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*Teaser Recent findings regarding utilization of intravitreally injected nanoparticles for the retinal-targeted delivery of all types of therapeutics are summarized. The respective pharmacokinetic model for intravitreal nanoparticles was also developed.*



# Intravitreal nanoparticles for retinal delivery

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**Intravitreal injection is one of the major administration routes for the treatment of posterior ocular diseases. Intravitreal therapeutics usually suffer from unsatisfactory efficacy owing to fast clearance from the vitreous humour and insufficient distribution into the retina. Engineered nanoparticles have been applied for specific tissue targeting over the past decades. In this review, we summarize the most recent research utilizing intravitreal nanoparticles to deliver therapeutics to the retina. Herein, the achievement made in preclinical research and challenges remaining in the field are highlighted. Parameters including size, charge, stability and choice of modified ligand on intraocular distribution and transport are also systematically discussed based on a proposed pharmacokinetic model. We provide insights for rational design principles for intravitreal nanoparticles for targeted retinal delivery.**

## Introduction

The retina lies in the inner layer of the eye and is responsible for light transmission. The distortion and malfunction of the retina can lead to temporary or even irreversible vision loss, which makes the retina a drug target for multiple ocular diseases [1], such as glaucoma, age-related macular degeneration (AMD) and diabetic retinopathy (DR). Although eyedrops remain the most widely used administration route for various ocular conditions because of the ease of use for topical formulations, the efficiency of drug delivery to the retina via this route is poor because of anatomical and dynamic barriers, which have been extensively reviewed [2,3]. The most direct and relatively safe method for retinal delivery is by intravitreal injection. With limited space but adjacent to the retina, injection into the vitreous humour has been considered as the most efficient administration route with multiple advantages such as increased drug concentration in the neural retina and decreased systemic side-effects [4,5]. Despite the advantages, the fast depletion of drug in the vitreous usually necessitates frequent injections to maintain the concentration within the therapeutic window. Higher risks of cataract formation, endophthalmitis, retinal detachment and vitreous hemorrhage are associated with frequent intravitreal injections. Hence, drug delivery systems such as hydrogels and implants have been designed for

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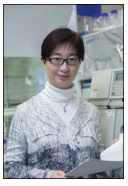

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improving residence times of drugs [6]. Other than temporal control, nanodrug delivery systems have been investigated for improving retinal penetration and achieving targeted intracellular delivery [7].

Nanoparticles used for drug delivery are colloidal systems with a size typically ranging from 20 to 500 nm. Nanoparticles are considered smart drug vehicles, because of their tunable stability, controllable release and programmable targeting. At least two nanoparticles have undergone clinical trials for treating ocular diseases. One is to treat metastatic melanoma of the eye, which cannot be removed by surgery, through intravenous injection of paclitaxel albumin-stabilized nanoparticles. (ClinicalTrials.gov identifier: NCT00738361). Another example is an eyedrop formulation containing dexamethasone-cyclodextrin via topical administration for treating diabetic macular edema (ClinicalTrials.gov identifier: NCT01523314). For the administration route of intravitreal injection, microparticles consisting of triamcinolone acetonide were investigated for the treatment of diabetic retinopathy (ClinicalTrials.gov identifier: NCT00407849). To our best knowledge, there has been no clinical trial to assess the use of intravitreal nanoparticles for treating posterior diseases, despite numerous reports of fundamental studies and preclinical investigations.

Intravitreally injected nanocarriers have been applied for retinal targeted delivery of small molecules [8], peptides [9], proteins [10,11] and nucleic acids [12]. Because the eye is relatively isolated from the blood circulation, targeting the retina with nanoparticles through intravitreal injection remains challenging. Generally, there are several main barriers including the vitreous, inner limiting membrane (ILM) and the retinal interaction including the extracellular binding and the off-target cellular internalization for intravitreal nanoparticles to cross before reaching the target cells. To cross these barriers, materials including polymers, proteins and lipids have been developed and formulated into nanovectors for delivering different drugs to treat various diseases of the retina. Nevertheless, to advance the field further, increasing the targeting efficiency to specific retinal tissues is important. Thus, it is beneficial to clearly outline the underlying rationale for engineering intravitreal nanoparticles for optimal ocular distribution. In this review, we summarize the most recent applications of retinal delivery via intravitreally injected nanoparticles and discuss the parameters of nanoparticles that affect the intraocular distribution and retinal localization. We plan to use a simplified pharmacokinetic model to provide insights into the design of smart nanoparticles with superior retinal delivery efficiency.

## Barriers to retinal targeting of intravitreal nanoparticles

Drug delivery to the retina remains challenging owing to the complicated anatomical and physiological barriers of the eye. Depending on the administration route, there are different barriers for the drug or drug delivery systems to overcome. For topical delivery, the corneal epithelium and long transport path from the administration site to the retinal tissues contribute as barriers for posterior segment targeting. These barriers can be overcome readily by direct injection to deposit the drug or drug carriers in the vitreous. Although in closer proximity with the retina, this route is not without challenges. For intravitreal injection, the vitreous, ILM and cellular binding or internalization and extracellular matrix account for the main biological barriers for efficient and targeted retinal delivery [13]. Drug molecules first need to cross the vitreous. The vitreous consists of water (98%), collagen, hyaluronan, proteoglycans, chondroitin sulfate and heparan sulfate [14]. It is responsible for protecting the lens from exposure to oxygen, preventing the retina from detachment and stabilizing fluid flow [15]. In the form of a gel, the vitreous prohibits nanodrug carriers from rapid diffusion to the retina. The diffusion coefficient of nanoparticles in the vitreous is influenced by their size [16]. The vitreous has a net negative charge, so the diffusion of nanoparticles is also affected by their surface charge [17]. Other than size and surface charge, the material properties contributing to colloidal stability and nonspecific interactions (e.g., binding with the protein and polysaccharide components of the vitreous) can alter the diffusion based on opposing retinal distribution generated from different materials [18,19]. Between the vitreous and retina, the ILM forms another barrier. The ILM is a basement membrane that consists of collagen fibers, proteoglycans, a plasma membrane of Müller cells and other retinal glial cells [20–23]. To date, the cut-off size for exogenous materials that can cross the ILM remains ambiguous, partly because of the interspecies variation [20,24]. For nanovectors, the carrier itself is clearly larger than the biomolecules (e.g., proteins), incapable therefore of penetrating the ILM. However, successful transport of nanoparticles across the ILM and the subsequent localization to the retina have been demonstrated in a number of reports [19,25,26]. Other factors that prevent a straightforward conclusion come from the use of nanoparticles with different characteristics such as surface charge and material composition, as well as the possibility of different transport mechanisms [17–19,26]. It was suggested that the ILM can be disrupted by enzymatic digestion. However, the need for this practice requires careful consideration, because different types of nanoparticles can interact with the ILM differently [27]. In other words, the ILM is only a significant barrier for some nanoparticles but not others.

In addition to the anatomical barriers, the convective flow in the eye poses a dynamic barrier for intravitreal nanoparticles. The aqueous flow generated from the ciliary body exits through the trabecular meshwork [28]. The bulk flow is expected to direct the unbound nanoparticles along the flow path toward the drainage system (Fig. 1). This flow away from the retina leads to elimination from the intraocular space and is a dynamic barrier for retinal targeting. Another convective flow mechanism in the vitreous toward the retina (Fig. 1) is caused by the hydraulic pressure gradient [29]. Compared with the aqueous outflow (2.2 μl/min in rabbits), this vitreous outflow is much slower (0.1 μl/min in rabbits) [30]. The effect of the vitreous outflow on intraocular transport and retinal distribution is thus more substantial for slow-diffusing nanoparticles [31].

The retina is composed of multiple cell layers. The malfunction of specific cell layers is related to the progression of specific ocular diseases. For example, the apoptosis of retinal ganglion cells (RGCs) is closely related to glaucoma; as such, therapeutics providing neuroprotection targeted to the RGCs offer a potential treatment for glaucoma [32]. Thus, the ability to target drugs to specific cells can improve the treatment efficacy, especially for drug molecules with intracellular targets. When transported into the retina, nonspecific uptake or binding will create a detour or

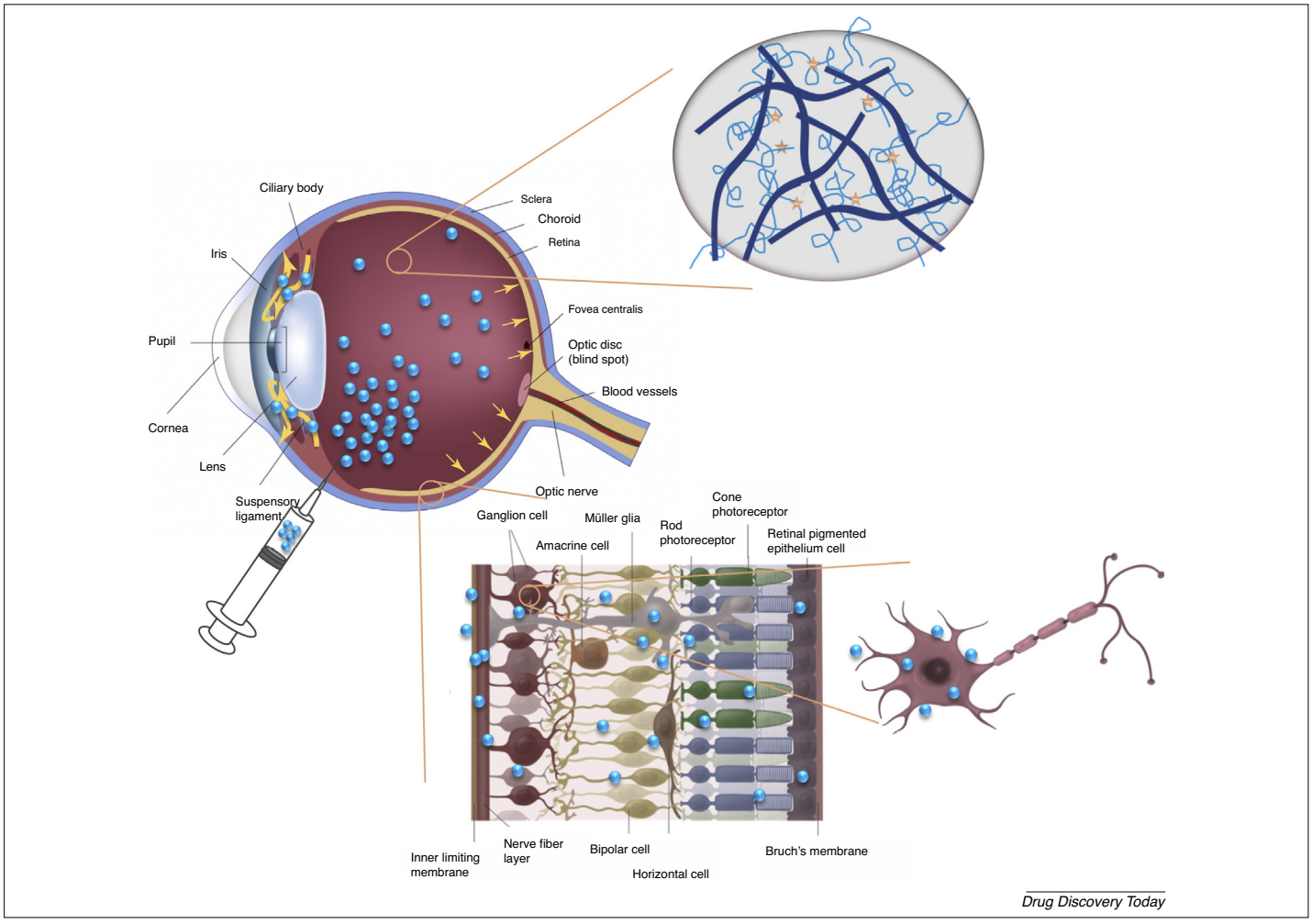


**FIGURE 1**

Schematic illustration of the barriers to efficient retinal delivery after intravitreal nanoparticle injection. Adapted, with permission, from [103].

even prevent the nanoparticles from reaching their cellular target. For example, whereas uptake of lipid nanoparticles by the inner retina is beneficial if RGCs are the target cells, the same characteristics will become disadvantageous if the outer retina [e.g., photoreceptor (PR) layer] is the target. Thus, interaction and internalization with off-target cells can jeopardize the intracellular delivery efficiency for intravitreal nanoparticles.

## Current status of intravitreal nanoparticles for retinal delivery

Intravitreal nanoparticles have been widely applied to deliver different types of therapeutics for treating different ocular conditions including inflammation, IOP elevation, angiogenesis and neurodegeneration [32–35]. Owing to the variation of physical and chemical properties of the drug molecules as well as different molecular targets and pharmacokinetics, the roles of the nanocarriers are diverse, from prolonging the residence time, protecting the drugs from enzymatic degradation to enhancing cellular uptake and intracellular trafficking. Herein, we summarize and discuss the current status of research regarding the application of retinal delivery via intravitreal nanoparticles with an overview given in Table 1.

### *Small molecules*

Many therapeutics used for treating posterior ocular diseases are small molecules. For example, dexamethasone is one of the most widely used anti-inflammatory drugs, whereas brimonidine has been utilized as an antiglaucoma drug for the purpose of IOP-lowering and neuroprotection. Because a great number of drugs including corticosteroids, angiogenetic inhibitors and IOP-lowering agents possess poor water solubility, one delivery strategy is the formation of a depot by allowing the drug molecules to aggregate in the vitreous after injection [36]. However, this method can result in adverse effects such as visual disturbance, unequal distribution and local retinal toxicity [37]. Nanoparticles can circumvent these disadvantages by encapsulating the drugs to achieve drug stability while avoiding unwanted aggregation [38]. Free drug molecules can be rapidly cleared from the vitreous either from the anterior pathway or posterior pathways owing to the smaller molecular size. The intravitreal half-life of small molecules ranges from ~1–30 h based on different molecular sizes and water solubility [39]. The half-life of intravitreally injected sodium fluorescein is ~2 h in rabbits [40]. Therefore, frequent injection is needed to maintain the concentration within the therapeutic window. However, repeated intravitreal injection commonly leads to dis-

**TABLE 1**

**Summary of current intravitreal nanoparticles application for retinal delivery of therapeutics**

| Materials | Characteristics | | | | Drug | Major observation | Refs |
|---|---|---|---|---|---|---|---|
| | *Size (nm)* | *Charge (mV)* | *EE (%)* | *Ligand* | | | |
| HSA-PEG | 267.5 | | 77.1 | | Apatinib | Significantly inhibited hyperpermeability at the cellular level; substantially reduced retinal vascular leakage in STZ-induced-diabetic mice | [38] |
| mPEG-PCL | 40 | −0.89 | | | Rapamycin | Micelles located in RPE layers for 14 days, with significant higher retinal drug concentration; abolished EAU detected at optimal concentration of rapamycin-loaded micelles. Therapeutic effects manifested locally instead of systemically | [48] |
| HSA | 152.8 | −29.7 | | | Brimonidine | Brimonidine-loaded HSA NPs significantly improved RGC survival and showed better protection effects than brimonidine solution at 14 days | [50] |
| HSPC, cholesterol, DSPE-PEG | 112.6 | −2.73 | 95 | YSA peptide | Doxorubicin | Enhanced uptake of DOX-loaded liposomes in ARPE-19 cells by conjugated YSA peptide; significant reduction in rat CNV area with no obvious retinal toxicity | [52] |
| Poly(γ-glutamic acid), L-phenylalanine | 180 | −25 | | | Dexamethasone | NPs accumulated in active microglia in a model of NMDA-damaged rat eyes; efficient suppression of RGC death and retinal detachment were observed after DEX-NPs injection | [49] |
| PLGA | 232 | | 56 | | Dexamethasone | Sustained DEX release in the vitreous for ~50 days with high constant vitreous concentrations for 30 days. Significant higher bioavailability of DEX-loaded NPs than the regular DEX solution | [8] |
| PLGA | 232.5 | | 52 | | Dexamethasone acetate | DA-loaded PLGA NPs resulted in a significant reduction of the CNV area compared with the control group at 14 days post injection. Dose-dependent effect observed in suppressing CNV at day 56 | [104] |
| PEG-PHDCA | 112 | | | | Tamoxifen | Significant and prolonged effect on treating EAU up to 3–9 days | [105] |
| DOPC, cholesterol, DOPG | <220 | | | | Ganciclovir | Liposomal ganciclovir formulation significantly protected the rabbit retina of HSV-1 induced retinitis compared to the regular ganciclovir solution or blank liposomes with no indication of ocular toxicity other than higher-dose triggered cataracts | [106] |
| HSA | 252.7 | −43.97 | | HA | Connexin43 mimetic peptide | Specifically targeted CD44-expressing retinal cells with prolonged retention and sustained release of the peptide, providing improved therapeutic effects of suppressing inflammation and preventing RGC loss | [25] |
| PLGA | | | | | Connexin43 mimetic peptide | NPs observed to target the RGC and choroid layer 30 min post injection; improved light sensitivity compared with saline-injected group, with preventing the loss of the photoreceptor as well as the suppressing inflammation | [107] |
| PLGA | 90 | | 80 | | Bevacizumab | NPs with bevacizumab encapsulated enhanced the anti-angiogenic efficiency for corneal and retinal neovascularization treatment with decreasing tissue toxicity *in vivo* | [108] |
| Albumin | 252.7 | −43.97 | | HA | Connexin43 mimetic peptide | With HA-coating, the targeting effect to the CD44 expressing cells in the neural retina and choroid was significantly improved. PLGA NPs also protected the peptide from degradation with prolonged release *in vitro* | [109] |
| Chitosan | 88.9 | 21.63 | | | Bevacizumab | Bevacizumab-chitosan NPs achieved prolonged inhibition of VEGF expression in the retina compared to the bevacizumab solution from 4 to 8 weeks | [110] |

**TABLE 1 (*Continued*)**

| Materials | Characteristics | | | | Drug | Major observation | Refs |
|---|---|---|---|---|---|---|---|
| | *Size (nm)* | *Charge (mV)* | *EE (%)* | *Ligand* | | | |
| PLA/PLA-PEO | 302 | −38.26 | 55.6 | | C16Y peptide | Sustained effects of CNV suppression by C16Y-NPs compared to theC16Y peptide solution with no retinal toxicity; effective penetration of the NPs into the retina and target to the RPE layer | [111] |
| DPPC, EPC, cholesterol | | | 11.1–45.5 | | Bevacizumab | Significantly increased the vitreal residence time of the free bevacizumab with the liposomal formulation | [112] |
| PC, cholesterol | 250–600 | −1.7 | 37 | | Vasoactive intestinal peptide | Significantly reduced ocular inflammation in EIU by VIP-loaded liposomes compared to the VIP free solution. Prolonged protection of the peptide by the liposomes were observed up to 14 days *in vivo* | [113] |
| DOTAP, DOPE | 286.2 | −41.1 | | HA | pDNA | HA modification increased transfection efficiency twofold *in vitro* with no toxicity as well as sixfold in the RPE cell layer in SD rats | [96] |
| DOTAP, DOPE | 95–847 | −20-54 | | HA | pDNA | HA modification improved the intravitreal mobility of the lipoplexes in an *ex vivo* model and improved the transfection efficiency by eightfold in ARPE-19 cells | [114] |
| bPEI | 259.8 | −41.2 | | HA | siRNA | Prolonged therapeutic effects of siVEGF nanoballs up to 2 weeks after intravitreal injection with highly efficient RPE and choroid targeting | [69] |
| bPEI | 260.7 | −4.98 | | | siRNA | Efficient laser-induced CNV inhibition after 1 and 7 days with no toxicity in the retina | [115] |
| DOTAP, DOPE, cholesterol | 80–120 | | | NLS/TAT peptide | pDNA | Intravitreal injection of the liposome protamine/DNA lipoplexes can specifically target and transfect RGCs | [116] |
| Precirol ATO5, DOTAP, Tween 80 Dextran, HA | 230 | 43 | | | pDNA | Higher transfection level was observed in PR and INL layers after HA-SLN injection; DX-SLN and HA-SLN promoted the structural improvement of the retina of Rs1h-deficienct mice significantly 2 weeks after intravitreal injection | [66] |
| Poly-lysine-PEG | | | | | pDNA with miRNA gene encoded | Significantly reduced VEGFR-2 levels and angiogenesis in aged $Ins2^{Akita}$ mice up to 3 months | [117] |
| DOTAP, squalene, Polysorbate 80 | 150 | | | | pDNA | Intravitreal injection of GFP-plasmid niosomes transfected through the inner layers of the retina and protein expression persisted for at least 1 month after the injection | [84] |
| 12-7NH-12, DOPE, DPPC | 150–180 | | | | Cy5-DNA | Intravitreally injected nanoparticles located in the NFL 4 h after injection | [118] |
| DOTAP squalene, Polysorbate 80 | 200 | 25 | | | pDNA | Niosomes protected the DNA from enzymatic digestion, resulting in successfully transfected HEK-293 and APRE-19 cells with no cytotoxicity. Intravitreal injection of the niosomes led to broad surface transfection in the inner layers of the retina | [67] |
| Chitosan | 256.5–67.3 | 10 | | | pDNA | Intravitreal injection of the nanoparticles transfected cells in the INL and IPL, but mainly in the RGC layer | [119] |
| DOTAP, cholesterol, PEG-DSPE | 131.9 | 19.73 | 95 | | siRNA | Nanoparticles facilitated the inhibition VEGFR1 expression in ARPE-19 cells, whereas intravitreal injection of the siRNA-loaded lipid nanoparticles reduced CNV area effectively with low toxicity | [120] |
| PLGA | | | | | pDNA, shRNA | GFP expression preferentially occurred after a single intravitreal injection of pDNA-loaded PLGA nanoparticles with persisting effects for 4 weeks; with the injection of pshHIF-1α nanoparticles, the mean thickness of the CNV lesions was significantly reduced with no signs of retinal malfunction | [121] |

**TABLE 1 (*Continued*)**

| Materials | Characteristics | | | | Drug | Major observation | Refs |
|---|---|---|---|---|---|---|---|
| | *Size (nm)* | *Charge (mV)* | *EE (%)* | *Ligand* | | | |
| PLGA | | | | | pDNA | Intravitreal injection of K5-plasmid nanoparticles resulted in high-level expression of K5 in the inner retina up to 4 weeks post injection, leading to significant reduction of retinal vascular leakage and retinal neovascularization in streptozotocin-induced diabetic rats with no obvious signs of the tissue toxicity | [122] |
| DOTMA, cholesterol, DOPE | | | | | pDNA | With plasmid DNA of 80 **μg**, the liposomes showed the highest transfection efficiency of luciferase activity in various ocular tissues and peaked at 3 days post injection | [123] |
| PC, cholesterol, PEG-DSPE | 150 | | | | Oligonucleotides | After intravitreal injection of liposome-encapsulated oligonucleotides, sustained release was observed in various tissues including the vitreous and retina-choroid, indicating that liposomes offered a protective effect for the oligonucleotides against enzymatic degradation | [124] |

Abbreviations: HSAhuman serum albumin; PEGpolyethylene glycol; mPEG-PCLmonomethoxy poly(ethylene glycol)-poly (ε-caprolactone); HSPCl-**α**-phosphatidylcholinehydrogenated (Soy); DSPE-PEG1,2-distearoyl-sn-glycero-3-phosphoethanolamine-*N*- [amino(polyethylene glycol)-2000]; PLGApoly(lactide-co-glycolide); PEG-PHDCApoly(methoxy poly(ethylene glycol) cyanoacrylate-co-hexadecyl cyanoacrylate]; DOPC1,2-dioleoyl-sn-glycero-3-phosphocholine; DOPG1,2-dioleoyl-sn-glycero-3-phospho-(1′-rac-glycerol); PLApoly-lactic-acid; PLA-PEOpoly-lactic-acid–polyethylene oxide; DPPC1,2-dipalmitoyl-sn-glycero-3-phosphocholine; EPCegg phosphatidylcholine; DOTAP1,2-dioleoyl-3-trimethylammonium-propane; DOPE1,2-dioleoyl-sn-glycero-3-phosphoethanolamine; bPEIbranched polyethylenimine; HAhyaluronic acid; VEGFvascular endothelial growth factor; DOTMA1,2-di-O-octadecenyl-3-trimethylammonium propane; CNVchoroidal neovascularization; siRNAsmall interfering RNA; pDNAplasmid DNA; SLNsolid lipid nanoparticles; NPsnanoparticles; DEXdexamethasone; DAdexamethasone acetate; VIPvasoactive intestinal peptide; EAUexperimental autoimmune uveitis; INLinner nuclear layer; IPLinner plexiform layer; RGCretinal ganglion cell; RPEretinal pigment epithelium; EIUendotoxin-induced uveitis; SDSprague–Dawley; STZstreptozotocin.

satisfactory patient compliance and increases the risks of endophthalmitis, vitreous hemorrhage, retinal detachment and cataract formation. Thus, one of the main roles for intravitreal nanoparticles is to increase the residence time in the eye. Compared with microparticles and hydrogels, the duration of release provided by nanoparticles is shorter because of the smaller dimensions. They are thus more useful for prolonged release in specific tissues in the retina rather than in the vitreous. Moreover, another important advantage of using intravitreal nanoparticles for delivering small molecules is to facilitate targeted delivery of the drug to a specific location in the retina. This design can potentially improve the therapeutic efficacy, because many small molecules possess drug targets on the cell membrane or in the cytoplasm [41,42]. Table 1 summarizes the application of nanoparticles for intravitreal delivery of small-molecule drugs. Some specific examples will be discussed further below.

As shown in Table 1, polymeric materials play a significant part in delivering small molecules via intravitreal nanoparticles. Polymers used possess multiple advantages, including good biocompatibility, chemical stability, tunable degradability and flexibility for engineering. One of the most commonly used materials, poly (lactic-co-glycolic acid) (PLGA) was approved by the FDA in 1984 and has been explored widely for controlled drug release purposes [43]. Zhang *et al.* studied the pharmacokinetics and tolerance of intravitreally injected dexamethasone (DEX)-loaded PLGA nanoparticles in rabbits [8]. With an average size of 232 nm, the PLGA nanoparticles provided sustained release of DEX for ~50 days in the vitreous with no abnormalities based on vitreal sampling and fundus observation. In addition, the area under the curve of DEX in vitreous, choroid-retina and plasma also suggested that PLGA nanoparticles significantly improved the bioavailability of the encapsulated DEX compared with the control group of regular DEX solution. Furthermore, in 2014, Ozurdex®, an intravitreal implant based on PLGA for sustained release of dexamethasone, was approved for the treatment of diabetic macular edema [44].

Other than PLGA [45], block copolymers such as poly(ethylene glycol)-poly(ε-caprolactone) (PEG-PCL) have also been broadly applied for nanoparticle fabrication [46]. Our group developed folate-modified PEG-PCL nanoparticles for targeted delivery of triamcinolone acetonide (TA), and anti-inflammatory and anti-angiogenic drugs, to the retinal-pigment epithelium (RPE), which is implicated for its role in AMD by secreting vascular endothelial growth factor (VEGF), thereby triggering choroidal neovascularization. Nanoparticles of ~130 nm were internalized effectively by ARPE-19 cells via folate-receptor-mediated endocytosis. Compared with a TA suspension at the same drug concentration, the nanoparticle formulation was much less toxic. As intracellular depots, the TA-containing nanoparticles prolonged the antiangiogenic effects up to 3 weeks by downregulating VEGF and upregulating pigment-epithelium-derived factor (PEDF) [47].

Rapamycin is commonly used for treating immune rejection and has been applied in the treatment of posterior uveitis following intravitreal injection. Because rapamycin is water insoluble, nanoparticles were used as drug carriers to increase the intraocular concentration with limited side effects. Wu *et al.* prepared rapamycin-loaded PEG-PCL micelles with a size of 40 nm and a neutral surface charge [48]. Results demonstrated that fluorescent micelles were distributed to the RPE layer 14 days post injection. PEG-PCL

micelles increased the concentration of rapamycin 1.5 times in the retinal tissues compared with the unconjugated drug in suspension. In a rat experimental autoimmune uveitis (EAU) model, slit lamp images of the clinical signs showed that intraocular inflammation was significantly suppressed after intravitreal injection of the rapamycin-loaded PEG-PCL micelles at the optimal concentration of 9 μg per eye. No systemic side effects were observed.

Ryu *et al.* developed polymeric nanoparticles made of amphiphilic poly(γ-glutamic acid) and investigated their potential to deliver dexamethasone for the treatment of retinal disorders by the suppression of phagocytic cells [49]. Intravitreally injected poly(γ-glutamic acid) nanoparticles with a size of 180 nm and a zeta potential of around −25 mV showed accumulation in activated microglia located in the inner plexiform layer in an *N*-methyl-D-aspartate (NMDA)-damaged retina model, in which most of the RGC layer was hampered. The nanoparticles encapsulating dexamethasone showed a significant effect in suppressing the death of RGCs as well as preventing retinal detachment.

Other than polymeric materials, protein, lipid and other materials have been used for preparing intravitreal nanoparticles. Human serum albumin (HSA) is one of the commonly used protein-based materials. Kim *et al.* investigated the neuroprotective effect of brimonidimine-loaded HSA nanoparticles on RGCs in an optic nerve crush model [50]. The confocal images of retinal cryosections showed that, after 6, 24 and 72 h, HSA nanoparticles containing brimonidimine were located around the RGC layer. Two weeks after intravitreal injection of the brimonidimine-loaded HSA nanoparticles, the density of surviving RGCs increased by threefold and twofold compared with the balanced salt and naked drug solution group in the rat optic nerve crush model, respectively. Doxorubicin is known as a chemotherapeutic, whereas it could also be used as an antiangiogenic agent [51]. Wang *et al.* explored the potential of YSA peptide [a 12–amino-acid peptide (YSAYPDSVPMMS) coded as YSA] as a targeting ligand to RPE cells by liposomes [52]. The results demonstrated that, with the conjugation of the YSA peptide, the cellular uptake efficiency of doxorubicin encapsulated into liposomes was significantly enhanced. The *in vivo* therapeutic efficacy of the YSA-liposomes in suppressing the choroidal neovascularization (CNV) in rats was significantly higher than the unmodified liposomes or the doxorubicin solution group. Low retinal toxicity was reported.

### *Proteins and peptides*

Over the past decades, the development of ophthalmic protein- and peptide-based biopharmaceutical drugs has accelerated rapidly, especially in the category of monoclonal antibodies for treating ocular conditions in the posterior segment. Drug delivery of proteins and peptides for ocular applications has been extensively discussed in previous reviews [10]. Significant challenges arise when delivering biopharmaceuticals like proteins and peptides owing to the large molecular size, slow diffusion and sensitivity to enzymatic degradation. When injected into the vitreous, the intrinsic metabolic instability and high hydrophilicity can result in fast clearance from the vitreous, with short half-life (typically 1 week), inadequate transport to and distribution in the retina. This has provided the motivation for innovating drug delivery systems. When a long-term depot is intended to reside in the vitreous, hydrogel or microparticles serve as better platforms [6,53]. Nanoparticles, by contrast, can be designed for the purpose of targeted delivery to cells and become an intracellular depot.

Connexin 43 mimetic peptide (Cx43 MP) is a therapeutic peptide that can prevent secondary damage following retinal ischemic and inflammatory disorders by blocking pathological Cx43 hemichannel opening in astrocytes and vascular endothelium [54]. Huang *et al.* found that the hyaluronic acid (HA)-modified human serum albumin (HSA) nanoparticles could effectively localize the Cx43 MP to CD44-receptor-expressing cell layers (PR and RPE) in injured rat eyes [25]. The intravitreally injected Cx43 MP-loaded nanoparticles significantly and protractedly prevented thinning of the retinal tissue layer and blood vessels. Significant downregulation of Cx43 and inflammation-responsive protein GFAP was observed 8 weeks after injection with Cx43-MP-loaded nanoparticles compared with the control groups of saline, free Cx43 MP and Cx43-MP-mixed nanoparticles without encapsulation. A high survival rate of 85.05% of RGCs at 8 weeks after administration demonstrated the sustained neuroprotective efficacy of the Cx43-MP-loaded nanoparticles.

Vasoactive intestinal peptide (VIP) is a peptide naturally produced in immunologic homeostasis of the ocular microenvironment. The introduction of the peptide can act as the treatment by disrupting the immune system [55]. Lajavardi *et al.* studied the biodistribution of fluorescently labeled liposomes and examined the therapeutic effects of VIP-loaded liposomes in an endotoxin-induced uveitis (EIU) rat model [9]. A day after injection, liposomes were observed to be distributed in the vitreous, ciliary body, conjunctiva, retina and sclera. At the same time, clinical characterization of EIU signs, including the expression of inflammatory cytokine and chemokine mRNA, were significantly reduced in the VIP-liposome-injected rats compared with the rats injected with saline, naked VIP or unloaded liposomes. The clearance pathway was evidenced by imaging the fluorescently labeled liposomes at the regional cervical lymph node.

### *Nucleic acids*

Nucleic acid drugs, including DNA, RNA, aptamers and oligonucleotides, are emerging therapeutics for treating posterior segment conditions [56–58]. Leber's congenital amaurosis (LCA), choroideremia, Usher syndrome, Stargardt's disease and X-linked retinoschisis (XLRS) are diseases that are related to mutations of specific genes (RPE65, REP1, etc.) and are thus general targets for gene-based therapy [59]. For RNAi-based therapy, neuroprotection treatment targeting glaucoma or antiangiogenesis therapy in AMD or DR are being intensively explored [58]. For gene-based therapy, viral vectors have shown better transfection efficiency than synthetic carriers in *in vivo* systems. In 2017, LUXTURNA™ (voretigene neparvovecrzyl), the first-ever ocular-viral-vector gene-therapy given as subretinal injection to target the RPE65 gene mutation in associated retinal dystrophy, was approved by the FDA [60]. However, even with viral vectors, it remains challenging to target specific retinal tissues through intravitreal injection; thus, utilizing a more invasive route (subretinal injection) for PR/RPE targeting might be required. These issues can be explained by the anatomical and dynamic barriers discussed in the previous sections. In addition, successful delivery of nucleic acid drugs must account for cell internalization and subcellular localization [61]. When injected into the vitreous nakedly, highly negatively

charged nucleic acid therapeutics generally suffer from low efficacy because of enzymatic degradation, fast clearance and inadequate targeted internalization by specific retinal cells. Incorporation into synthetic nanocarriers, by contrast, can protect nucleic acids from degradation and can target them to the liver, intestine or tumors [62–64]. In recent years, the ocular distribution of intravitreally injected nanoparticles containing nucleic acids has been investigated and their therapeutic effects have been shown in various animal models (Table 1). The mutation of the retinaoschisin-encoding *Rs1* gene is associated with XLRS, which is the leading cause of macular degeneration in males [65]. Apaolaza *et al.* developed solid-lipid nanoparticles (SLNs) with a lipidic core (Precirol® ATO5), lipidic surface (DOTAP) and a polymer core (HA or dextran) to condense plasmid DNA encoding human *RS1* and GFP genes [66]. After intravitreal administration into mice, SLNs condensed with either HA (HA-SLN) or dextran (DX-SLN) showed GFP expression in multiple layers of the retina, whereas HA-SLN showed higher transfection levels compared with DX-SLN in the inner nuclear layer (INL) and PR. Two weeks after injection, intravitreal *RS1* plasmid-loaded SLNs significantly improved the retinal structure by increasing the thickness and decreasing the cavities characteristic of Rs1h-deficient mice.

Puras *et al.* constructed lipoplexes based on positive lipids and surfactants for retinal delivery of plasmid DNA [67]. DNA-loaded lipoplexes with a size of 200 nm and a zeta potential of 25 mV showed successful condensation and protection of plasmid DNA from the enzymatic degradation *in vitro*. In *in vitro* transfection studies, lipoplexes demonstrated high transfection efficiency in HEK293 and ARPE19 cells without obvious cytotoxicity. Via intravitreal injection in Sprague–Dawley (SD) rats, gene transfection of EGFP expression in multiple cell layers was achieved in the retina including RGC, INL and the bipolar cells and Müller cells. Tai *et al.* used penetratin-modified hydroxyl-terminated polyamidoamine (PAMAM) dendrimers to deliver antisense oligonucleotides [68]. The results showed that the dendrimer formulation could significantly improve the permeability of the antisense oligonucleotides and prolong the retinal distribution in RGC, INL, outer plexiform layer (OPL) and RPE up to 8 h post-injection.

RNAi therapeutics have been utilized in retinal gene regulation. Ryoo *et al.* manufactured novel siRNA-based nanoballs (siVEGF NB) by condensing anti-VEGF siRNA with positively charged and branched polyethyleneimine (bPEI) in the core while coating the surface with negatively charged HA using electrostatic interactions [69]. A week post intravitreal injection, the siVEGF NB had accumulated in the RPE layers. The angiogenesis effects of the novel nanosystem were tested in the laser-induced CNV mouse model. The area of CNV, as well as the VEGF mRNA, was reduced for up to 2 weeks with intravitreally injected siVEGF NB. This delivery vehicle is potentially useful for the treatment of AMD.

## Pharmacokinetics aspects for design principles

Based on the physiological and dynamic barriers discussed in the previous sections, we propose here a simplified compartmental model to illustrate the transport and partition of intravitreally injected nanoparticles (Fig. 2). Although nanoparticles are injected into the vitreous proximal to the retina, there are several barriers to overcome. Meanwhile, controlling the release of the therapeutic in the right location, not only at the tissue level

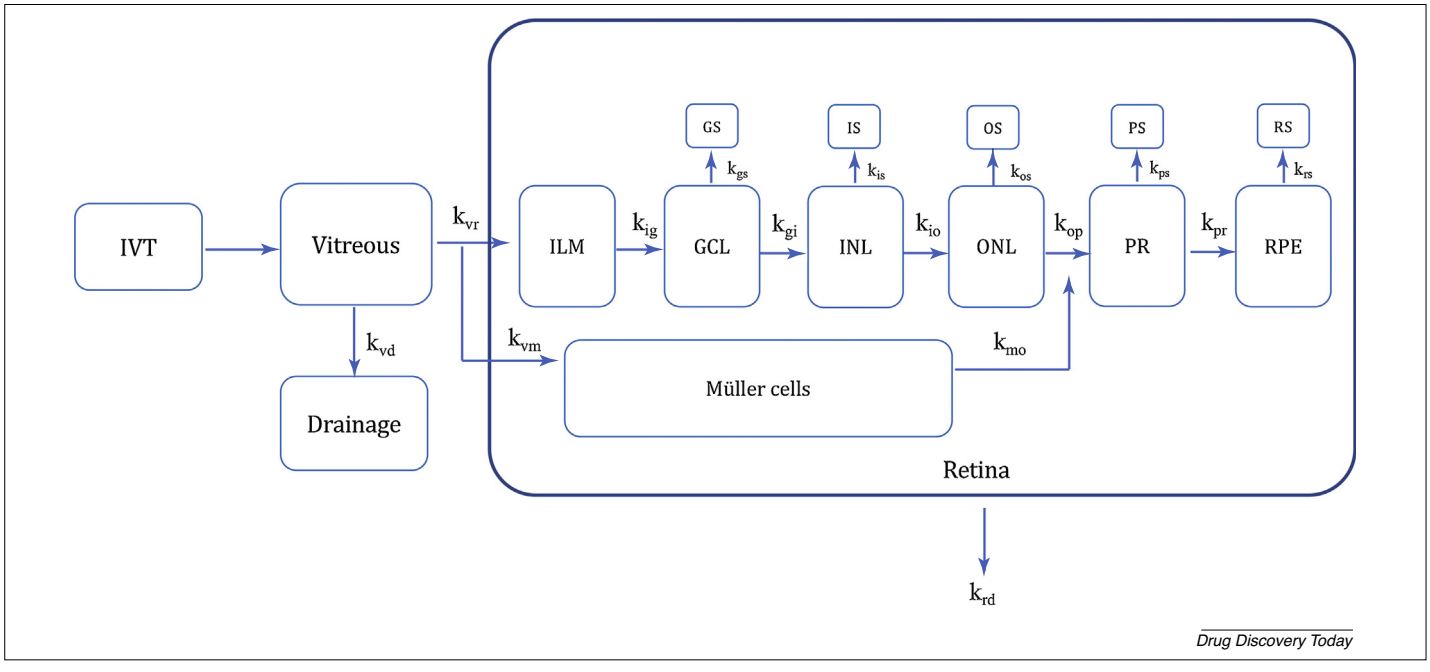


**FIGURE 2**

Simplified pharmacokinetic model for the intraocular pathway of intravitreally injected nanoparticles. Abbreviations: IVT, intravitreal injection; ILM, inner limiting membrane; GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; PR, photoreceptor; RPE, retinal pigment epithelium; $k_{vd}$, elimination rate constant from the eye through aqueous outflow drainage; $k_{vr}$, transport rate constant from the vitreous to the retina; $k_{ig}$, $k_{gi}$, $k_{io}$, $k_{op}$, $k_{pr}$, uptake rate constant to the different cell layers (GCL, INL, ONL, PR and RPE); $k_{vm}$, uptake rate constant of Mülller cells; $k_{mo}$, exocytosis rate constant of Müller cells; $k_{rd}$, clearance rate constant from the retina; $k_{gs}$, $k_{is}$, $k_{os}$, $k_{ps}$, $k_{rs}$ rate constant of nanoparticles distributing into a certain subcellular compartments of cells in the GCL, INL, ONL, PR and RPE, respectively.

but down to the subcellular level, is desirable for improving the drug effect. To breakdown the complexity, we have divided the intraocular tissues into the main layers and have further distinguished the extracellular and intracellular spaces. The movement of nanocarriers from one compartment to another is dependent on the net diffusion, convection, binding to the extracellular matrix and cell components, and cell membrane activity (e.g., receptor-mediated endocytosis), as described in the previous section. In this model, transfer between compartments is characterized by a lumped rate constant. We assume that the main trajectory of the nanocarriers radiates outward from the point of injection. Nevertheless, we have included the possibility of Müller-cell-mediated transport from the vitreous to the outer retina, which bypasses the inner retina [17]. Because the physiochemical properties of the nanoparticle can affect more than one rate constant in this model, parameters must be optimized by considering the goal of delivery and the balance and influence of different processes (Fig. 3). We will use this model to shed light on the effects of different parameters (size, surface charge, stability) on the observed ocular distribution, which will lead to a better understanding for the rational design of intravitreal nanoparticles.

### *Size*

All rate constants shown in the model are influenced by the size of the nanocarrier. It has been demonstrated that size generally affects the half-life of intravitreally injected nanoparticles. Sakura *et al.* showed that intravitreal polystyrene nanoparticles with sizes of 2 μm, 200 nm and 50 nm had half-lives of 5.4, 8.6 and 10.1 days in rabbit eyes, respectively [70]. For rate constants characterizing the process out of the vitreous, $k_{vd}$ is facilitated by the aqueous outflow whereas $k_{vr}$ is mainly dependent on the diffusion in the vitreous and partially on the vitreous outflow toward the retina [16,31]. When the size of the nanoparticles is >1000 nm, the large size hinders the nanocarriers from leaving the vitreous, which is a prerequisite for reaching the retina. Xu *et al.* showed that polystyrene nanoparticles of 1190 nm could not diffuse freely in bovine vitreous [16]. Sakurai *et al.* also observed that polystyrene particles with an extra-large size of 2 μm remained in the vitreous up to 1

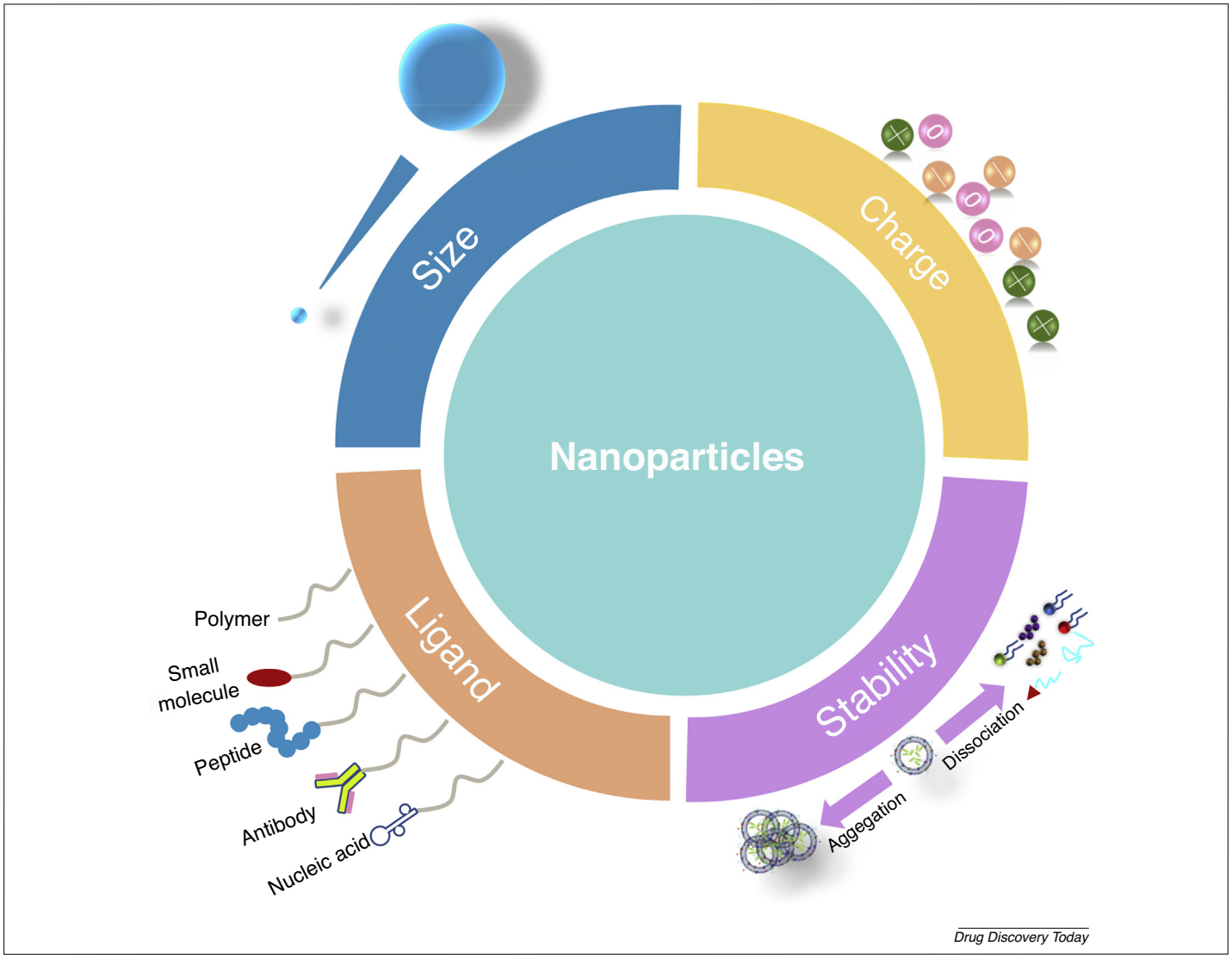


**FIGURE 3**

Parameters of intravitreal nanoparticles that affect their intraocular distribution and elimination. Adapted, with permission, from [98].

month after injection [70]. Theses carriers becoming trapped in the vitreous could explain why $k_{vd}$ and $k_{vr}$ have low values. By contrast, a large particle size can be advantageous when a long-term depot localized inside the vitreous is desirable. For example, a composite of gel-encapsulated nanoparticles, as well as a large conglomerate of aggregated nanoparticles, effectively enlarged the size of the carrier [71,72]. In the absence of, or before, degradation, carriers remain in the vitreous with minimal clearance. This strategy provides a prolonged and sustained supply of nanoparticles (or the released drug) in the vitreous. By contrast, when the size decreases, $k_{vr}$ and $k_{vd}$ increase. For particle sizes below 1000 nm, $k_{vr}$ can outcompete $k_{vd}$ such that nanoparticles can transport into the retina. It should be noted, however, that parameters other than size, such as charge interaction and ligand binding, will also influence $k_{vr}$ and $k_{vd}$, and will be further discussed below. For delivery to specific retinal tissues, the rate constants of internalization by nontargeted cells need to be minimized, whereas those of targeted cells should be maximized. Size alone can be used to regulate these rate constants. Generally, endocytosis takes place for nanoparticles with a size <500 nm [73]. By contrast, phagocytosis happens for nanoparticles ranging from 1000 to 2000 nm [74].

After passing the ILM, a smaller size that is more amenable for uptake will direct the nanoparticles to the inner layers where endocytosis mostly happens; whereas larger particles (e.g., 0.4–2.0 μm) will end up in the RPE layer through phagocytosis [75]. This has been exemplified by intravitreally injected magnetic nanoparticles destined for the RPE layer of *Xenopus* embryos and zebrafish [76]. Because the particle size can increase owing to aggregation *in vivo*, colloidal stability, covered in the next section, is another important factor in controlling the rate constants. Other than penetrating across the ILM, there is another pathway for nanoparticles to enter the retina from the vitreous (Fig. 2). Koo *et al.* showed that HSA nanoparticles with a size of 326 nm could enter the retina through endocytosis by Müller cells and were exocytosed into the outer layers such as the ONL and PR layers [17]. In this case, $k_{vm}$ should outcompete $k_{vd}$. The path mediated by Müller cells needs to be further validated. It could provide alternatives for nanoparticles to bypass the ILM barrier to directly target outer retinal layers. It should also be noted that the cut-off size for substances penetrating across the vitreous, entering the retina and crossing the ILM varies with species [20].

As well as the composition, anatomical geometry and structure of the respective ocular tissues vary drastically among species, especially from animal models to humans. The dimension and space limitation of the vitreous as well as the proximity of the vitreous to the retina make it more difficult to translate the research results based on rodents to clinical trials for human use. Mice [77] and rats [78] are two major animal models for intravitreal delivery. However, these clearly possess low vitreous volume and thicker retina compared with humans, making the intraocular transport and distribution of drug and vectors an insufficient prediction for application in humans [79]. One way to resolve this issue is simulating. Missel-developed models for the prediction of drug clearance for different species with more anatomically accurate geometric models among rabbit, monkey and human [31]. However, there is limited research regarding the clearance and intraocular distribution of intravitreal nanovectors, especially regarding comparison and justification of the results among different species. In this case, it remains difficult to translate these drug delivery systems into clinical application.

### *Stability*

The stability of nanoparticles has a significant influence on biodistribution. Unstable nanoparticles tend to aggregate or disassemble. Generally, polymeric nanoparticles possess better stability than liposomes and lipid-based nanoparticles owing to the facile disassembly, composition exchange and aggregation for materials comprising the latter [80]. For systemic delivery, the half-life of liposomes in the blood for rodents is 14–18 h [81], whereas the half-life of polymeric nanoparticles can be up to hundreds of hours [82]. If nanoparticles injected into the vitreous are unstable, the values of $k_{vd}$ and $k_{vr}$ are expected to change over time, because the actual particle size varies upon disassembly or aggregation. When nanoparticles fall apart, the disassociated components can be rapidly eliminated from the eye because $k_{vd}$ increases drastically with decreased molecular size [39]. This can be illustrated by comparing the half-life of intravitreal liposomes and polymeric nanoparticles, because liposomes are well known to disassociate *in vivo*. In rabbits, the half-life of intravitreally injected liposomal carriers ranges from tens of hours to ~100 h [83], whereas the half-life of intravitreal polystyrene nanoparticles of similar size (~50 nm) is ~10 days [70]. By contrast, when aggregated in the vitreous, the elimination of nanoparticles is hampered (low $k_{vd}$) and only limited diffusion in the vitreous toward the retina is possible (low $k_{vr}$). This leads to deposition of nanoparticles in the vitreous with insufficient partitioning into the retina. After entering the retina, nanoparticles pass through one retinal cell layer to the next, progressing from the inner to the outer retina. To pass through the GCL to the INL effectively, $k_{gi} > k_{ig}$ is needed for nanoparticles. If dissociation of nanoparticles happens in this process, $k_{rd}$ could increase significantly for the dissociated carrier components, leading to rapid elimination through the retina without further penetration into the outer retinal layers [39].

When constructed with lipids, nanoparticles show preferred distribution and thus efficacy in the inner retinal layers including the ILM, GLC and INL instead of the PR and the RPE [19,84,85]. Lee *et al.* showed retinal penetration of intravitreal injected phosphatidylcholine (PC)-based lipidic nanoparticles with significantly more accumulation in the ILM, GCL, INL and ONL layers than the PR and RPE layers [19]. By contrast, effective nanoparticle-mediated distribution or therapy in the outmost layers including PR and RPE via intravitreal injection can be commonly realized by polymeric materials [26] or polymer-condensed systems [66]. Bourges *et al.* discovered that Rh-6G-loaded poly(D,L-lactide) nanoparticles remained in the RPE layers for >4 months after intravitreal injection [26]. By contrast, aggregation of nanoparticles can decrease the internalization rate constants of inner retinal cell layers, thus favoring the disposition in the RPE layer. Giannaccini *et al.* demonstrated that intravitreal magnetic nanoparticles could be utilized for targeted and sustained delivery in RPE cells in zebrafish. Besides the suitable clearance rate constants, magnetic nanoparticle accumulation in the RPE was explained by attenuated internalization rate constants in off-targeted cells (RGC, INL, etc.) owing to the *in vivo* aggregation [76]. All of these studies have shown the stability–size impact on intraocular distribution and targeted delivery to specific retinal cells.

After internalization by specific cells, particle stability remains an important consideration for subcellular release, notably for gene therapy. Almutairi's group designed a light-responsive polymeric nanoparticle that allowed UV-triggered release of nintedanib to target retinal angiogenesis. With UV irradiation [(365 nm wavelength, 8 mW/cm$^2$) immediately after intravitreal injection of nintedanib-loaded polymeric nanoparticles], the extent of laser-induced CNV in rats was significantly suppressed not only straight after the injection but even after 10 weeks post injection owing to sustained drug release [86,87]. For gene and RNAi-based therapeutics, subcellular release and nuclear targeting are essential for improving the transfection efficiency. In this case, less stable nanoparticles can be favorable after entering the target cells. Major efforts have been made to facilitate endosomal escape and subcellular release by utilizing the proton sponge effect (development of lipidoids), redox-triggered release and ligand-targeted nuclear localization [88–90]. With all of these strategies, the stability of the nanoparticles is disrupted and the rate constants of distributing into subcellular compartments are significantly improved for maximizing the transfection efficiency.

### *Surface charge*

Surface charge affects the *in vivo* biodistribution of nanoparticles by two processes: binding with biological tissues via electrostatic interaction as well as destabilization via dynamic exchange of the nanoparticle components. As discussed in the previous section, the vitreous has a negative net charge, and the ILM is composed of negatively charged proteoglycans. In addition, the membranes of retinal cells also consist of negatively charged phospholipids. Thus, electrostatic interactions and binding between intravitreally injected nanoparticles and these ocular tissues affect and determine the rate constants of drainage ($k_{vd}$), the distribution into the retina ($k_{vr}$) and cellular internalization rate constants in each of the retinal layers. $k_{vd}$ and $k_{vr}$ increase (or decrease) together with the change of surface charge. In general, $k_{vd}$ and $k_{vr}$ decrease to some extent with the increase of positive charge because electrostatic interaction between positive and negative charges slowed down the diffusion and convective movement and thus increased the retarded effects of nanoparticles in the vitreous. The fate of nanoparticles depends on which rate constant will be affected more. It is also noted that the surface charge can interplay with other factors, such as the size and particle stability, such that the outcome of distribution is not purely because of the difference in the initial surface charge of the nanoparticles. As such, there have been contradictory observations with regard to the effect of charge on the ocular distribution of intravitreal nanoparticles. Koo *et al.* showed that negatively charged nanoparticles made of HSA or HA successfully reached the retina, whereas positively charged nanoparticles composed of PEI or glycol chitosan either stayed inside the vitreous or were blocked by the ILM [17]. By contrast, Lee *et al.* engineered liposomes with a neutral charge or positive surface charge of different zeta potentials, and showed that mildly positive liposomes with a zeta potential of ~20 mV could diffuse out of the vitreous and achieve effective retinal penetration [19]. We examined the charge impact on intraocular distribution of lipid-based nanoparticles and reached a similar conclusion [85]. We found that nanoparticles of negative and neutral charge were rapidly eliminated from the eye. Positively charged nanoparticles with a zeta potential of ~30 mV were capable of distributing across the retina with less binding in the vitreous than nanoparticles with a zeta potential of ~45 mV. For nanoparticles with a negative surface charge, $k_{vr}$ and $k_{vd}$ are relatively high. For negative or neutral lipid nanoparticles that were rapidly eliminated off the eyes, the charge interaction with the vitreous further destabilizes the particle into molecular components, leading to a higher $k_{vd}$ and faster drainage [19,85]. On the other hand, positive nanoparticles penetrated the retina. We explain this by the effect of electrostatic interaction in lowering the value of $k_{vd}$, with a magnitude larger than its effect on $k_{vr}$ [17,85]. When nanoparticles carry excessive positive charge, they will be trapped in the vitreous resulting in no retinal penetration [17,19]. However, this explanation might not apply to all types of nanoparticles when other parameters (ligand or stability) can interfere. It has been found that negatively charged HA-coated PEG-siRNA nanoballs were capable of distribution in the entire retina up to 7 days post intravitreal injection [69]. This shows that the targeting ligand must have an impact on the intraocular transport of nanoparticles.

### *Ligand*

Ligand-targeted delivery, or active targeting, takes advantage of the specific interaction with certain binding sites in the extracellular matrix or receptors on the targeted cell surface. This strategy has been widely investigated for the design of drug delivery systems against tumors [91–93]. Other than material composition, ligand modification alters the surface charge of the nanoparticles. For ocular application, it serves as a favorable method for targeted delivery to specific retinal cells. However, the application of ligand-targeted delivery via intravitreal nanoparticles has yet to be fully explored (Table 1). HA as a ligand has been investigated for retinal targeting. CD44 expressed on Müller cells can bind with HA [94]. In an EAU mouse model, CD44 expression in the RPE layer was upregulated. This provided the motivation to use HA-nanoparticles for targeted delivery [95]. When facilitated by receptor-mediated endocytosis, the internalization rate constant of the specific cell layer ($k_{ig}$, $k_{gi}$, $k_{io}$, $k_{op}$ or $k_{pr}$) significantly increases, resulting in improved uptake efficiency by the targeted cells. HA-modified HSA nanoparticles with encapsulated apatinib have shown significant improvement with regard to hyperpermeability inhibition on a cellular level with substantial reduction of retinal vascular leakage in streptozotocin (STZ)-induced diabetic mice [38]. For enhanced green fluorescent protein (EGFP) DNA-loaded liposomes, HA modification increased the transfection efficiency by twofold *in vitro* with no toxicity, as well as sixfold in the RPE cell layer of SD rats [96]. Unlike other ligands, HA is one of the natural components presented in the vitreous. Other than the electrostatic interaction between nanoparticles and the vitreous components, changes of the vitreous can have an influence [97]. This process can lead to an unknown binding effect of HA-nanoparticles in the vitreous with downregulated $k_{vd}$ despite the high net negative charge on the surface.

Other ligands have been explored but to a lesser extent. Folate has been commonly used for targeted delivery to tumors [99]. Suen and Chau proved that cellular uptake of folate-modified PEG-PCL nanoparticles was significantly enhanced compared with the non-modified nanoparticles in ARPE-19 cells [47]. YSA peptide conjugated onto liposomes for doxorubicin delivery significantly

reduced the area of CNV with little retinal toxicity [52]. After intravitreal injection into zebrafish, magnetic nanoparticles were shown to be taken up by RPE cells. With the recombinant VEGF grafted on the surface, the transcytosis through the RPE layer was promoted and nanoparticles were transported all the way to the choroid [100]. With all of these successful examples illustrated above, ligand-modified nanoparticles offer a promising platform for targeted retinal delivery. In the future, more focus and effort should be put on the discovery and development of potent ligands, including cell-penetrating peptides for cellular internationalization, and growth-factor-derived peptides for neuronal cells [101,102].

## Concluding remarks and future perspectives

Intravitreal nanoparticles, composed of polymers, proteins or lipids, have been widely explored for the delivery of all types of therapeutics to the retina. In this review, the most up-to-date research has been summarized. To provide insights for the rational design of nanoparticles for maximizing delivery efficiency to the retina down to the cellular level, a simple pharmacokinetic model has been proposed to help understand the influence of nanoparticle properties on intraocular transport, retinal penetration and elimination. However, the lack of quantitative assessments of retinal distribution and intraocular clearance of the particles currently limit the analysis to be qualitative. To gain a better understanding and optimize intravitreal drug delivery, more-quantitative results regarding the intraocular distribution and subcellular location in specific cells will be necessary. To design optimal nanocarriers for retinal delivery, the combined effects of all the parameters (size, surface charge, stability, ligand) and their change *in vivo* must be considered. Moreover, targeting ligands should be further explored to facilitate gene and RNAi-based delivery with maximized therapeutic efficacy in the retina.

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