, low cerebrospinal fluid pressure-mediated translaminar cribrosa pressure gradient, excitotoxicity, and oxidative stress are also proposed to be involved in glaucomatous optic nerve damage.[31,32]

Despite multiple factors other than IOP being related to the pathogenesis of glaucoma, current treatments are mainly concentrated on decreasing the IOP. Effective reduction of IOP significantly prevents glaucoma progression in most patients in clinical trials, including patients with normal tension glaucoma.[33] There are several methods available for reducing the IOP in patients with glaucoma. The first and most commonly used method for IOP reduction is medical therapy.[34] Topical eye drops lower IOP via two mechanisms. Topical beta-blockers, alpha-agonists, and carbonic anhydrase inhibitors reduce the production of aqueous humor in the ciliary body.[35] Conversely, topical cholinergic drugs, such as pilocarpine, enhance the outflow of aqueous humor through the TM-SC pathway.[28] Prostaglandins can also decrease IOP by increasing the outflow of aqueous humor through the
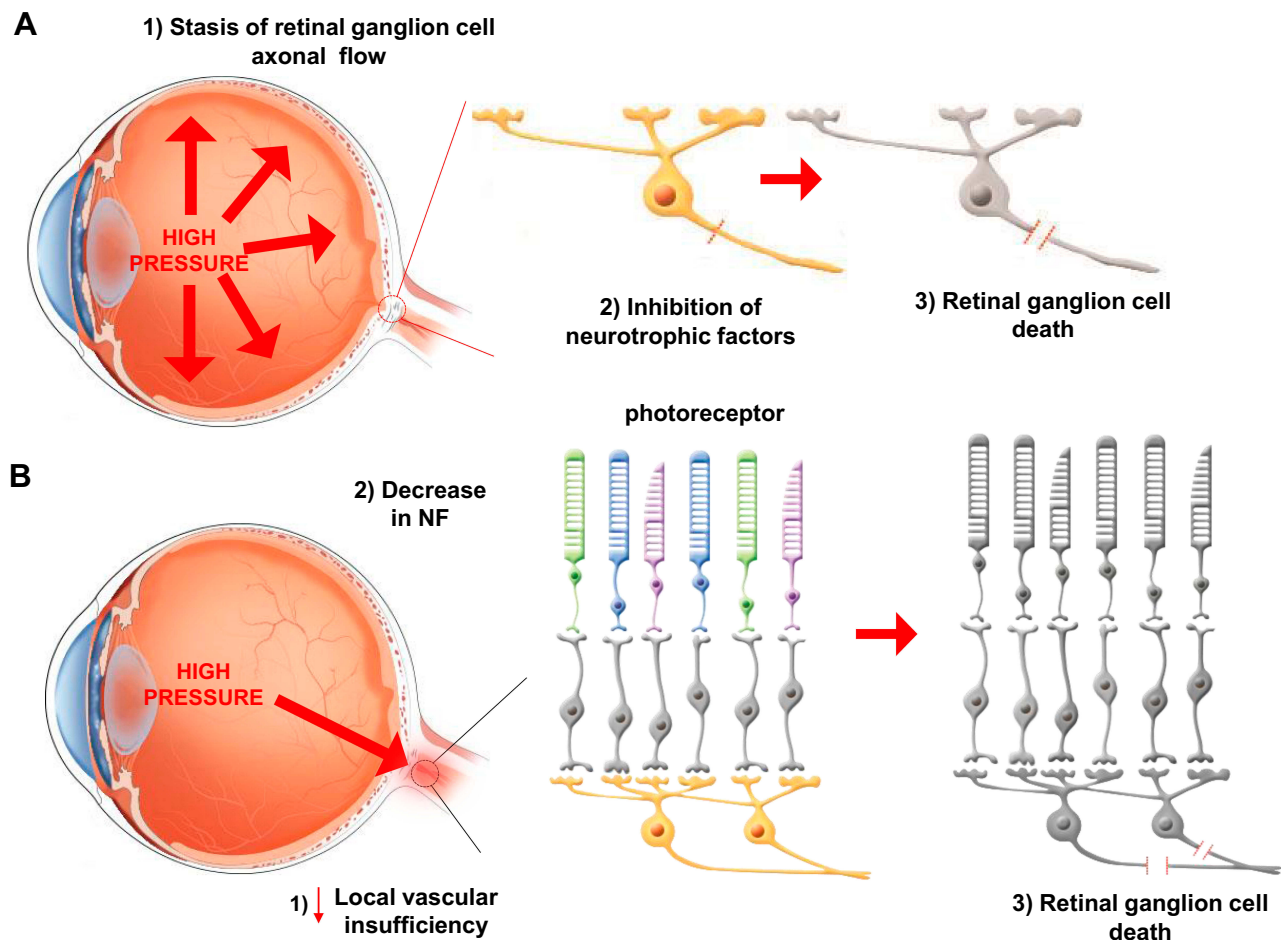

Figure 1 Two main mechanisms of glaucomatous optic nerve damage. (**A**) The elevation of IOP and (**B**) deficiency of vascular result in the blockage of neurotropic factors and proteins to induce the death of RGCs.

uveoscleral pathway.[36,37] When antiglaucoma medication is not enough, the aqueous humor can be drained through an extraocular site via trabeculectomy or drainage devices.[38]

Limitations of Current Glaucoma Treatment

Despite their effectiveness, there are several disadvantages of using topical eye drops. First, most topical eye drops that are currently used should be administered 1–3 times per day. Ocular discomfort when dropping the eye drops and frequent administration cycles results in poor patient compliance. Low compliance is an especially important issue in the elderly population, as it renders the medical treatment ineffective with the patients having to undergo surgery (Table 2).[39] Second, the bioavailability of topical eye drops inside the eye is very low.[40] The volume of commercial drop dispensers (25–50 μL) generally exceeds the capacity of the conjunctival sac (10 μL), so that the major portion of the liquid drains out of the eye and onto the eyelids and cheeks, where further absorption may occur. The capacity of the conjunctival sac depends on several factors, such as blink rate, position (applying when standing or lying down), and the means of application. Therefore, bioavailability has to be classified as extremely low and is reported in the literature to be in the order of 5–10%.[41,42] The small volume of eye drops that remains in the conjunctival sac is absorbed via two routes. Small lipophilic drugs are absorbed via the transcorneal route.[43] Conversely, large hydrophilic drugs are absorbed via the transconjunctival and transscleral routes.[43] Prostaglandins have been reported to enter the eyeball through the sclera rather than cornea.[44] In the transcorneal route, the drugs first meet the precorneal tear film constituted of a deep mucous layer and superficial aqueous layer.

The ocular system is protected by effective clearing mechanisms, including lacrimal secretion in the precorneal tear film for removal of irritants and blinking reflex. As a result, the half-life of a topical drop in the precorneal tear film is about 1 min, which is the only time available to the drug for penetrating the cornea and accessing the aqueous humor.[45] The next barrier, corneal epithelium, which contains multiple desmosomes and tight junctions, prevents molecules larger than 500 Da to penetrate the cornea.[46] As a result, 80% of the delivered drug cannot penetrate cornea, and may be absorbed into the blood vessels of the conjunctiva.[47] Only less than 10% of the drug is absorbed into the eye and approximately 1% of that reaches the aqueous humor.[48] Moreover, in transconjunctival and transscleral routes, over half dosage of the drugs is absorbed into systemic circulation through the vessels of conjunctiva and sclera.[49] A few drug molecules that can penetrate cornea are quickly filtered through the TM, where their half-life is less than 2 hours. This makes it difficult for the drug molecules to reach their target tissue.[50] Low compliance, maintaining sustained drug levels, and effects at their targets are important problems to be solved in glaucoma medical treatment.

Surgical drainage devices are used in glaucoma when IOP-lowering medications fail.[51] These devices provide a new route to the aqueous humor from the anterior chamber of the eye to the collection plate beneath the conjunctiva. Currently, the major commonly used surgical drainage devices are Ahmed, Molteno, Krupin, and Baerveldt.[52] After implantation of these devices, the major cause of surgical failure is fibrosis around these devices. It decreases the overall surgical success rate to about 40–50%. Intraoperative or postoperative antimetabolite injections like mitomycin C (MMC) or 5-fluorouracil (5-FU) can prevent fibrosis around these devices.[53] However, these antimetabolite injections can increase the risk of infection around the bleb. However, if the drainage device is coated with nanomaterial, it may lead to a more successful surgical outcome without side effects.[54]

Another fundamental limitation is that visual loss from glaucoma has not been shown to be reversible with any current treatment. Furthermore, RGC loss may continue to progress in spite of IOP reduction in some patients with glaucoma.[55] In this regard, neuroprotective strategy has been proposed.[56] This review is focused on the application of nanotechnology-based strategies to overcome these current limitations in glaucoma treatment.

Nanotechnology Approach in Medicine
Nanomedicine

Nanotechnology, as defined by the National Nanotechnology Initiative, is the "science, engineering, and technology

Table 2 Limitations of Current Glaucoma Treatment

Low compliance of medical treatment	• Ocular discomfort • Frequent administration cycles
Low bioavailability of medicine	• Only less than 10% of drug absorbed into the eye • Only 1% of drug reaches the aqueous humor
Surgical treatment	• Failure due to fibrosis of surgical site
Irreversibility of glaucoma-induced vision loss	• The retinal ganglion cell death is not reversible under current treatment

conducted at the nanoscale, which is about 1–100 nm".[57] In 1959, Richard Feynman, known as the father of nanotechnology, proposed the use of nanoscale machines in molecular and atomic modifications.[57] Scientists first visualized the nanoscale using a scanning tunneling microscope in 1981.[58] Thereafter, our understanding and ability to manipulate matter at molecular and atomic scales became tremendously enhanced. There are two definitions of nanomedicine. The first is the technology providing molecular aid for treatment and diagnostics using already existing knowledge on the human body, further described as using nanostructures with therapeutic effects.[59] The second is "the comprehensive monitoring, control, construction, repair, defense, and improvement of human biological systems at the molecular level, using engineered nanodevices and nanostructures that operate massively in parallel at the single-cell level, ultimately to achieve medical benefit".[60]

The size of nanoparticles is the essential feature of nanotechnology.[61] While matter interactions at macroscopic scales can be predicted in classic physics, nanoscale interactions can be predicted through quantum mechanics in nanotechnology.[62] Thus, nanomaterials have unique chemical and physical characteristics that differ from materials at the macroscopic scale. As the size of particles becomes smaller, surface-to-volume proportion of the particles becomes larger. A nano-scale material will offer a larger number of locales for synthetic responses than a macro-scale material at per-unit size of a given material.[62] Because of these characteristics of nanomaterial, medical network has great interest in nanotechnology. Many nanomaterials provide interesting features, including electrical conductivity, biocompatibility, magnetic properties, and biodegradability.[59]

Nanomedicine as Novel Drug Delivery System

Glaucoma is a type of ocular disease characterized by gradual degeneration and functional exacerbation of optic nerve, which progressively decreases visual sensitivity, and may lead to blindness. In open-angle glaucoma, the outflow of aqueous humor is blocked internally, resulting in the gradual elevation of intraocular pressure, whereas in closed-angle glaucoma, trabecular meshwork outflow pathway is blocked by iris which leads to rapid elevation of intraocular pressure.[2] Unlike open- or closed-angle glaucoma, the eye pressure of normal-tension glaucoma does not exceed the normal range of intraocular pressure. Both secondary open- and closed-angle glaucoma refer to any form of glaucoma with identifiable cause of elevated

intraocular pressure. Uveitic glaucoma is associated with uveitis, and childhood glaucoma is early-onset glaucoma which could be attributed heredity.[17]

The primary treatment for glaucoma is topical medication or eye drops. Several therapeutic classes, including prostaglandin analogs, β-blockers, carbonic anhydrase inhibitors, α-2 agonists, and cholinergic agents, were developed in the form of eye drops to relieve the IOP. Proper administration of eye drops can prevent glaucoma progression through reduction of IOP; however, it requires manual dexterity, which is found to be challenging in the elderly population. Furthermore, poor adherence and short residence time requiring high frequency of dosing can lead to missing of proper dosing time. A new drug delivery system that allows gradual release of the drug for a few months with a single administration needs to be developed.

Numerous methods are available for developing hollow, solid, or porous nanoparticles with various shapes and sizes.[57] Molecules like drug compounds, DNA, RNA, or antibodies can be included as components or encapsulated within nanoparticles. Widely used nanodelivery systems are nanoparticles, nanosuspension, nanodiamonds (NDs), nanocrystals, liposomes, niosomes, dendrimers, cyclodextrins, and other devices (Figure 2).[63] The advantages and disadvantages of nanomaterials are summarized in Table 3. These nanomaterials can incorporate the drugs in two ways: through encapsulation inside the nanomaterials or conjugation on the surface of nanomaterials. The encapsulated drug is released as the nanomaterial disassembles at the target site, while the nanomaterial-conjugated drug is released after the bond between the nanomaterial and drug is cleaved at the target site.[64] These drug delivery strategies of nanomaterials can further enhance and compensate the limitations of conventional treatment for glaucoma. In addition, inorganic nanoparticles can be incorporated into a hydrogel to mimic the mechanical property of contact lens for sustained release.[65]

The selection of an appropriate system depends on the drug type (hydrophobicity, size, stability), target tissue, and route of administration. Nanodelivery systems can provide more targeted delivery, sustained release, bioavailability, dose accuracy, minimal tissue irritation, longer shelf life, and better solubility.[66,67]

Most treatments for glaucoma are designed to lower IOP, and the most common route of administration is topical administration. However, low bioavailability and poor corneal permeability of the administered drug remain as
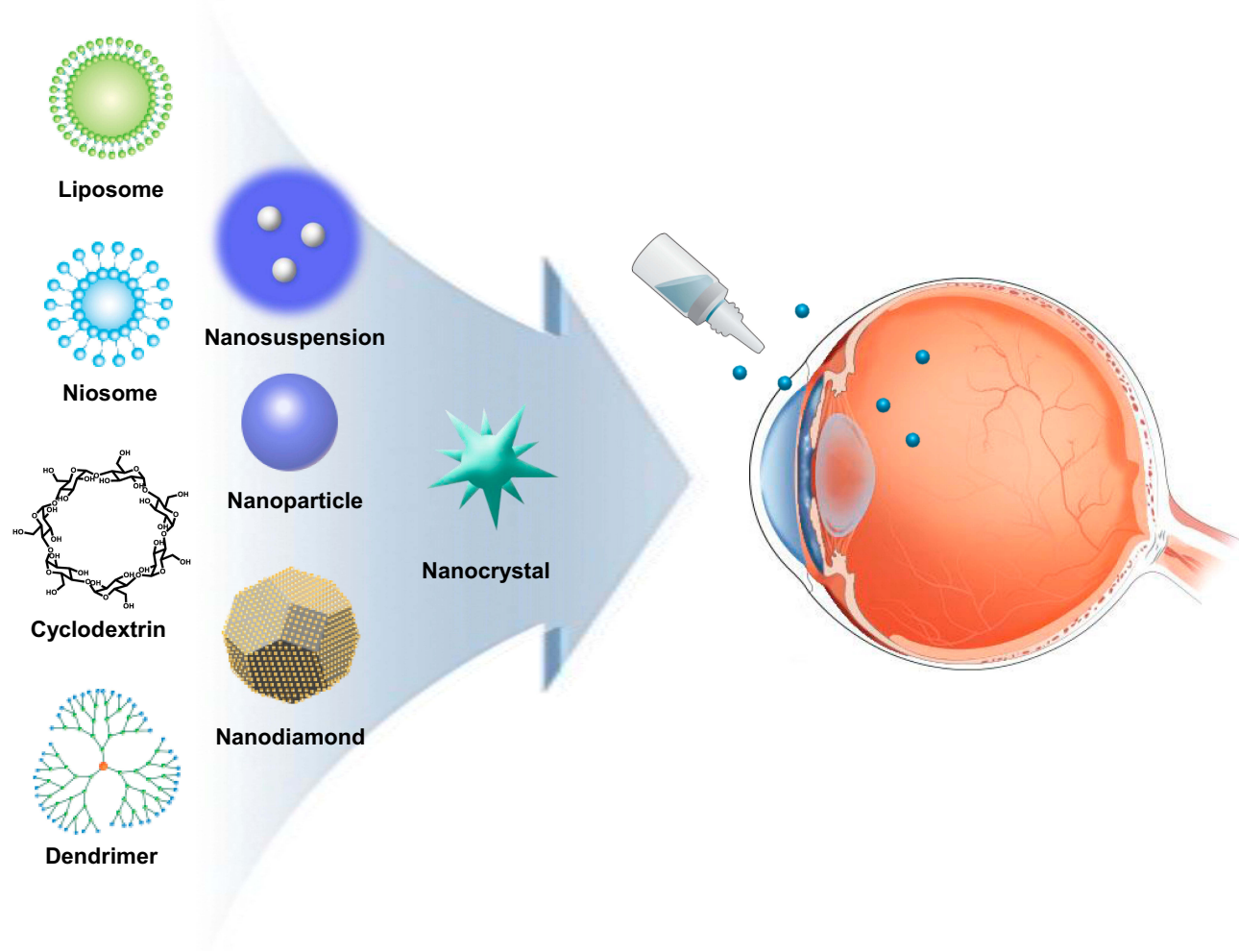
Figure 2 Delivery route of nanomedicine. Topical administration of various types of nanomaterials to reduce IOP.

challenges for the improved therapeutic treatment. Nanotechnology-based drug delivery system can provide sustained release and better bioavailability without irritation.[68] Furthermore, the surface of nanoparticles can be coated to specifically target the desired site for drug delivery. Nanotechnology-based drug delivery is very similar to that of conventional treatment, but with improved efficacy at the same dosage through appropriate nanotechnology.

Desired drugs are administered through four routes, including topical, subconjunctival, systemic, and intracameral, to reach the anterior segment.[69] Administered drugs need to overcome specific barriers depending on the administration route. In general, the drug-loaded nanoparticles are formulated in the form of eye drops for topical administration, so the limitation of topically administered drug will briefly be discussed.[70,71] For better efficacy of topically administered drugs, two factors need to be considered: tear film and cornea.[69] Drugs are quickly cleared due to

blinking reflex and lacrimal secretion in the tear film and the cornea provides a physical barrier to the penetration of drug.

Nanoparticles need to circumvent the existing barriers by increasing their precorneal retention time through surface functionalization.[72] Then, most nanoparticles can enter cells through endocytic pathway. Although the uptake pathway of each nanoparticle is unique, the factors affecting the corneal penetration of nanoparticles remain the same and mainly include size, shape, and charge.[73] For instance, 35–200 nm sized indomethacin nanoparticles can penetrate corneal epithelium through energy-dependent endocytosis pathway including clathrin- and caveolae-endocytosis, and micropinocytosis.[74]

Toxicity of nanomaterials also needs to be considered. Many studies have extensively evaluated the toxicity of nanoparticles on main organs, whereas only a few studies have examined the potential toxicity of nanomedicine regarding their exposure time or size on the eye.[75]

Table 3 Advantages and Disadvantages of Nanomaterials

	Advantages	Disadvantages
Liposome	• Low toxicity • Prolonged half-life of drug • Biodegradable	• Limited storage condition • Blurring after intravitreal injection
Niosomes	• Non-ionic surfactants • Low toxicity • Non-immunogenic • Can encapsulate hydrophilic, lipophilic, and amphiphilic drugs	• Inefficient drug loading • Aggregation • Physical instability
Inorganic nanoparticles	• Low cost • Stability • Biocompatibility • Ease in surface modification • Uniform in size	• Possible toxicity • Low clearance
Dendrimer	• Tunable size • Tunable physical property	• Inefficient drug loading • Possible toxicity
Nanosuspension	• Rapid dissolution through IV administration • Less irritation • Higher bioavailability	• Physical instability
Cyclodextrin complexes	• Chemical stability • High aqueous solubility	• May cause irritation • Safety concern
Nanocrystals	• Tunable particle size • Sustained release of drug • Improved dose proportionality • Applicability to all administration routes	• Physiochemical instability • Uniform dosage cannot be achieved
Nanodiamonds	• Chemical stability • Small size • Large surface area • High absorption capacity • Stable drug complex	• Potential toxicity (difficult to remove all organic solvent) • No sustained release of drug

Furthermore, each nanoparticle has unique physiochemical properties including chemical composition, surface area, and charge that can affect its toxicity on the eye.[75,76]

Various biological models have been utilized for determining potential toxicity of nanoparticles in the eyes. Most biological models (including in vitro and in vivo models) when treated with inorganic nanoparticles exhibited oxidative stress and damaged ocular system.[75,77] The toxicity of nanoparticles needs to be considered before using them for safe and effective glaucoma treatment.

Liposomes
Liposomes are artificial lipid bilayers of phospholipids that are biocompatible with the human body.[78] Liposomes provide a potential delivery system that can carry both hydrophilic and hydrophobic drugs. They can encapsulate and protect solutes while delivering drug molecules until they reach the target structures.[79] Liposomes can be designed to have more bioavailability, bioefficacy, and sustained release of drug molecules.[80] Another interesting characteristic of liposomes is their responsiveness to modifiable triggers such as thermosensitivity, electromagnetic waves, and pH environment. Thus, release of the entrapped molecules can be strictly controlled.[81] Besides of the above characteristics, liposomes can reduce the elimination of the drug substance from the body and increase drug dosage at the targeted site. This factor may increase patient compliance by decreasing the dosing frequency.[82] In an in vitro drug release study, timolol-loaded liposome formulations showed a 1.93-fold increase in permeability coefficients.

They also reduced IOP from 30 to 300 min after instillation (minimum IOP = 11.96 ± 0.74 mmHg at 1 hour), and decreased IOP from 30 to 180 min (minimum IOP = 13.61 ± 0.95 mmHg at 2 hours) when used in the form of eye drops in albino rabbit eyes.[83]

Surface charge of liposomes is an important factor of drug contact time at corneal epithelial surface. The corneal epithelium has negatively charged mucinous membrane, which provides a good binding surface to positively charged liposomes. Because of prolonged contact time at corneal surface, positively charged liposomes demonstrate better corneal permeation than neutral or negatively charged liposomes.[46,84] In addition, positively charged liposomes demonstrated higher encapsulation efficiency than neutral and negatively charged liposomes in an in vitro study.[46] The entrapment efficiency would increase because of electrostatic attraction between anionic drug and positively charged liposomes. Charge repulsion between drug anion and negatively charged liposomes may induce low entrapment efficiency and may suppress drug-loading efficiency.[84] Meanwhile, electrostatic repulsion may occur between drug anion and negatively charged liposomes, resulting in a higher percentage of drug release. After drug release from the liposomal vesicles, drug molecules only rely on passive diffusion to cross the corneal barrier for intraocular absorption. Thus, the longer the contact time at the corneal surface in the encapsulated state, the higher the bioavailability of the drug.[85] Positively charged liposomes produce high binding affinity between the negatively charged mucin of corneal epithelium and liposomal vesicles, enhancing the contact time of positively charged liposomes.[86]

Positively charged acetazolamide (ACZ)-sterylamine liposomes demonstrated more IOP reduction than neutral and negative-charged liposomes in rabbit eyes (−7.80 ±1.04 vs −5.50 ± 1.65 vs −3.70 ±2.18 3 hours after topical administration). The effect of IOP reduction persists for longer period in positively charged ACZ-sterylamine liposomes than neutral and negatively charged liposomes in rabbit eyes (8 hours vs 6 hours vs 3 hours). Moreover, it showed higher encapsulation efficiency compared with neutral and negative liposomes (48.27 ± 1.01 vs 39.73 ± 0.44 vs 25.55 ± 1.50) in an in vitro study.[85]

Neutrally charged vesicles of L-a-dipalmitoyl phosphatidylcholine (DPPC):pilocarpine hydrochloride showed a prolonged drug release up to 600 minutes in rabbit eyes, which is greater than that of negatively charged liposomes or free-drug eye drops.[80] These prolonged release profiles and high bioavailability of surface charged liposomes indicate the possibility for better patient compliance through reduction of dosing frequency.

The conventional liposomes have some limitations in terms of clinical uses. They tend to aggregateand may cause leakage of entrapped drug, and are susceptible to phagocytosis. The modification of surface characteristics of liposomes has helped to overcome these drawbacks of conventional liposomes. Bioadhesive polymers are used to coat liposomes. Coating with bioadhesive polymers inhibits the aggregation of liposomes and increases their viscosity and hence their corneal residence time.[87] Poly L-lysine-modified liposomes can not only enhance the efficacy of eye drop formulations, but also deliver drugs at the retinal site where RGC death occurs.[88] Chitosan (mucoadhesive polysaccharide)-modified liposomes increase the residual time of the drug formulation in ocular tissue by enhancing the viscosity of the solution.[89] Timolol maleate chitosan-coated liposomes (TMCHL) produced a 3.18-fold increase in the corneal permeability coefficient in an in vitro drug release study. In a study on transcorneal permeation of New Zealand rabbits, TMCHL was found to be more effective in lowering IOP than timolol eye drops (final IOP = 19.67 ± 1.14 mmHg vs 23.80 ± 1.49 mmHg, respectively).[90] Latanoprost-loaded Egg-phosphatidylcholine (EggPC) liposomes developed using a thin film hydration technique enhance the stability of phospholipids in liposomes, effectively lowering the IOP in rabbit eyes for at least 90 days; this effect was significantly greater than that of once-daily topical latanoprost controls.[91]

Microbubbles are contrast agents used for medical ultrasound imaging. They improve the transfection efficiency after ultrasound-induced cavitation.[92,93] However, microbubbles are unstable and are too large in size (1–6 μm) to be used in intravascular clinical application.[94] The size of liposomes can be controlled easily and they can be used as drug, antigen, and gene carriers, and can be transferred to targeted organ.[95] Thus, bubble liposomes are a new and promising strategy for the delivery of genes through ultrasonication, which intensifies the penetration of genes.[96]

Recently, some clinical studies have explored the effect and safety of drug-loaded nanoliposomes in glaucoma treatment. Dorzolamide (DRZ)-loaded nanoliposome demonstrated better IOP reduction than commercially available DRZ formulation in rabbit.[97] IOP lowering observed at 2 weeks was 23.26 ± 9.24%, 9.25 ± 5.76%, 32.60 ± 7.90%, and 17.48 ± 7.62% for the DRZ-loaded nanoliposome group and the commercially available DRZ

formulation group.[98] Latanoprost/thymoquinone-loaded liposomes and latanoprost-loaded liposomes were able to significantly reduce the IOP in glaucomatous white albino rabbits, which lasted for 8 hours. Conversely, the effect of the free latanoprost did not persist for more than 24 hours.[99]

For evaluating the safety and efficacy of nanoliposomes in human eye, liposomal latanoprost was injected in subconjunctival space once in six subjects who were diagnosed with either ocular hypertension (OHT) or primary open-angle glaucoma (POAG). Subconjunctival injection of liposomal latanoprost was well tolerated by all the six subjects. From a baseline IOP of 27.55±3.25 mmHg, the mean IOP decreased within 1 h to 14.52±3.31 mmHg (range 10–18 mmHg). This represented a mean decrease of 13.03± 2.88 mmHg (range 9–17 mmHg), or 47.43±10.05% (range 37–63%). A clinically and statistically significant IOP reduction (≥20% IOP reduction, P=0.001 to 0.049) was observed for 3 months after injection.[100]

Niosomes

Niosomes are spherical, closed bilayer structures of non-ionic amphiphiles. Niosomes provide a potential delivery system that can carry both hydrophilic and hydrophobic drugs simultaneously, which has possible usage in combined drug therapies.[101] Niosomes can provide longer time period to enhance bioavailability, and have positive therapeutic responses at low cost. However, the major advantage of niosomes is the non-toxic nature of non-ionic surfactants.[102] Timolol maleate-loaded niosomes using chitosan have shown a prolonged effect in reducing IOP by releasing the timolol for longer time intervals.[103] In glaucoma rabbits, timolol maleate niosomes coated with chitosan were effective for over 8 hours, and IOP reduction was longer than the conventional dosage form, which lasts up to 2 hours.[104] Niosomes of ACZ show better and prolonged drug release in the ocular tissue, which can eliminate the need for oral medication.[105] Some types of niosomes, such as multilamellar niosomes, can entrap a higher dose of drug and can release it for more longer period.[106]

Polymeric Nanoparticles

Polymeric nanoparticles have spherical shapes with sizes in the nanometer range. Because of their nanometer size, nanoparticles easily pass through biological membrane barrier systems, and can easily carry drugs to the target organ.[107] The smaller the size of nanoparticles, the larger the surface area, and higher the drug-loading capacity.[108] There are three types of nanoparticles. First-generation nanoparticles are simple polymer matrix–type particles. Second-generation nanoparticles are simple nanoparticles with polymer coating. This polymer coating can enhance adhesion of nanoparticles to target site. Third-generation nanoparticles are antibody-attached nanoparticles, which can bind with specific group of cells and release the drug at the particular target site. Nanoparticles of propoxylated glyceryl triacylate (PGT) with timolol in 1:1 ratio can release the timolol for longer duration.[109] Additionally, Poly(butyl)-cyano acrylate nanoparticles of pilocarpine can significantly decrease IOP without any side effects.[110]

ACZ-loaded Eudragit nanoparticles displayed better permeability and flow across corneal tissue than a conventional drug suspension in an in vitro transcorneal permeability study. ACZ loaded Eudragit nanoparticles and ocular insert demonstrated substantial reduction in IOP and improved ocular tolerability when compared to ACZ suspension in an in vivo study.[111]

Ex vivo transcorneal permeation study displayed higher ACZ permeation at 8 hours with nanoparticle-in situ gel (74.50 ± 2.20 mg/cm^2) than with ACZ eye drops (20.08 ± 3.12 mg/cm^2) and ACZ suspension (16.03 ± 2.14 mg/cm^2). In addition, nanoparticle-in situ gel did not display harmful effects on any corneal layer. Moreover, 1% of PLGA nanoparticle-in situ gel exhibited greater IOP-lowering effect 1 hour after administration, which was sustained for up to 8 hours, while 1% ACZ eye drops sustained their action for approximately 2 hours in normotensive rabbit eyes.[112]

Dendrimers

Dendrimers are polymeric materials with excessive flexible branching, resulting in a large surface area. Dendrimers can provide enough space for drugs inside the excessively flexible branching.[113] Evidently, dendrimers also have also biocompatible and nonimmunogenic characteristics similar to other nanomaterials. Dendrimers with quaternary ammonium group can also diminish the need for benzalkonium chloride (BAK) as a preservative.[114] Polyamidoamine (PAMAM) dendrimers showed longer residual time of pilocarpine, for up to 5 hours. In addition, PAMAM dendrimers significantly lower ocular irritation and have greater bioavailability than commercially available Carbopol eye drop formulations.[115] In a study, dendrimers of carteolol showed longer residual duration, which can decrease daily eye drop frequency, in glaucoma

rabbit eyes.[114] Topically administered hybrid dendrimer hydrogel/poly(lactic-co-glycolic acid) nanoparticle showed greater concentration of brimonidine and timolol than saline control in aqueous humor for 7 days.[116]

In vitro investigation of cytotoxicity and cell viability of ACZ-loaded water-soluble mucoadhesive carbosilane dendrimers revealed that generations 1 and 2 of cationic dendrimers and all 3 generations of anionic dendrimers are well tolerated at 10 µM, with higher than 80% cell survival for all of them, except for G3-C (from the 3rd generation of carbosilane cationic dendrimers). G3 cationic carbosilane dendrimers (5 µM) demonstrated rapid (1 hour post-instillation) and sustained (up to 7 hour) hypotensive effect, reaching a peak 22.6% IOP reduction in normotensive rabbit eyes.[117]

Nanosuspension

Using high-pressure homogenization or different milling methods, dispersed solid particles in the liquid phase can be incorporated into a colloidal drug delivery system (nanosuspension). This system can be easily integrated with other delivery systems, such as hydrogels, because of non-water soluble characteristics. Nanosuspensions are especially effective in delivery of lipophilic drugs, and can enhance their bioavailability.[63] In situ gel-forming nanosuspension of forskolin (coleonol) lowered IOP by 31%, and the drug efficacy lasted for 12 hours, which is significantly longer than that achieved with other conventional delivery systems.[118] A pilocarpine-loaded chitosan-poly(acrylic acid) nanosuspension showed zero-order release kinetics of pilocarpine.[108]

When molecules of drug are transported in the body, they encounter several ions. There can be several electrostatic interactions between drug molecules and ionic medium. This can reduce the stability of drug molecules. Ion Exchange Resins (IERs) are polymers containing appropriately substituted acidic groups, such as carboxylic and sulfonic groups for cation exchangers, or basic groups, such as quaternary ammonium group for anion exchangers. IER protects ionic drugs through shielding and competitive binding effect. It can be synthesized or purchased depending on its application.

Betaxolol-loaded nanoparticles using Ion Exchange Resin (IER) complex suspension have been approved and are commercially available in the US market as Betoptic S for use as ophthalmic drug delivery system. The cationic exchange resin containing 0.25% betaxolol provides microscopic beads of 5 µm diameter and its residence time in the cul-de-sac is increased with the addition of polyacrylic polymer.[119] In a multicenter, double-masked parallel study of 352 patients with primary open-angle glaucoma or ocular hypertension, no significant difference was found between 0.5% betaxolol solution and Betoptic S in terms of IOP reduction, whereas prevalence of ocular discomfort after instillation was significantly lower for the Betoptic S.[120] As such, nanosuspension will be an attractive drug delivery system for lipophilic antiglaucoma drugs, such as carbonic anhydrase inhibitors, in the future.

Cyclodextrin Complexes

Cyclodextrins are cyclic oligosaccharides in which sugar molecules are arranged in a ring-like pattern. Based on the sugar molecules in the ring structure, cyclodextrins are categorized in three main classes: α–cyclodextrins, β–cyclodextrins, and γ–cyclodextrins containing 6-, 7-, and 8-membered sugar rings in the cyclic structure.[121] The drug molecules are enclosed within cage-like ring structure. Primary advantage of these structures is delivery of the drug compounds without changing their molecular structure. Because of hydrophilic surface characteristics of cyclodextrins, lipophilic drug enclosed in cyclodextrins can easily pass through the hydrophilic barrier of the external eye and can safely reach the lipophilic corneal surface. Finally, drug compounds can be released in the aqueous humor.[122] The sulfobutyl ether of betacyclodextrin (SBE7-beta-CD) complex of pilocarpine prodrug showed effective protective effect on pilocarpine before reaching the target area, and decreased the strong irritation caused by pilocarpine by preventing its rapid absorption.[123] The multicomponent complex of hydroxypropyl β–cyclodextrin and triethanamine with ACZ can release ACZ for up to 4 hours and yields a reduction in IOP by approximately 30% without any irritation.[124] Dorzolamide with γ cyclodextrin showed prolonged residual time in the aqueous humor, and can be a great potential system for a once-daily formulation.[125]

BZL-hydropropyl-ß-cyclodextrin (HP-ß-CD) inclusion complex (HP-ß-CD/BZL) into nanoliposomes ("HP-ß-CD /BZL-loaded nanoliposomes", BCL) demonstrated a 9.36-fold increase in the permeability coefficient compared with commercially available BZL. BCL formulation reduced IOP in less than 1 hour, reached peak efficacy (32.3%) at 2 hours, and showed a sustained release effect for 12 hours. From 2 to 12 hours after instillation, BCL resulted in significantly lower IOP when compared with BZL suspension.[126]

In vitro analyses of stability and phase solubility of Latanoprost-propylamino-ß cyclodextrin (CD) demonstrated a significant improvement in its solubility and stability. In vivo evaluation of ocular tolerability revealed that ocular irritation

was 15.5% with the commercially marketed formulation of latanoprost and 9.5% with the latanoprost-propylamino-ß-CD formulation. Microscopic evaluation of ocular tissues showed that commercially marketed formulation of latanoprost induced higher inflammatory mixed cell infiltrates than lata-noprost-propylamino-ß-CD formulation.[127]

Nanocrystals

Nanocrystals may be defined as pure solid particles with a mean diameter of less than 1 μm and a crystalline structure. Nanocrystals are composed entirely of drug itself with crystalline coating.[128] Nanocrystals can provide a large effective surface volume area and have a good bioavailability without need for carriers, such as other nanosubstances. Nanocrystals have fast initial dissolution within 1 hour, indicating better bioavailability.[129]

Nanocrystals of brinzolamide (BZL) showed higher IOP-lowering efficacy than the commercially available market product (75% vs 49%).[130] Pilocarpine hydrochlor-ide loaded nanocomposite formulations (cellulose nano-crystals and triblockpoloxamer copolymer) showed higher sustained drug release and less toxicity than topical formulation of in vitro gel.[131] Trimethyl lock BZL prodrug nanoparticles showed similar efficacy as commercial BZL at 1/5th the molar concentration without toxic effects on the cornea in normotensive rats.[132]

Nanodiamonds

Nanodiamonds are 2–10 nm large carbon nanoparticles with a truncated octahedral structure.[133] Nanodiamonds have tailorable surface structure. Many functional groups are covalently or non-covalently conjugated on the surface of nanodiamonds. The surface of nanodiamonds coated with polyethyleneimine (PEI) can be conjugated with enzyme-cleavable N-acetylated chitosan along with timo-lol maleate to prepare a nanogel in the form of contact lens. Therefore, the sustained release of timolol maleate can be achieved in the presence of lysozyme as the chit-osan dissociates. Nanodiamonds also provide the mechan-ical support of contact lens.

Ophthalmic Devices Using Nanotechnology
Contact Lenses

The use of nanoparticles as drug carriers can pave the way for the sustained release of a drug. Nanotechnology can be applied to contact lenses for slow and controlled release of drugs for a long duration (Figure 3A).[134] Using solvent impregnation method, both hydrophilic and hydrophobic drugs can be easily incorporated in the lenses without the issue of solubility, which is a major drawback of many currently used drug delivery systems.[135] The drug embedded in contact lenses through nanotechnology can result in high drug-loading capacity and controlled release of the drug over a long time, which would result in better patient compliance.[136] Various studies have explored the efficacy of nanoparticles embedded in contact lens for potential glaucoma therapy. Silicone contact lenses, ACUVUE® TruEye™ containing timolol significantly reduced IOP.[137] With contact lenses, only 20% of the dose is required to produce a similar therapeutic effect compared with the full dose needed in currently used eye drops.[137] Contact lenses with timolol containing PGT nanoparticles released timolol for 1 month in Beagle dogs. In these type of lenses, the ratio of timolol to PGT can be modified.[109] Timolol, when loaded onto ethylene glycol dimethacrylate and PGT-based nanoparticles, not only exhibits prolonged drug release time of up to 4 weeks but also remains well encapsulated under refrigerated conditions for up to 5 months.[138] Another study has found that hyaluronic acid and chitosan-based polymeric nanoparticles loaded with timolol showed a significant reduction in IOP compared to conventional timolol solution.[139] Many promising results from studies have gained interest, which has lead to the development of various nanoparticle-drug formulations for glaucoma treatment. However, most developed drugs are currently in the preclinical stage.[140]

The traditional IOP measurement can only be performed at the hospitals, and, thus, patients cannot check their IOP on a daily basis.[141] Because the fluctuation of IOP may be a risk factor for glaucoma progression, daily monitoring of IOP could be clinically useful for some patients in whom glaucoma progress despite stable IOPs in the clinic. Nanotechnology has been used to develop contact lens for continuous monitoring of IOP.[142] These contact lenses are composed of a platinum-titanium gauge sensor, which is 170 nm thick and is embedded in two layers, a gold antenna, microprocessor, and an integrated circuit for turning on IOP monitor. If the stress in the gauge is increased, other mechanical forces are compressed, which alters the electri-cal forces present in the gauge. These contact lenses showed reproducible intraocular pressure in the range 15–30 mm Hg in enucleated pig eyes.[143]

Based on the development of nano or microelectrome-chanical system, silicone-based Triggerfish® was developed with reliable IOP measurements and was approved by
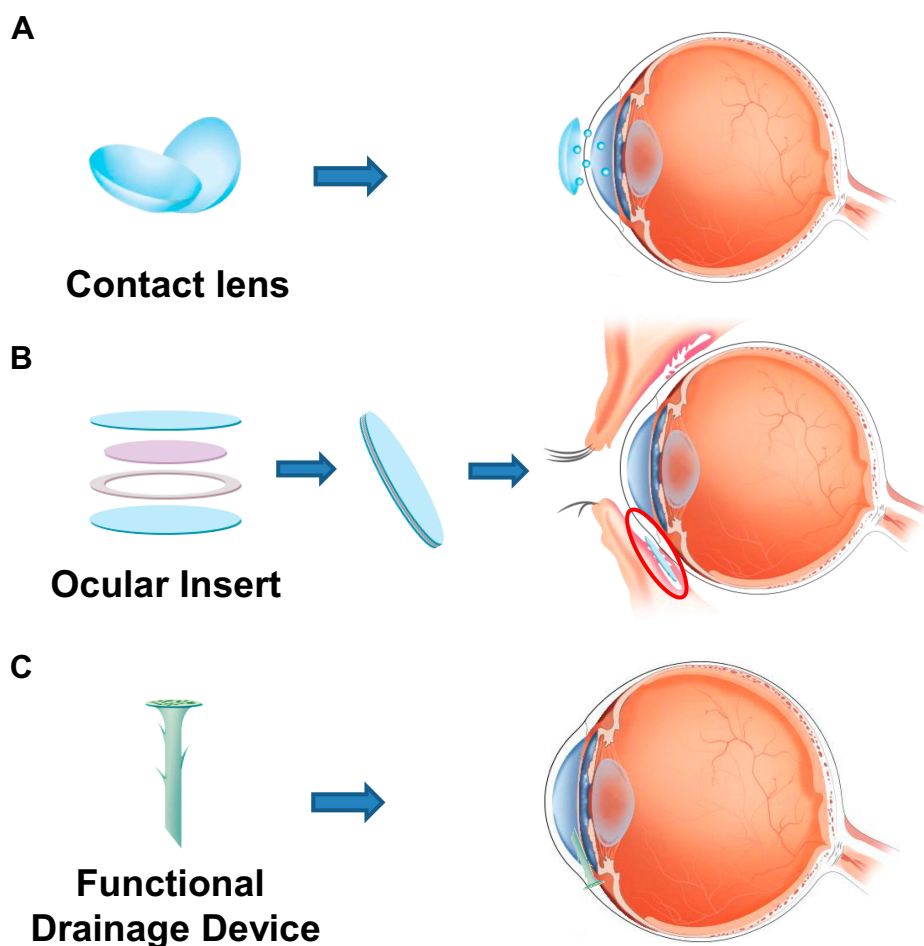
Figure 3 The application of nanotechnology to ophthalmic devices. The sustained release of drug through (**A**) contact lens and (**B**) ocular insert. (**C**) The coating of functional drainage device.

FDA.[144] In addition, a permanent implant Eyemate® for continuous IOP monitoring is under human clinical trials for commercial use.[145] As of today, iCare® home tonometer is commercially available as self IOP measurement device but it cannot provide data continuously for 24 hours.[146] In this aspect, nanotechnology supported the development of various fields including IOP monitoring, surgical devices, drug delivery systems for glaucoma treatment.

Hydrogel

Hydrogels are three-dimensional networks of hydrophilic polymers, either synthetic or natural, and can be applied to various fields including drug delivery system, regenerative medicine, and cell encapsulation. Hydrogels can be used for treatment of ocular diseases including glaucoma, corneal abrasion, and cataracts.[147] Hydrogel is mainly used as a sealant after incision. The mechanical property of hydrogel can be manipulated to meet the requirements of

a sealant, and PEG-based hydrogel, known as ReSure® has been approved by the FDA.[148] Today, hydrogel is widely used as a sealant in ocular incision due to better leakage prevention than the conventional suture.[149] Therefore, many studies have extensively explored the application of PEG-based hydrogel in corneal attachment and retinal attachment.[150]

Nanoparticles can be incorporated into a type of hydrogel, known as a nanogel, for drug delivery system. In glaucoma treatment, conventional medication including timolol maleate, pilocarpine, and xalatan shows low bioavailability and short retention time; hence incorporating these drugs into hydrogel can increase their retention time through improved bioavailability.[109] For instance, corneal penetration of timolol maleate loaded chitosan-alginate nanoparticle was twice than that of conventional timolol maleate, indicating the extended retention time. The use of hydrogel or nanogel as a sealant and drug carrier can

accelerate the healing process and limit bacterial infections after incision by incorporating growth factors or antibiotics.

Ocular Inserts

Ocular inserts are thin drug-impregnated devices of solid or semisolid consistency. Using ocular inserts, a drug can be released for a long period and in a controlled manner (Figure 3B). Ocular inserts are usually placed in the conjunctival cul-de-sac.[48] Ocular inserts provide the most accurate dosing at a constant rate for a long time, thus they can increase the bioavailability of drugs with better pharmacotherapeutic effect. In addition, through mucoadhesive polymers, ocular inserts can slowly release the drug in the ocular compartment and can reduce systemic drug absorption, thus prevent systemic side effects.[122] Ocular inserts of levobunolol HCl were showed to release levobunolol hydrochloride for longer time in a controlled manner, thus providing more effective glaucoma treatment.[151]

The OTX-TP (Ocular Therapeutix) consisting of PEG-based hydrogel with embedded travoprost-loaded PLA microspheres delivers travoprost to the ocular surface via an intracanalicular punctal plug for up to 3 months, as well as resorbs and drains through the nasolacrimal system. These microspheres slowly degrade and show a sustained drug release over a period of 30 days. Ten days after implantation, IOP was reduced by 5.4–7.5 mmHg with 100% retention rate. It also demonstrated enhanced therapeutic benefit for 90 days with a consistent 90% retention rate.[152] The Phase II trial did not find any serious adverse effects and showed only a slightly less hypotensive effect as compared with timolol.

Latanoprost Punctal Plug Delivery System (L-PPDS) is another punctal plug drug delivery system. In a study on L-PPDS in patients with open-angle glaucoma, IOP was reduced by 5.7 mmHg. After implantation of L-PPDS, 60% of subjects in the study showed IOP reduction by at least 5 mmHg or more and 47% of the subjects showed IOP reduction of at least 6 mmHg. A statistically significant mean change in IOP (22.3%) was recorded in the subjects treated with L-PPDS when compared with controls.[153]

Surgical Nanodevices

Studies have been reported on the improvement of glaucoma drainage devices using nanotechnology. A two-layer film of polylactic-co-glycolic acid (PLGA) loaded with 5-FU and MMC showed continuous drug release at a constant rate in vitro. This film showed continuous release

of 5-FU until day 28, with a delay in the first 3 to 5 days.[54] Currently, commonly used surgical devices have other postoperative complications. The devices are implanted in the post equator area of the eye and occupy large spaces. This can effect the function of levator muscle of the eyelid, and may result in ptosis.[154] Implantable nanodevices may solve these issues. Nanodrainage implant known as ANDI, composed of a polymeric shaft and a nanoporous membrane, has been invented. Microelectromechanical systems and nanofabrication technologies were used for developing ANDI. Major problem of ANDI was over-efficient drainage, which can lead to hypotony (Figure 3C).[155]

In addition, glaucoma devices with ferrofluid nanoparticles were invented. Because of its supramagnetic characteristics, ferrofluid plays a role like that of a valve. Valves are opened only when liquid flow pressure is more than the magnetic pressure of ferrofluid. After implantation of these ferrofluid drainage device in rabbit eyes, IOP was more reduced in the valves compared with contralateral control eye.[156] A biodegradable plug filter, with polylactic acid (PLA) or PLGA, was invented to prevent postoperative hypotony. Plugs are approximately 500 μm long and a hole with diameter of 44 μm is laser-drilled in the center of the plug. These plugs could prevent abrupt IOP reduction and stabilize gradual IOP reduction. However, this plug is only effective for about 15 days because of its biodegradable characteristics. It showed effective IOP reduction for 15 days in rabbit eyes.[157] Using microelectromechanical systems, an antifouling glaucoma drainage device was invented. This device was designed as an array of parallel microchannels composed of PEG-214 and PEG-4000. This polymer provides stability against swelling and therefore provides resistance to channel clogging due to biofouling. In addition, in vitro studies indicated that the polymer has lower nonspecific protein adsorption than glass, polypropylene, polydimethylsiloxane, and polymethylmethacrylate. Finally, the design was proven to be resistant to intrusion by *Escherichia coli* in a week-long experiment. However, additional studies with most common microbes and in vivo animal studies are yet to be completed to validate the efficiency of the device.[158]

Trabecular Meshwork Regeneration and Neuroprotection Using Mesenchymal Stem Cells

Although reduction of IOP can delay disease progression, the completely damaged optic nerve system cannot be recovered. Therefore, a new neuroprotective agent is

required to improve the patients' quality of life. Due to their potential to differentiate into multiple lineages and secretion of paracrine factors, mesenchymal stem cells can be a breakthrough in the regeneration of TM and damaged RGCs. TM is located near the cornea and is associated with IOP.[159] Increased outflow resistance of aqueous humor at TM gradually increases IOP,[160] and therefore treatment of the TM can temporarily relieve IOP. The regeneration of TM can, therefore, reduce IOP.[161] Bone marrow-derived mesenchymal stem cells (BMMSCs) and paracrine factors secreted by BMMSCs after they are released in the TM through laser therapy can regenerate ocular tissue. In addition to ocular regeneration, although MSCs cannot differentiate into RGCs, they can secrete factors that can help the dysfunctional RGCs to survive.[162]

Research on treatment of corneal wound healing and regeneration using stem cells is underway.[163] This cell-based approach is being tried as another potential therapeutic strategy for glaucoma treatment because decreased cellularity in the TM increases the outflow resistance.[164,165]

If successful, it could be a new method of increasing the physiological aqueous outflow by targeting the TM. Du et al demonstrated that after injection of human TM stem cells into mouse anterior chamber, they homed to the TM and differentiated into functional TM cells.[166] In the first week, they expressed TM marker protein CHI3L1 and maintained the TM for 4 months. Snider et al reported improved stem cell delivery to the TM using magnetic nanoparticles.[167] MSCs were labeled using magnetic nanoparticles. The prussian blue nanocubes labeled MSCs could be delivered to the entire circumference of the TM, which was not achieved without magnetic steering.

Dillinger et al reported hyaluronan coated nanoparticles could deliver small interfering RNA against connective tissue growth factor (CTGF), which is a mediator of pathologic effects in TM and SC, by binding to those cells via CD44. Gene silencing in human TM cells lead to a significant reduction of CTGF expression. Thus, HA-coated nanoparticles combined with RNA interference may be a novel, causative therapeutic modality for glaucoma.[168] Studies on nanotechnology and tissue engineering have been conducted to provide in vitro disease model for glaucoma. The biomimetic SC inner wall has been reported to be engineered with microfabrication technique.[169,170] More recently, bioengineered glaucomatous 3D human TM has also been developed as an in vitro disease model.[171,172] Thus, the models will offer a new

platform for studying aqueous outflow physiology and drug screening.

Since the deprivation of NFs is a critical mechanism in pathophysiology of glaucoma, the induction of various NFs has been investigated as a modality for the protection of optic nerve damage in glaucoma. Previous studies have showed that injection of microsphere containing glial cell-derived neurotrophic factor (GDNF) into vitreous increased RGC survival in vitro and in vivo.[173–176] Ciliary neurotrophic factor (CNTF) also has been shown to have neuroprotective effect on central nervous system. For sustained delivery of CNTF, encapsulated CNTF nanospheres provided neural stem cell differentiation without loss of potency compared to the unencapsulated growth factor.[177]

Additionally, damaged RGCs are not capable of self-repairing, but specific cell types, including Müller glia cells, could constitute neurogenic progenitors under pathological conditions. Müller glia cells are found in the retina of all species, and are characterized as retina-derived stem cells in cold-blood vertebrates.[178] In mammals, Müller glia cells can undergo reactive gliosis through morphological changes, dedifferentiation, and upregulation of markers. However, these cells can re-enter the cell cycle to regenerate rod photoreceptors.[179] Furthermore, these cells can express mature retinal neuronal markers under specific culture conditions (Figure 4).[180] Thus, manipulation of Müller glia cells could lead to the development of a new regenerative treatment for ocular tissues damaged by glaucoma.

Discussion and Perspectives

Despite being the second leading cause of blindness, glaucoma remains an incurable disease with effective therapeutic treatment. The current treatment of glaucoma is mainly focused on delaying the disease progression. Furthermore, regardless of the technological success in the medicinal field, low compliance and short therapeutic time remain as challenges for pharmacologic treatment of glaucoma. Patients need to be dexterous with both hands and be aware of dosing times for the proper and effective administration of the drug, which is challenging in the elderly population. Thus, the development of a new platform with improved therapeutic time is required.

The advances in nanotechnology can overcome the limitations of current glaucoma treatments. Efficacies of topically administered drug for glaucoma treatment are limited since only a small proportion of administered drug can reach the target sites, whereas most of the drug is washed away. Nanotechnology can be utilized to enable the sustained release

IOP Reduction

Retinal Regeneration

BM-MSC & Secretomes

BDNF
NGF
EGF
bFGF
CNTF
PDGF

Activated Müller cells

Retinal ganglion cell regeneration
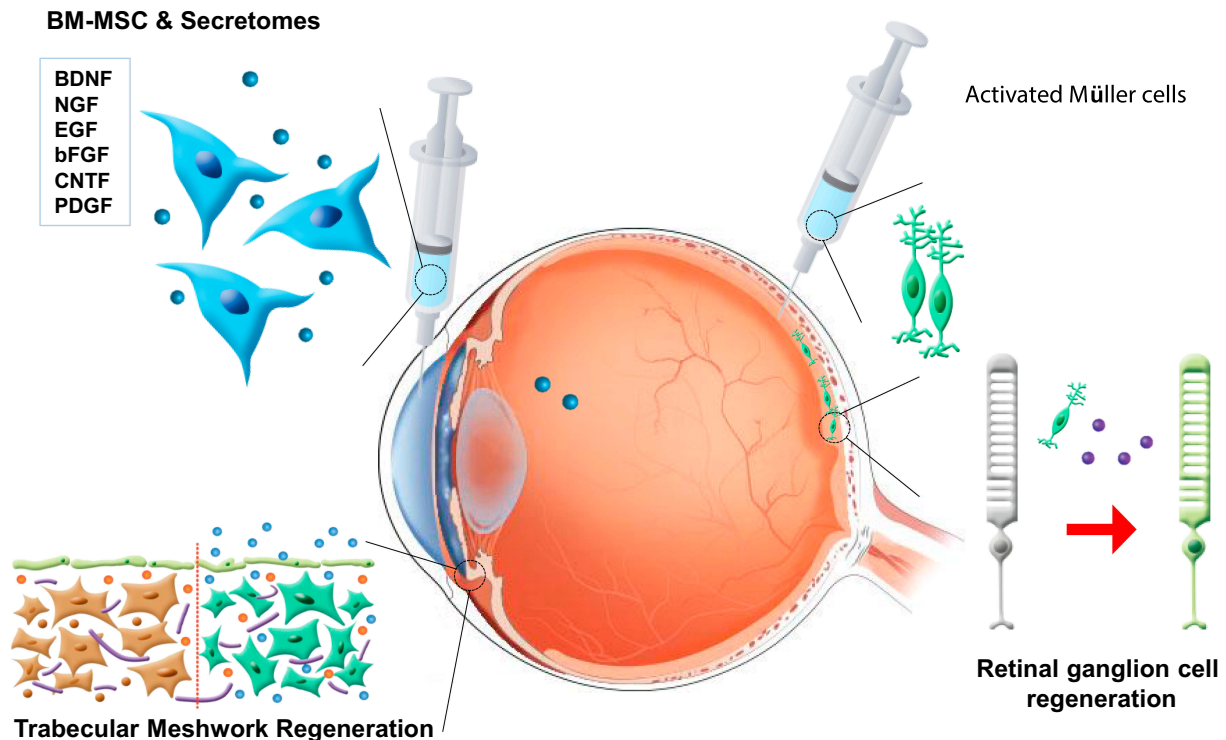
Trabecular Meshwork Regeneration

Figure 4 Stem cell use for ocular protection and regeneration. Neurotropic factors released by bone marrow-derived mesenchymal stem cells and Müller cells induce the regeneration of the trabecular meshwork and retinal ganglion cells.

of the conventional drug by increasing its bioavailability. Various nanoparticles including liposomes, polymers, and nanocrystals are currently under clinical trials for their efficacies. Effective glaucoma treatment can be achieved through the encapsulation or conjugation of desired drugs to appropriate nanoparticles to maximize the possibility of reaching the desired sites and to prolong the residual time. The surface of nanoparticles can be coated such that the particles can endure the physiological conditions of precorneal tear film. Therefore, nanoparticles, regardless of type, shape, or size, allow the sustained release of drugs unlike free drugs, resulting in the reduction of IOP. These results can be translated into decreased drug administration frequency and, thus improved compliance.

Nanoparticles can also be applied in the form of ophthalmic devices to treat glaucoma. Nanoparticles can be easily embedded in the polymer of contact lenses or ocular inserts for sustained release without major drawbacks, and the encapsulated drug in the contact lenses can remain stable under refrigerated conditions, similar to topical

administration of nanoparticles. Furthermore, the mechanical property of contact lens can be manipulated with nanoparticles to prevent potential damage after insertion in the eye. In addition, the encapsulated drug in the contact lens can be released under physiological cues, such as lysozyme, for controlled release. Controlled release of drug can reduce the dosing frequency. Furthermore, nanotechnology applications are varied, including the coating of a surgical device and IOP diagnosis. Recently, an IOP monitoring device has been developed in the form of contact lens to measure IOP of patients for 24 hours. Despite these merits, nanoparticles may induce toxicity upon administration; hence, appropriate dosage needs to be cautiously determined before the initiation of treatment to minimize the side effects. While the use of nanoparticles has improved the glaucoma treatment when compared to the traditional treatment method, stem cells can be used to protect and possibly regenerate the TM and damaged RGCs.

Stem cells are widely known for their multi-lineage differentiation and secretion of NFs. These cells can

differentiate into TM to relieve IOP and secrete NPFs to protect damaged ocular tissues from apoptosis. For ocular regeneration, based on the replacement of damaged RGCs, Müller cells could be used because they can express mature retinal markers under certain conditions.

Co-treatment with appropriate stem cells and nanoparticles can synergistically help to protect and regenerate the damaged RGCs. In addition, the targeting ability of stem cells can be enhanced through the usage of nanoparticles, which not only provide a prolonged retention time in physiological conditions, but also reduce the side effects. Despite these beneficial effects of co-treatment, caution is required when using nanoparticles since each type of nanoparticle possesses unique mechanical and physiochemical properties. This review highlighted the usage of drug-loaded nanoparticles and stem cells to effectively halt the disease progression and possibly regenerate the damaged tissue.

Conclusion

In recent years, conventional glaucoma treatment has been equipped with stem cells and nanoparticles due to the advances in nanotechnology and understanding of stem cells. Nanoparticles can help in reaching the desired target sites and provide sustained release to delay the progression of glaucoma. Then, stem cells can assist in glaucoma treatment by regenerating trabecular meshwork for lowering IOP and potentially regenerate damaged retinal ganglion cells. However, the potential adverse effects of nanomedicine need to be determined before using them in the treatment.

Acknowledgments

Disclosure

The authors declare that they have no conflicts of interest.

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