

Nanocarriers for treatment of ocular neovascularization in the back of the eye: new vehicles for ophthalmic drug delivery

Pathologic neovascularization of the retina is a major cause of substantial and irreversible loss of vision. Drugs are difficult to deliver to the lesions in the back of the eye and this is a major obstacle for the therapeutics. Current pharmacological approach involves an intravitreal injection of anti-VEGF agents to prevent aberrant growth of blood vessels, but it has limitations including therapeutic efficacy and side-effects associated with systemic exposure and invasive surgery. Nanotechnology provides novel opportunities to overcome the limitations of conventional delivery system to reach the back of the eye through fabrication of nanostructures capable of encapsulating and delivering small molecules. This review article introduces various forms of nanocarrier that can be adopted by ocular drug delivery systems to improve current therapy. The application of nanotechnology in medicine brings new hope for ocular drug delivery in the back of the eye to manage the major causes of blindness associated with ocular neovascularization.

Keywords: drug delivery • eye • nanocarriers • ocular neovascularization

The majority of diseases that cause substantial and irreversible vision-loss result from pathologic ocular neovascularization, such as in wet age-related macular degeneration (AMD), myopic choroidal neovascularization (myopic CNV), diabetic retinopathy (DR), retinopathy of prematurity (ROP), retinal vein occlusion and ocular tumors [1–4]. As neovascularization is the common pathway to blindness in these highly prevalent conditions, the development of cost-effective therapeutics for treatment of pathologic ocular neovascularization to restore and preserve vision are a priority in ophthalmology [2].

Due to the anatomy and physiology of barriers in the eye, the treatment and management of pathologic ocular neovascularization in the back of the eye is a challenging task [3]. Advanced ocular drug delivery systems or vehicles are needed to optimize and control delivery of ocular therapeutics to the target sites either by increasing their penetration or by prolonging contact time. Recent advances in nanotechnology provide novel

opportunities to overcome the limitations of conventional drug delivery systems through the fabrication of nanostructures capable of encapsulating and delivering small molecules. Nanoparticles have small sizes ranging from 1 to 200 nm which can be fabricated bespoke through chemical processes to control the release of therapeutic agents and enhance the penetration through different biological barriers of the eye [3]. For example, particulate nanocarrier-based delivery systems have improved the pharmacokinetic and pharmacodynamic properties of therapeutic agents for the eye [4]. Advancements in material designs and formulations for new nanoparticles have offered exciting possibilities to deliver drugs to the retina [5]. Nanocarrier-based drug delivery system can be employed for treatment of ocular diseases in the posterior segment to overcome the issue of frequent intravitreal injections of large drug molecules such as anti-VEGF agents.

In this review, we provide an overview of various nanomaterials with the potential for

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use in drug delivery to the eye, and the current development of novel nanocarriers for treatment of ocular neovascularization.

Pathological neovascularization in the back of the eye

Pathological neovascularization that occurs in the posterior segment of the eye, which includes the retina and choroid, is a major cause of vision impairment in ocular diseases. The two main types of peri-retinal neovascularization in the posterior segment of the eye are retinal neovascularization (RNV) and CNV. Table 1 summarizes the features and current treatment options for pathological neovascularization in the posterior segment of the eye.

Pathologic RNV

There are several diseases in which closure of retinal vessels occurs including DR, ROP, central or branch retinal vein occlusion and vasculitis. Hypoxia in the retina is the key element of RNV. This form of aberrant vessel growth is characterized by an initial phase of pre-existing vessel loss or blunted vessel development that triggers a subsequent phase of hypoxia-induced neovascularization. Developing and normal microvessels in the retina are vulnerable to small changes in the homeostasis of oxygen, glucose and blood pressure that may lead to focal ischemia in the retina; that is, premature babies and diabetic patients are at risk of developing RNV [6]. Under these circumstances, physiologic wound repair responses are directed at growing collateral vessels to re-establish oxygen supply to the ischaemic region of the retina, but these neovessels are immature and lack endothelial tight junctions. Consequently, they are prone to serious leakage that distorts normal retinal topography and increases the susceptibility of the retina to inflammation [7]. Accumulation

of plasma and innervation of pre-retinal vessels in the vitreous chamber leads to collapse, degeneration and contraction of the vitreous, macular edema and retinal detachment, ultimately compromising vision [8]. Vascular endothelial growth factor, a proangiogenic factor, is well known as a crucial mediator in RNV because of its angiogenic, hyperpermeability and proinflammatory properties [9]. Moreover, VEGF is always found to be significantly higher in ocular tissues and fluids from patients with proliferative retinopathy than in unaffected individuals [10]. Ocular injection of anti-VEGF compounds is a pharmacological treatment available to patients with proliferative DR [11].

Choroidal neovascularization

Rupture and defects in Bruch’s membrane, a complex five-layered extracellular matrix tissue that separates the retinal pigment epithelium (RPE) from the choriocapillaris is the main cause of CNV. The resulting new choroid vessels can grow from the choriocapillaris into the subretinal space and subretinal pigment or form a retinal–choroidal anastomosis that spans between the deep capillary bed of the retina and the photoreceptor layer of the choroid [8,11]. These new and immature choroidal vessels allow leakage of blood or fluid into the subretinal pigment epithelial space that disrupts the photoreceptor layer and distorts vision. Aging tends to compromise the integrity of Bruch’s membrane and is therefore a risk factor for the development of CNV, which is a main feature of neovascular AMD and myopic CNV [12]. It occurs as a result of an accumulation of membrane lipoproteins that can instigate inflammation and remodeling of membrane extracellular matrix protein [12], thereby inducing structural changes in Bruch’s membrane. Proinflammatory and proangiogenic VEGF is also implicated in neovascular AMD [11] and intraocular injections of anti-VEGF

Table 1. Characteristics of pathological neovascularization in the back of the eye.			
Region of ocular neovascularization	Features	Risk factors/associated ocular diseases	Treatment
Retina	Protrusion of preretinal vessels into vitreous	Retinopathy of prematurity, retinal vein occlusion, diabetic proliferative retinopathy	Laser photocoagulation, intraocular injection of steroid and intraocular injection of anti-VEGF agents
Choroid	Sprouting of vessels from the choriocapillaris into the subretinal space and subretinal pigment Formation of a retinal–choroidal anastomosis across the deep capillary bed of the retina and the photoreceptor layer of the choroid	Aging, neovascular age-related macular degeneration and myopic choroidal neovascularization	Intraocular injection of anti-VEGF agents

agents remain the preferred treatment option for neovascular AMD [13], as well as in myopic CNV [14].

Challenges to conventional therapy

The large molecular size of the anti-VEGF agents, which are antibodies and the unique anatomy and physiology of the blood–retina barrier (BRB), restrict access of such drugs to the retina and choroid. Therefore, such drugs need to be administered by an ophthalmic surgeon as an intravitreal injection. Ocular barriers such as vitreous, inner limiting membrane, BRB and anterior clearance pathway are some of the factors that influence the absorption of drugs given intravitreally (review by Thakur *et al.* [15]). The need to perform frequent injection of anti-VEGF agents to maintain therapeutic efficacy also increases the risk of sight threatening complications associated with this invasive mode of drug delivery. Indeed, a monthly rather than a bi-monthly intravitreal injection of anti-VEGF agent like ranibizumab is required to maintain sufficient level of ranibizumab in suppressing vitreal level of VEGF in a group of patients with AMD [16]. The other downside of anti-VEGF therapy is systemic exposure following intravitreal injection [17]. Anti-VEGF agents like bevacizumab and aflibercept were found to decrease plasma level of free VEGF as early as 3 h following intravitreal injection and the inhibitory activity could last for up to 7 days in patients with AMD [17]. The resulting systemic VEGF inhibition may explain the adverse events associated with intravitreal injection of anti-VEGF [17,18]. Some of the challenges of current anti-VEGF therapy are therefore to minimize both the frequency of drug injection and systemic exposure. This may be achieved by prolonging the contact time, improving the penetration and confining the exposure of drug to the lesions. The clinically approved application of intravitreal implants of slow release of glucocorticoids for the treatment of macular edema [19] may be adopted to improve current anti-VEGF therapy. There remain limitations associated with this intravitreal implant as discussed in the following section ‘Potential nanocarriers for ophthalmic drug delivery’. The other way to improve current anti-VEGF therapy may use nanotechnology as a drug carrier to increase drug efficacy by overcoming ocular barriers. Here we introduce the type of nanocarriers available for ocular drug delivery and discuss the advantages and disadvantages associated with potential routes of drug administration for the posterior segment of the eye.

Potential nanocarriers for ophthalmic drug delivery

Nanoparticles are defined as small objects with a diameter of generally less than 100 nm, but can be extended

up to 200 nm. Because of the diverse size range, they offer more options for various applications in areas of biomedicine, food science and water industries. Several different types of nanocarriers have also been investigated for their use in ocular applications. Based on their chemical properties, we have divided the discussion into three sections: lipid-based nanoparticles, polymeric nanoparticles, inorganic nanoparticles and implant devices. Table 2 summarizes the advantages and disadvantages of different types of nanocarriers for ophthalmic drug delivery.

Lipid-based nanoparticles

Amphiphiles such as lipids or surfactants consist of one or more hydrophobic chains and a hydrophilic head group. The amphiphilic nature of some lipids allows them to form different phases such as cubic, hexagonal or lamellar phases. Israelachvili *et al.* [20] and Tanford *et al.* [21] developed a simple method to semiquantitatively study the relationship between single lipid/surfactant molecule shape and their phase change. Briefly, the shape of molecules with the dimensionless packing parameter, P , is defined as: $P = V/la$; where ‘ V ’ is the molecular volume of the hydrophobic region, ‘ l ’ is the hydrophobic chain length and ‘ a ’ is defined as the cross-sectional area of the polar head group. As described in Figure 1, when $P = 1$, the amphiphiles have zero mean curvature and can assemble as a flat bilayer with a lamellar liquid crystalline phase and their dispersions can form vesicles. When $P < 1$, amphiphiles can form oil-in-water self-assembly structures, such as micelles and hexagonal phases. When $P \geq 1$, amphiphiles self-assemble as reverse phase (water-in-oil) and several interesting structures have been observed such as reversed micelles or reversed hexagonal structure [22], inverted bicontinuous cubic phase [23].

Lipid-based nanoparticles have been widely studied for the application as drug carriers in many biomedical areas, such as AIDS therapy [24], cancer treatments [25] and dermatology [26] because they are biocompatible, biodegradable, nontoxic, flexible and nonimmunogenic. Liposomes and micelles have successfully been used as a vehicle for a range of drugs and vaccines. [27–32] However, there remain limitations in the development of liposomes as drug carriers, such as high cost, rapid removal from blood by cells, as well as leakage and fusion of encapsulated drug. Nonetheless, their potential to assist targeted delivery of drugs to the eye has been a point of interest in recent times. Rajala *et al.* investigated the use of liposome–protamine–DNA complex (LPD) nanotechnology in gene delivery via a subretinal route [28]. An artificial virus was created using LPD, modified with a cell permeable peptide and a nuclear localization signaling peptide, to deliver the

Table 2. The advantages and disadvantages of different types of nanocarriers for ophthalmic drug delivery.

Type of particle	Advantages	Disadvantages
Lipid-based nanoparticles	Biocompatible, biodegradable, nontoxic and nonimmunogenic Prolong half-life of drugs High carrying capacity	Can affect vision after intraocular injection High cost Rapid removal from blood and fusion of encapsulated drug
Polymeric nanoparticles	Easy for engineering Prolong half-life of drugs High carrying capacity with multiple drug molecules	Can affect vision after intraocular injection Rapid removal from blood
Inorganic/metal nanoparticles	Safe and low toxic Good tissue penetration High carrying capacity	Nondegradable Rapid uptake by cells or removal from blood Difficult for engineering
Implant device	Controlled release of drug over long periods of time (nondegradable) Can be fabricated into various shapes for injection (degradable)	Requires surgical implantation or injection

retinal pigment epithelium protein 65 (*Rpe65*) gene for treatment of eye disease in mice. It is known that *Rpe65* is the key enzyme that regulates the photochemical, 11-cis-retinal, which allows for our ability to see light. The study found that LPD promoted efficient delivery of the *Rpe65* gene in a cell-specific manner, and facilitated the long-term expression of the *Rpe65* gene in *Rpe65* knockout mice, leading to *in vivo* correction of their blindness.

Polymeric nanoparticles
Micelle-like nanoparticles

Micelles can be formed in water from hydrophobic and hydrophilic layers. They are usually prepared from certain types of copolymers such as amphiphilic diblock (hydrophilic–hydrophobic) or triblock (hydrophilic–hydrophobic–hydrophilic) copolymers which can achieve a very narrow size range containing unique core–shell architecture (Figure 2A). The hydrophobic segments (red color) surround the hydrophilic inner core (black color), separating it from the aqueous exterior, thus allowing for various drugs to be protected in the hydrophilic core. The hydrophobic shell also provides a chance to install active molecules, attached with functional molecules, which can reconstitute the nanocarrier with multiple functions such as specific targeting and controlled release. Moreover, polymeric micelles, in general, show better kinetic stability, greater solubilization capacity and less cytotoxicity than surfactant-based micelles.

Due to their unique structure, micelle-like nanoparticles have been investigated in recent years for their

potential application in ocular medicine. Li *et al.* compared the delivery of diclofenac with rabbit eyes using diclofenac-loaded methoxyPEG)-poly(ε-caprolactone) (MPEG-PCL) micelle formulations versus diclofenac phosphate buffered saline (PBS) solution eye drops [29]. *In vitro* penetration studies across the rabbit cornea demonstrated a 17-fold increase in penetration with the micelle formulations compared with that of the PBS solution eye drops. Moreover, the AUC 0–24 h (mg/l/h) was twofold greater in the diclofenac-loaded MPEG-PCL micelles than the diclofenac PBS solution eye drops. These results suggest that the bioavailability of ocular drugs may be improved through the use of micelle formulations.

Dendrimer-based nanoparticles

Unlike the classical polymers mentioned above, dendrimers are a new class of nanoparticles that are hyperbranched, star-shaped structures, which contain many arms arranged in a highly regular branching pattern, typically symmetrically around a central core (Figure 2B). They also have unique molecular weights and a well-defined number of exterior surfaces with functional surface groups. The controlled multivalence of dendrimers allows for attachment of multiple drug molecules, enabling a high drug payload. Moreover, the functional surface groups can be modified further with targeting groups or solubilizing groups, allowing the enhancement of the dendrimers’ interaction with biological membranes.

Yang *et al.* showed that the hybrid PAMAM dendrimer hydrogel/poly(lactic-co-glycolic acid) (PLGA) formulation enhanced the bioavailability of antiglau-

coma drugs in the cornea of adult male rabbits [30]. There was also sustained effective reduction in intraocular pressure following a single topical administration of the drug-containing dendrimer formulation. Thus, this new drug platform may allow for greatly reduced dosing frequency of conventional topical formulations for the eye.

Inorganic/metal nanoparticles

Gold nanoparticles

Typical gold nanoparticles used for drug delivery contain an inert gold core and an active outer layer. The particle size ranges from 1 to 150 nm with limited dispersity. The active outer layer can conjugate with desired drugs or molecules. The core of gold nanoparticles used for drug delivery is essentially inert and is safe and approved for internal medicine [31]. Through thiol linkages, it is possible to introduce a functional group to bind drugs (Figure 2C). Moreover, their photo-

physical properties can trigger drug release at remote places [32]. The disadvantage of gold nanoparticles in the application of drug delivery is that the gold core is not degradable, and the excretion of these carriers takes a longer time through the cell cycle.

Interestingly, however, intravenously administered gold nanoparticles of a specific size have been shown to pass through the BRB, and can be distributed throughout all the retinal layers without cytotoxicity. Kim *et al.* demonstrated that after intravenous injection of gold nanoparticles into C57Bl/6 mice, 20 nm nanoparticles were found to pass through the BRB and were detected in all the retinal layers [33]. Importantly, the retinal cells containing nanoparticles did not show any structural abnormality or increase in cell death compared with cells without nanoparticles. These findings raise the possibility for the use of small gold nanoparticles (20 nm) as a drug delivery option across the BRB in ocular disease.

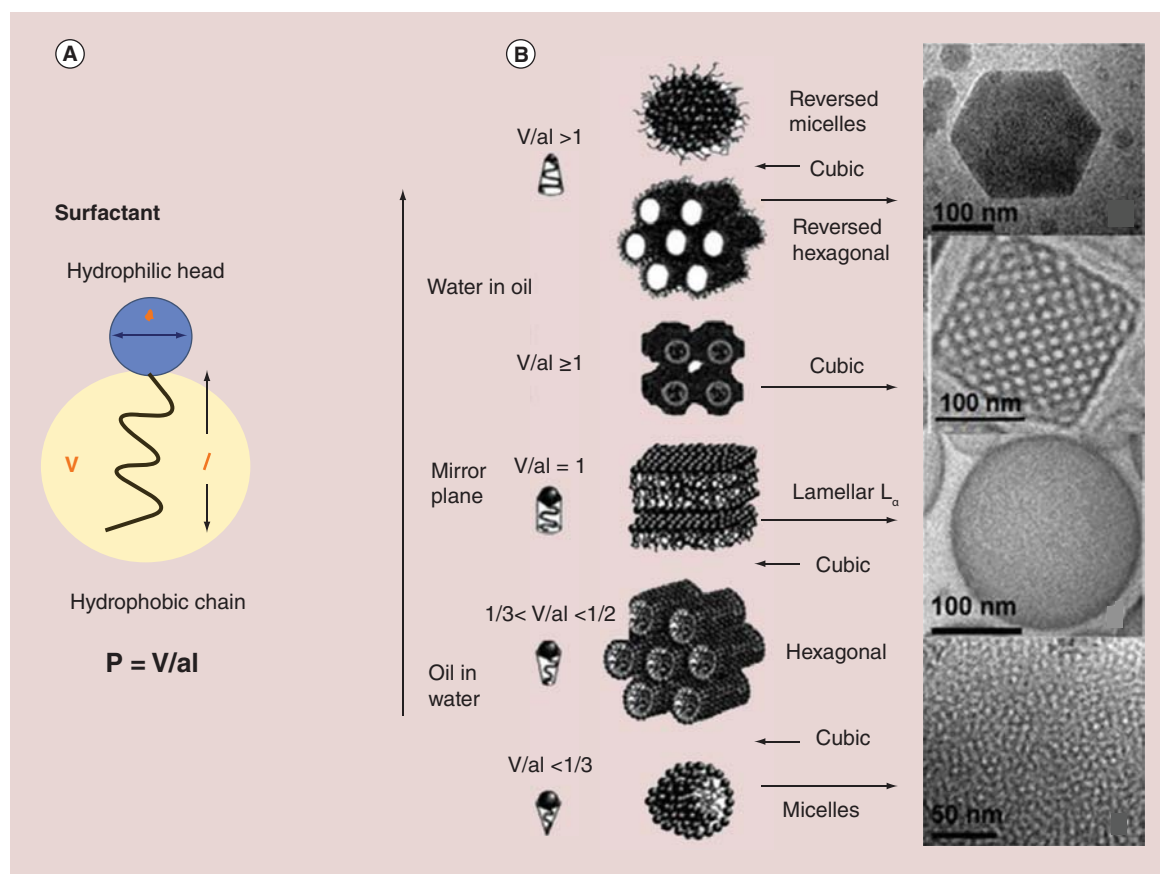


Figure 1. Relationship between single amphiphilic molecule shape and their phases. (A) P is packing parameter which is defined as $P = V/al$. (B) When $P \geq 1$, amphiphiles self-assemble as water-in-oil structure such as reversed micelles, reversed hexagonal structure or inverted bicontinuous cubic phase. When $P = 1$, the amphiphiles can assemble as a flat bilayer with a lamellar liquid crystalline phase. When $P < 1$, amphiphiles can form oil-in-water self-assembly structures, such as micelles and hexagonal phases. $P = V/al$; ' V ' is the molecular volume of the hydrophobic region; ' l ' is the hydrophobic chain length; ' a ' is defined as the cross-sectional area of the polar head group.

For color figures, please see online at www.futuremedicine.com/doi/full/10.2217/NNM.15.47

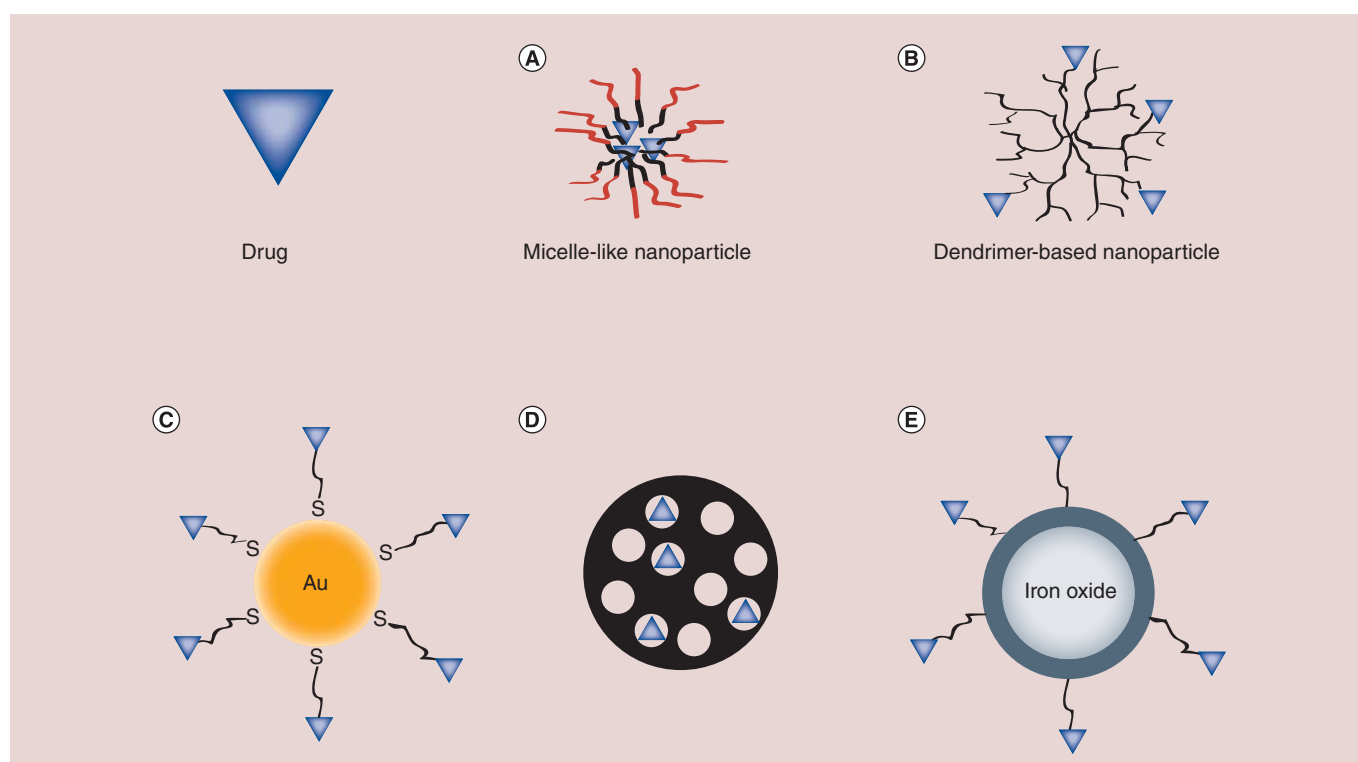


Figure 2. Various formulations of engineered nanoparticles for drugs carriers. (A) Micelle-like nanoparticles. (B) Dendrimer-based nanoparticles. (C) Gold nanoparticles. (D) Mesoporous silica nanoparticles. (E) Magnetic iron oxide nanoparticles.

Mesoporous silica nanoparticles

Mesoporous silica nanoparticles (MSNPs) are perfect substrates to carry biomolecules. The hollow structure of MSNPs results in a high surface area for adsorption of cargo and high pore volume to absorb and encapsulate relatively large amounts of bioactive molecules (Figure 2D). The silica chemical composition, which is biocompatible, facilitates the delivery [34–41]. With various engineering on the surface and inside walls of MSNPs, the nanoparticles can be controlled for multiple functions, such as cell penetration, magnetic targeting [35] and fluorescent visualization [36]. Notably, like gold nanoparticles, silica is not biodegradable and thus, after cellular internalization, MSNPs will stay in the cells for a long time until diluted by cell proliferation.

To date, there have been no documented studies that have investigated the use of MSNPs for ocular applications. However, given their potential to carry a high drug load and to facilitate controlled release of these drugs, more research to evaluate the use of MSNPs in ocular drug delivery may be of value.

Magnetic iron oxide nanoparticles

Magnetic iron oxide nanoparticles are iron oxide particles with the wide size range from 1 to 100 nm. They contain a core–shell structure, where the core is a magnetite (Fe_3O_4) or maghemite ($\gamma\text{Fe}_2\text{O}_3$) and

the shell is generally a layer of polymer or functional groups. Functional groups could be a wide range of molecules such as carboxyl, antibodies, amines, biotin and streptavidin, and such functional groups can be attached via disulfide cross-linkers (Figure 2E) [37–46]. Drug molecules are usually conjugated to the shell of magnetic nanoparticles and then introduced into the body. By means of an external magnetic field, drug-loaded magnetic particles can be concentrated in the therapeutic target area to limit damage to other tissues. Additionally, magnetic iron oxide nanoparticles also have the ability to provide irradiation from radioactive microspheres, and to introduce hyperthermia, providing more potential magnetic treatment options [38]. The main advantages of magnetic iron oxide nanoparticles are that the nanoparticles can be visualized by magnetic resonance imaging, and the drug-loaded nanoparticles can be guided or held in place by means of a magnetic field. However, there are limitations to the application of magnetic iron oxide nanoparticles in drug delivery; first, the magnetic gradient cannot be concentrated in three dimensions, and second, it is a challenge to maintain the magnetic particles' direction and keep them in a targeted organ once the magnetic field is removed from outside [39].

More recently, magnetic iron oxide nanoparticles have also been investigated for its use in magnetically targeting cells to facilitate their delivery to diseased tis-

sue in the retina. Yanai *et al.* magnetized rat mesenchymal stem cells (MSCs) using fluidMAG-D, which is a superparamagnetic iron oxide nanoparticle [40]. *In vitro* studies showed that magnetization of cells with fluidMAG-D was well tolerated, for cells remained viable and retained their ability to differentiate. FluidMAG-D labeled MSCs were then either injected intravitreally or intravenously via the tail vein of the S334ter-4 transgenic rat model of retinal degeneration, with or without placing a gold-plated neodymium disc magnet within the orbit, but outside the eye. The results showed that intravenous injection of the fluidMAG-D labeled MSCs achieved similar retinal localization as the intravitreal injected cells, but was notably associated with a tenfold increase in magnetic MSC delivery to the retina. Moreover, magnetic MSC treatment with orbital magnet resulted in significantly higher retinal concentrations of anti-inflammatory molecules IL-10 and hepatocyte growth factor. This suggests that not only does intravenous MSC therapy deliver a higher drug load to the site of interest; it also produces therapeutically useful biochemical changes in the dystrophic retina. This approach may be of optimal benefit in diseases of the outer retina, such as AMD, where controlled delivery to focal cells is required.

Implant device

Long-lasting and controlled release of drug can be achieved by implants made from polymeric materials [41]. According to the feature of materials, the implants can be classified into two types, biodegradable and nonbiodegradable devices. Nonbiodegradable implants have been shown to have more accurate control of drug release and longer release periods as compared with the biodegradable devices, but surgical removal of the device poses a risk to a patient [42]. Depending on the places of implantation or injection (intraocular or periocular implants), the ocular release of the drug ranges from 5 weeks to 6 months. In clinical application, several types of commercial implants currently are available for treatment of ocular disease, including Ozurdex® (Allergan, Inc., CA, USA; dexamethasone biodegradable implant), Kenalog® (Bristol-Myers Squibb, NJ, USA; triamcinolone acetonide suspension), Trivaris® (Allergan, Inc.; triamcinolone acetonide suspension), Triesence® (Alcon, TX, USA; triamcinolone acetonide suspension), Retisert® (Bausch & Lomb, Inc., NY, USA; fluocinolone acetonide nonbiodegradable implant) and Iluvien® (Alimera Sciences, Inc., GA, USA; fluocinolone acetonide nonbiodegradable implant). These sustained-release systems have showed promise to prolong drug retention for management of ocular diseases such as macular edema.

Routes for nanocarriers-based drug delivery to the back of the eye

Routes for ocular drug delivery to reach the posterior segment of the eye includes topical, systemic (e.g., intravenous), intraocular (e.g., intravitreal) and periocular (e.g., subconjunctival) and are summarized in Figure 3.

Topical drug delivery

Topical drug delivery would be an ideal approach for the treatment of retinal and choroidal neovascularization. Several earlier studies aimed at eye drop delivery showed poor clinical success in the posterior segment due to the presence of physiological ocular barriers and elimination pathways in the cornea. Drug absorption appears to occur through corneal and noncorneal pathways [5]. After topical administration of eye drops, most of the drugs are absorbed across the cornea into the anterior chamber. The noncorneal route of absorption is normally via conjunctiva and sclera. Drug molecules can penetrate through the conjunctiva and trans-scleral pathways, but the BRB remains a major obstacle that limits the availability of drug reaching the lesion. Typically, only a low fraction of drugs reaches the intraocular tissues [43] and this is mostly diluted by blood flow around the conjunctival and nasal mucosa [44]. Although a wide range of nanocarriers have been developed for topical application [45], limitations of drug penetration to deeper sites remain to be improved.

Systemic drug delivery

Targeting drugs to the posterior segments of the eye can be achieved by systemic drug delivery such as intravenous administration, but this is still limited by the BRB. After systemic administration, drugs can penetrate through any leaky vessels into the choroid and diffuse into the posterior chamber. Numerous fenestrations are present in the endothelium of the choriocapillaris resulting in very little resistance to the transport of systemic solutes into the choroid. Only a hydrophilic molecules can penetrate into the posterior chamber through ready access across the BRB [46]. Thus, application of nanocarriers via systemic administration may improve efficacy in the posterior segments by enhancing the biodistribution to the disease target site and potentially minimize dose requirements. Singh *et al.* investigated the inhibitory efficacy of PLGA nanoparticles with surface-engineered transferrin, an RGD peptide (arginine-glycine-aspartic acid) on CNV in a laser-induced model. After systemic administration, these RGD-engineered PLGA nanoparticles were able to penetrate and accumulate in the lasered eye but not in the normal eye [47]. Thus, enhancement of drug tar-

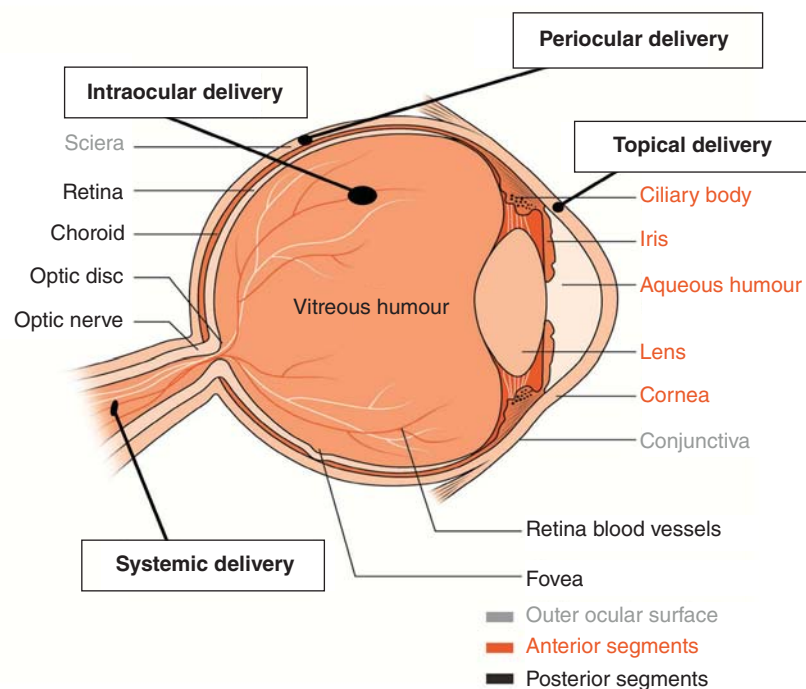


Figure 3. Routes of administration (topical, introcular, periocular and systemic) for delivering of drug to the posterior segment of the eye.

getting to disease sites in the posterior segment can be improved by nanocarriers but systemic drug delivery still poses off-target side-effects.

Intraocular drug delivery

Intraocular drug delivery route by intravitreal injection has a long history in clinical practice. This delivery route was originally employed in the treatment of endophthalmitis and retinitis. Currently, this method is becoming widely used in the treatment of neovascular AMD and macular edema secondary to occlusive vascular disease and diabetes. This approach has a number of disadvantages including the high frequency of injections required (usually monthly), potentially serious side-effects and importantly, they can be tissue destructive [48] as well as can increase intraocular pressure after intravitreal administration [49]. Therefore, although intravitreal injections allow the benefit of delivering drugs close to the site of disease, the potential downsides and risks must also be considered.

Nanocarrier-based drug delivery aims to localize the drug to the target tissues as well as prolong drug release, thereby reducing the need for repeated injections. Behar-Cohen *et al.* showed that polylactide nanoparticles were able to penetrate into the RPE layer after intravitreal administration in rats, and the nanoparticles were detectable at vitreous for 4 months after a single intravitreal injection [50]. Another study

conducted by Kompella *et al.* also shows that polylactide microparticles are retained for more than 3 months after intravitreal injection in rabbit eyes [51]. Therefore, the intraocular route combined with nanocarriers has received considerable attention due to its advantages such as specific targeting and prolonged drug release, and has shown promise for treatment of eye disease in the posterior segments.

Periocular drug delivery

Periocular administration includes subconjunctival, subtenon, retrobulbar, peribulbar and posterior juxta-scleral, is also a potential route for drug delivery [52]. The drugs given by these routes can be delivered to the different layers of the eye, in that order, depending on source concentration and the barrier properties of these and other intermediate layers between this site of administration and target side. Periocular routes of delivery have been noticed as potentially safer alternatives for delivering drugs to the retina, avoiding the risks of intraocular damage posed by intravitreal injection. There is an increased risk of systemic drug exposure, but it is still considerably less when compared with systemic or topical drug delivery. Periocular delivery therefore might be capable of delivering the drug to the desired site in a large concentration while avoiding most of the systemic side-effects, with a relatively long duration of action.

There are still some limitations to the periocular route such as lower bioavailability of the drugs in the retina than by the intravitreal route. Some studies have indicated that the fraction of drug absorbed is reduced by a rapid loss of drug from the periocular sites into systemic circulation [53–64]. To improve the penetration of drug into intraocular tissues, various absorption enhancement approaches with nanocarriers have been attempted. Amrite and Kompella *et al.* [54] found that subconjunctivally administered 200-nm and larger particles (negatively charged carboxylated fluorescent polystyrene) can be almost completely retained at the site of administration for at least 2 months, whereas 20-nm particles were cleared rapidly via the circulation after 7 days. Therefore, larger particles are considered as more appropriate drug carriers for sustained delivery by the subconjunctival route in compared with smaller particles.

Applications of nanocarriers for the treatment of pathological neovascularization in the back of the eye

Topical administration would seem as an ideal route to deliver drugs to the back of the eye, but less than 5% of administered drug enters the eye when given topically. The low penetration of ophthalmic formulation is largely due to anatomic barriers in the human cornea [55]. The incorporation of drugs into nanoparticles provides opportunities to overcome the limitations of conventional eye drops. Most of the nanoparticles that have been developed for drug delivery target ocular sites. For specific ocular neovascularization, a few types of nanoparticles commonly used for drug delivery such as gold nanoparticles [56,57], silica nanoparticles and silver nanoparticles [58–60] have been shown to act as inhibitors of neovascularization even without carrying drugs (Table 3). Therefore these particles might synergise with the activity of antiangiogenic compound when they are incorporated together. Other nanoparticles such as PLGA [47] may improve the mobility of drug across physical barriers in the eye to improve the penetration of drug to reach the lesions.

Gold nanoparticles

Kim *et al.* injected gold nanoparticles into a mouse model of ROP. This study shows that RNV was significantly inhibited by gold nanoparticles via an intravitreal injection [56]. Furthermore, gold nanoparticles effectively suppressed VEGF-induced *in vitro* angiogenesis of retinal microvascular endothelial cells by limiting the angiogenic activity. Gold nanoparticles also blocked VEGF-induced autophosphorylation of VEGFR-2 to inhibit activation of extracellular regulated protein kinases 1/2. This has been further con-

firmed by Cho *et al.* [57] that gold nanoparticles can inhibit the extracellular signal-regulated kinase in a mouse model of ocular neovascularization.

Silica nanoparticles

Silica nanoparticles also show the same effect as gold nanoparticles that inhibit VEGF-induced RNV and block ERK 1/2 activation via regulation of VEGFR-2 phosphorylation [61]. Silica nanoparticles demonstrated no toxic effect on retinal endothelial cells by histological analysis in mice subjected to oxygen-induced retinopathy. Furthermore, silica nanoparticles can effectively increase the penetration into the cornea and provide further access to vitreous area due to their small size (5–50 nm). In addition, silica nanoparticles are a promising carrier to be used for topical administration to treat CNV. Despite these advantages, biodegradation and biodistribution of silica nanoparticles have to be addressed in their clinical applications.

Silver nanoparticles

Silver nanoparticles were found to affect the cellular functions of VEGF [58]. The proliferation and migration of bovine retinal endothelial cells (BRECs) in the presence of VEGF were blocked by treatment with silver nanoparticles. In BRECs, the Akt phosphorylation were blocked by treatment of silver nanoparticles which enhanced the activity of caspase-3 and formation of DNA ladders. The results suggest that silver nanoparticles induced apoptosis in BRECs and blocked cell survival via PI3K/Akt dependent pathway. Silver nanoparticles have been suggested a cost economic alternative for retinal therapies. However, the smaller size of silver nanoparticles will need to be modified to enhance the penetration of ocular tissue for targeted drug delivery.

Engineered nanoparticles

Several engineered nanoparticles have been developed to deliver drugs for ocular neovascularization treatments in the eye. These include liposomes [16,62], micelles [63–76] and polymeric nanoparticles [64–82] which are described in Table 4. The most popular nanocarrier is the polymeric nanoparticle. Particularly, PLGA-based block copolymer is one of the most attractive candidates for ocular drug delivery. This is because PLGA is approved by US FDA as a clinically applicable material and is completely degradable, biocompatible, nontoxic and easy to be engineered with various types of drugs. It also offers protection of drug from degradation and the possibility of sustained release. PLGA-based copolymers are amphiphilic, which allows them to self-assemble with a hydrophobic core and a hydrophilic corona structure in aqueous environments as

Table 3. Inhibition of ocular neovascularization by nanoparticles.

Study (year)	Type of particles	Type of NV	Mechanism	Ref.
Kim JH <i>et al.</i> (2011)	Gold nanoparticles	RNV	Inhibit VEGF-induced autophosphorylation of VEGFR-2 and Src phosphorylation	[56]
Cho WK <i>et al.</i> (2015)	Gold nanoparticles	RNV	Inhibit VEGF-2 receptor and ERK	[57]
Jo DH <i>et al.</i> (2012)	Silica nanoparticles	RNV	Inhibit VEGF-induced autophosphorylation of VEGFR-2 and ERK 1/2 activation	[61]
Kalishwaralal K <i>et al.</i> (2009)	Silver nanoparticles	RNV	Inhibit VEGF-induced Src and AKT/PI3K pathway	[58]
Curunathan S <i>et al.</i> (2009)	Silver nanoparticles	RNV	Inhibit VEGF-induced AKT/PI3K pathway	[60]
Kalishwaralal K <i>et al.</i> (2010)	Silver nanoparticles	RNV	Inhibit VEGF-induced Src and AKT/PI3K pathway	[59]

RNV: Retinal neovascularization.

described in [Figure 4](#). The hydrophobic internal core is capable of incorporating hydrophobic drugs allowing for improved loading of water insoluble drugs and the hydrophilic shell serves as a membrane which aids in controlling the release of drugs.

Plasmid DNA has been successfully encapsulated into biodegradable PLGA nanoparticles to be used for gene delivery in many applications. Park *et al.* demonstrated that a natural angiogenic inhibitor, plasminogen kringle 5 (K5), was encapsulated with PLGA polymer forming K5 nanoparticles and these had no effect on retinal structure and function [64,70]. Such engineered K5 nanoparticles allowed high level of expression of K5 in the rat retina as determined by western blot analysis and immunohistochemistry. An intravitreal injection of K5 nanoparticles reduced retinal leakage and retinal expression of inflammation factors in diabetic rats and K5 also inhibited retinal neovascularisation in rats with oxygen-induced retinopathy.

Xu *et al.* investigated the inhibitory efficacy of intravitreally injected PLGA nanoparticles loaded with steroids (dexamethasone acetate) in laser-induced CNV model [69]. By using solvent evaporation techniques, an approximate 52% of dexamethasone acetate was encapsulated into PLGA nanoparticles to form an oil-in-water emulsion. An intravitreal injection of PLGA-dexamethasone acetate emulsions to laser-injured eye did not induce retinal toxicity. The release of dexamethasone acetate in the vitreous was then measured by LC in combination with fluorescein angiography, a fundamental imaging technique in the eye, to evaluate the incidence of CNV at 14 and 56 days after laser photocoagulation. The results suggested that PLGA-dexamethasone acetate emulsions can inhibit the development of experimental CNV at

a concentration-dependent manner. LC demonstrates that 50% of dexamethasone acetate was released from the PLGA-dexamethasone acetate emulsions after 14 days of the single injection and this is followed by a constant and sustained release of the remaining dexamethasone over 40 days. Interestingly, an inhibitory effect of dexamethasone acetate was sustained for more than 2 months even dexamethasone acetate could no longer be detected. Overall, the release of dexamethasone from an intravitreal injection of the PLGA-dexamethasone acetate complex in the eyes with CNV revealed a triphasic pattern, consisting of an initial burst (1–3 days), a log phase with relatively permanent release rate (3–21 days) and final burst phase (21–56 days).

Conclusion & future perspective

Eye disease in the posterior segment such as neovascular AMD and proliferative DR are the leading causes of substantial and irreversible vision loss resulting from pathological ocular neovascularization. Frequent intravitreal injection of anti-VEGF agents, the current standard treatment for sealing off leaky blood vessels or preventing neovascularization, is an invasive intraocular procedure with the attendant risks of infection, retinal detachment, cataract and inflammation with the risk of permanent vision loss. These limitations impose considerable costs on patient quality of life and have an enormous economic impact on the health care system. The advent of nanocarriers provides opportunities to overcome the limitations of barriers *in vivo*, and to reduce the risk of severe complications that can enhance bioactivity and prolong bioavailability of therapeutic agents in the retina. Most of the nanocarriers that have been developed for drug delivery can potentially be used in the eye. Liposomes have been

Table 4. Summary of engineered nanocarriers to treat pathological neovascularization in the eye.

Study (year)	Type of nanocarriers	Drug/gene	Type of NV	Ref.
Gross N <i>et al.</i> (2013)	Liposomes (cationic)	Paclitaxel and verteporfin	CNV	[62]
Kim H <i>et al.</i> (2012)	Micelles	Flt1 peptide	RNV	[63]
Iriyama A <i>et al.</i> (2011)	Micelles	Plasmid DNA	CNV	[65]
Di Tommaso C <i>et al.</i> (2012)	Micelles	Cyclosporin A	CNV	[66]
Shmueli RB <i>et al.</i> (2013)	Polymeric nanoparticles	Serpin-derived peptide	CNV	[67]
Iwase T <i>et al.</i> (2013)	Polymeric nanoparticles	Doxorubicin and daunorubicin	CNV and RNV	[68]
Xu J <i>et al.</i> (2007)	Polymeric nanoparticles	Dexamethasone acetate	CNV	[69]
Park K <i>et al.</i> (2009)	Polymeric nanoparticles	Plasminogen kringle 5 plasmid	RNV	[64]
Luo L <i>et al.</i> (2013)	Polymeric nanoparticles	Flt23k plasmid	CNV	[70]
Wang Z <i>et al.</i> (2015)	Polymeric nanoparticles	Low-density lipoprotein receptor extracellular domain	CNV	[71]

CNV: Choroidal neovascularization; RNV: Retinal neovascularization.

routinely explored in ocular drug delivery but they are of relatively high cost and are rapidly removed from blood by cells. Inorganic and metal nanoparticles are usually not biodegradable and the excretion of these carriers will rely on the cell cycle, which results in increased lengths of time for the carriers to be removed from eyes. Polymeric nanoparticles are currently promising candidates for ocular drug delivery because they are completely degradable, nontoxic and easy to be functionalized with various types of drugs: PLGA-based polymers especially have been successfully applied in ocular drug delivery systems. Future

development of nanoparticles focuses on improving the target specificity and longer duration of use with minimal adverse side-effects. Nanotechnology introduced into pharmaceuticals provides new approaches for treatment of posterior segment eye diseases with enhanced benefits and minimal risks.

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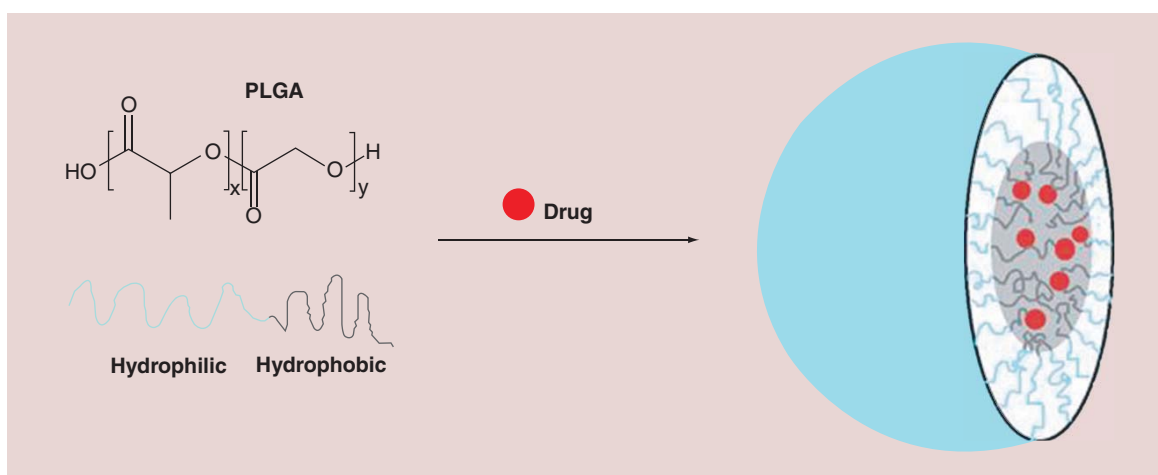


Figure 4. The poly(lactic-co-glycolic acid)-based core-shell nanoparticles are formed through self-association of hydrophilic and hydrophobic block copolymers. The hydrophobic internal core is capable of incorporating hydrophobic molecules, making it a candidate for drug delivery carrier applications for taking in drugs with poor water solubility. PLGA: Poly(lactic-co-glycolic acid).

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Executive summary

Pathological neovascularization in the back of the eye

- Posterior segment eye diseases (the back of the eye) such as age-related macular degeneration, diabetic retinopathy are the leading causes of substantial and irreversible vision loss resulting from pathologic ocular neovascularization.
- Treatment of pathologic ocular neovascularization in posterior segment of the eye is a challenging task due to the anatomy and physiology of ocular barriers.
- Current treatments for pathological neovascularization in posterior segment of the eye suffer from significant disadvantages including the side-effects related to the frequent injections.

Nanocarriers for drug delivery applications for management of pathological neovascularization in the back of the eye

- The key routes for nanocarriers-based drug delivery into the posterior segment of the eye include topical, systemic, periocular, intraocular and impale routes.
- Nanoparticles that have been developed for drug delivery can be applied to ocular drug delivery. Some types of particles such as gold, silver and silicate nanoparticles can act as inhibitors of neovascularization even without carrying the drugs.

Conclusion & future perspective

- Nanocarriers provide novel opportunities to overcome the limitations of barriers *in vivo* and to reduce the risk of severe complications that enhance bioactivity and prolong bioavailability of therapeutic agents in the posterior segment of the eye.
- Polymeric nanoparticles are currently a promising candidate for ocular drug delivery because they are completely degradable, nontoxic and easy to be functionalized with various types of drugs.

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