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REVIEW ARTICLE

Ocular barriers as a double-edged sword: preventing and facilitating drug delivery to the retina

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Abstract

In recent decades, the growing of the aging population in the world brings increasingly heavy burden of vision-threatening retinal diseases. One of the biggest challenges in the treatment of retinal diseases is the effective drug delivery to the diseased area. Due to the existence of multiple anatomical and physiological barriers of the eye, commonly used oral drugs or topical eye drops cannot effectively reach the retinal lesions. Innovations in new drug formulations and delivery routes have been continuously applied to improve current drug delivery to the back of the eye. Unique ocular anatomical structures or physiological activities on these ocular barriers, in turn, can facilitate drug delivery to the retina if compatible formulations or delivery routes are properly designed or selected. This paper focuses on key barrier structures of the eye and summarizes advances of corresponding drug delivery means to the retina, including various local drug delivery routes by invasive approaches, as well as systemic eye drug delivery by non-invasive approaches.

Keywords Retina · Blood-retinal barrier · Ocular drug delivery · Prodrug · Sustained release formulations

Chronic retinal diseases (fundus diseases) are rapidly increasing as the lifespan extends in the global population [1, 2]. The leading contributors to moderate and severe vision impairment of people aged over 50 years include age-related macular degeneration (AMD, 6 million), glaucoma (3 million), and diabetic retinopathy (DR, 3 million) according to the Global Vision Database [3]. The chronic nature and hardly accessible location of retinal diseases bring challenges to sufficient and/or durable exposure of medications to the retina [4]. Up to today, most intraocular drugs are delivered invasively and repeatedly. More innovations are needed to decrease the repetitiveness of invasive drug delivery and increase the retention time of intraocular drugs at the diseased site.

The retina as a tiny sensory organ is located as the innermost layer of the eye and has complex light to electronic conversion and transmission functions. The retina is strictly protected by multiple anatomical and physiological barriers, which maintain the homeostasis of retinal neurons and avoid the entry of unnecessary substrates into the environment. The tight protection also makes drug molecules difficult to access the retina. Multiple delivery routes and advanced formulations have been explored to improve drug delivery efficacy and drug retention time. Commonly used eye drops are currently not suitable for the treatment of retinal diseases. Most of the new topical formulations are still in the early animal testing phase (see our recent review) [5]. Innovations in systemic ocular formulations and new injection routes are two areas that progress rapidly to meet the needs of novel therapeutics such as macromolecular drugs, gene therapy, and cell therapy.

Recently, plenty of works have summarized updates in retinal drug delivery from different perspectives, which are rich in detailed advancement of animal or clinical exploration results [6–10]. From the view of ophthalmologists and ophthalmic drug developers, a clear understanding of the mechanisms of drug delivery to the retina by taking advantage of different ocular anatomical and physiological properties is vital to boost future innovations and open new possible drug delivery means, which lacks sufficient discussion in previous reviews. These unique anatomical and physiological features, which are conventionally regarded as "ocular drug barriers," are increasingly recognized as a double-edged sword that can facilitate drug entry into the retina with the development of new drug delivery means

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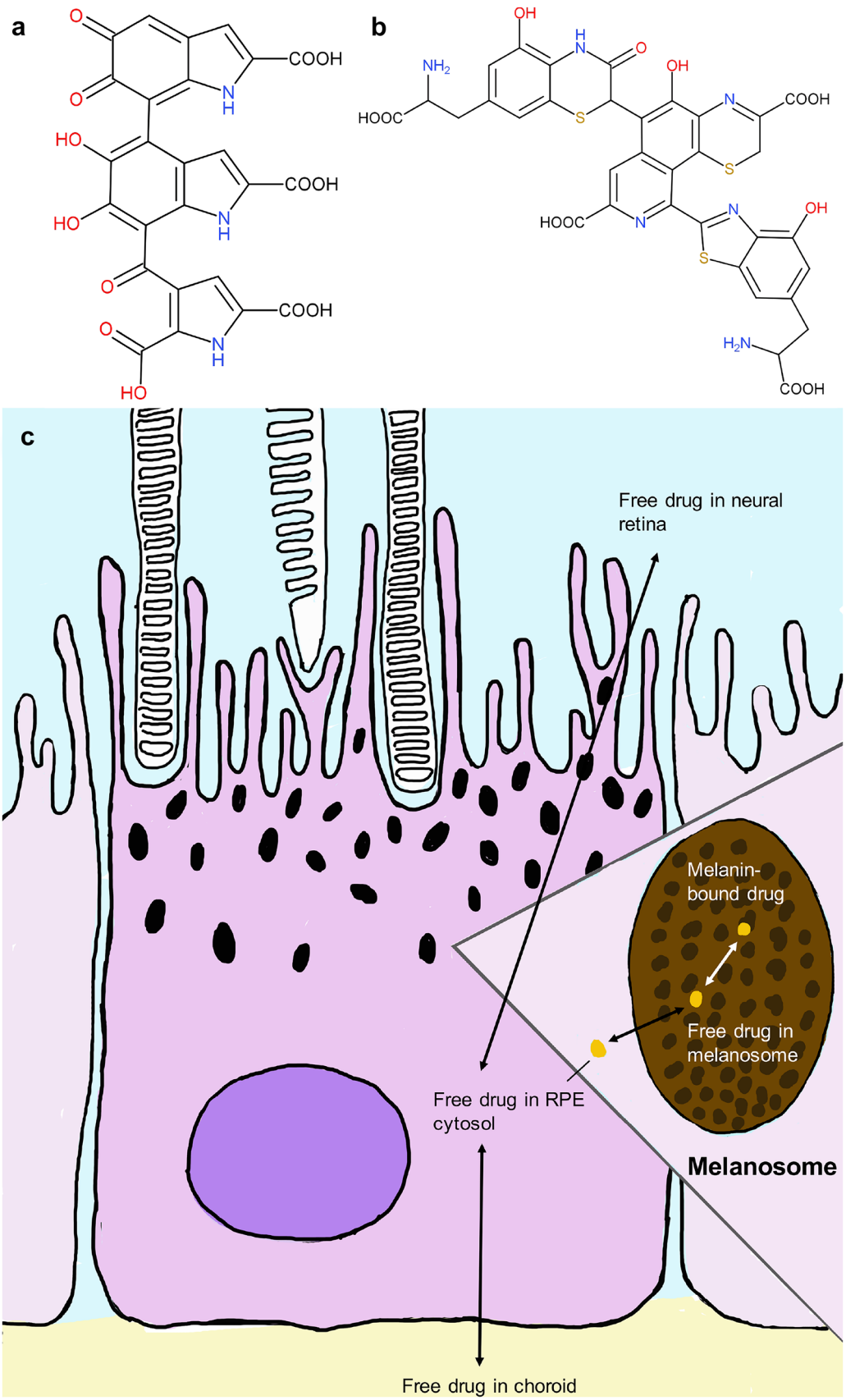
a
b
c
Free drug in neural retina
Melanin-bound drug
Free drug in melanosome
Free drug in RPE cytosol
Melanosome
Free drug in choroid

◂**Fig. 1** Schematic diagram of drug binding to melanin in RPE. Melanin is a hydrophobic molecule with multiple carboxyl groups. **a** The chemical structure of eumelanin and **b** the structure of pheomelanin. **c** The interaction of a drug with melanin in the RPE. A drug needs to enter a RPE cell and then melanosome. The binding of melanin facilitates the enrichment of drug molecules in RPE area

that take advantage of these properties. In addition, the basic researches on drug distribution, tissue remodeling, ocular inflammation, and molecular mechanisms associated with drug delivery are insufficient to guide clinical explorations and need further study. In our review, we aim to link unique ocular barriers with new retinal drug delivery means and discuss their unmet challenges and outlooks in clinical translation.

Blood-retinal barriers (BRBs) and transporter-targeted prodrug approach

The retina is a 10-layered membrane with a mean thickness of 249 μm in humans [11] and has specific BRB structure to protect itself from unneeded molecules from the blood such as endobiotics and xenobiotics [12]. The inner BRB (iBRB) is formed by non-fenestrated endothelial cells, pericytes, and glial cells, and is responsible for the homeostasis of the inner two-thirds of the retina. The outer BRB (oBRB) is formed by retinal pigment epithelial (RPE) cells and their tight junctions, which block the free movement of molecules between the choroid and retina [13]. However, BRB has its own transporters on the cells that selectively control the movement of circulating molecules into the retina [14]. Two types of transporters have been extensively studied in the retina, i.e., the ATP-binding cassette (ABC) transporters that efflux intracellular wastes and xenobiotics and the solute carrier (SLC) transporters that influx various nutrients or efflux organic molecular drugs [15]. When a drug molecule interacts with these transporters, its bioavailability and retention time in the retina can be affected. For example, fluorescein has a short retention time in the retina after the fluorescein angiography test, which is largely due to its excretion by the synergic actions of ABC transporters and SLC transporters [16, 17].

It has been found that when a drug molecule is linked to nutritional molecules, such as amino acids, glucose, oligopeptides, ions, vitamins, choline, or nucleoside, the modified drugs have increased efficiency to cross cells via the influx transportation system [18, 19]. These nutrition-bound drug molecules are a type of prodrugs, and the original drug molecules can be released after the attached nutrient part is degraded after entering the intracellular space. Some early work demonstrates promising results of nutrition-bound prodrugs in trans-corneal and trans-scleral transportation. Dipeptide glycine-valine-linked Ganciclovir had increased trans-scleral permeability in a rabbit model by 2-folds [20]. As similar transporters are expressed on iBRB and oBRB, this transporter-targeted prodrug approach has been suggested to assist circulating prodrugs to cross the BRB as well [21, 22]. One example is dipeptide-Glycylsarcosine which effectively enhanced the concentration of the original drug in the retina and vitreous humor after systemic administration in a rabbit model, with the percentage of drug uptake in the vitreous and retina being $1.00 \pm 0.10\%$ and $0.13 \pm 0.03\%$, respectively [23].

Although the prodrug approach might be a potential way to improve the retinal distribution of drugs administrated systemically, some concerns are present about their usefulness. Certain drug transporters present on the BRB are also present on the blood–brain barrier (BBB) [24], and therefore, unwanted effects on the central nervous system may present and potential risks have to be closely investigated and monitored. Understanding of expression of useful drug transporters on the BBB and BRB, such as L-type amino acid transporter 1 (LAT1), may provide valuable information on the drug efficacy and safety in humans, as some transporter-targeted prodrugs have already been approved for CNS diseases, such as L-DOPA and gabapentin [25]. Besides, prodrugs may become competitive substrates to the nutritional molecules and may interfere with the normal metabolism of the retina. During drug design, one should also consider metabolizing enzymes such as cytochrome P450 which may degrade prodrugs in the ocular tissues before they enter the target cells [26]. In the preclinical phase, the selection of appropriate animal species is also important as some nutrient transporters may express differently in human and laboratory animals [27, 28]. Taking together, the prodrug approach has been a promising strategy for small molecular drugs. Over 30 different prodrugs have been approved by FDA, accounting for more than 12% of all approved small molecular drug entities [29]. As more selective transporters on the BRBs are identified and characterized, the nutrient prodrug approach can be an alternative approach for fundus drug delivery.

RPE pigment and the melanin-binding approach

Melanin is a pigment molecule and is present in the RPE and pigmented epithelial cells of the uveal tract (the iris, ciliary body, and choroid) and some cells in the trabecular meshwork [30]. There are two kinds of melanin molecules (eumelanin and pheomelanin) in the eye, which are both hydrophobic and negatively charged with multiple carboxyl groups (Fig. 1). Therefore, melanin has a high binding affinity to hydrophobic and basic drugs, such as Chloroquine,

Timolol, Thiopyridazine, Tamoxifen, and more [31, 32]. Quantitative measurement of cellular uptake of melanin-binding drugs such as Chloroquine, Propranolol, Timolol, Diclofenaca, and Methotrexate confirmed that the presence of melanin was the major factor that changed the fraction of cellular bound and unbound drug fractions by a range of 3–4 orders of magnitude [33]. The melanin-binding ability of a drug molecule affects its distribution in the eye but can induce ocular toxicity when drug concentration is too high in the retina. Therefore, chemical drugs often need to be tested for the potential risk by in vitro melanin-binding tests, in vivo animal studies [34], or even in silico models [35, 36]. The melanin-binding affinity might be useful as a method of drug delivery into the eye.

Drugs with high melanin-binding ability can be selectively enriched in the melanin-containing RPE cells and choroid in the eye. A cyclooxygenase-2 inhibitor Celecoxib was administrated into pigmented Brown Norway (BN) rats and non-pigmented Sprague Dawley (SD) rats to compare their pharmacokinetic profiles. The BN group had 1.5 times higher area under the curve (AUC) in the RPE and choroid, but 1.5 times lower AUC in the retina and vitreous compared to that of SD rats after periocular injection, indicating that Celecoxib was selectively enriched in melanin-rich cells, like pigmented cells in the retina and choroid [37]. Danicopan, an inhibitor of the alternative pathway of complement activation, was found to be enriched in the choroid, RPE, iris, and ciliary body after oral dosing in rabbits [38]. The molecule could be detected in the uveal tract and retina as long as 240 h after the last dose, despite an undetectable plasma drug level after 96 h. These data suggested that melanin binding could extend the drug retention time in pigmented ocular tissues.

The study of two vascular endothelial growth factor (VEGF)/platelet-derived growth factor (PDGF) receptor tyrosine kinase inhibitors was a practical case of applying the melanin-binding approach in ocular drug development [39]. Pazopanib and GW771806, which showed a strong binding affinity to melanin in vitro experiments, were given to pigmented Long-Evans rats and albino SD rats separately by a single oral dose. The concentration of these two compounds was significantly higher in pigmented eyes than that in non-pigmented eyes 3 days after administration. The ocular half-lives of Pazopanib and GW771806 reached 439 and 442 h, respectively. Further mass spectrometry analysis confirmed that the original form of the compounds could be released from the melanin-bound form. In a preclinical efficacy study, mice receiving GW771806 eye drops had a reduction of the pathological changes of choroidal neovascularization (CNV) at the high dose (5 mg/mL), and prevention of disease progression at the low dose (2 mg/mL). Pazopanib administered orally as a single dose before CNV induction showed an inhibitory effect on the development of CNV in mice, despite the plasma level of the drug remaining below the detection limit. Sunitinib, a protein kinase inhibitor with the melanin-binding property, was administrated as the topical gel formulation and had significant protection of retinal ganglion cells in a glaucoma animal model. Danicopan (ALXN2040), an orally administrated complement factor D inhibitor, had a sufficiently high retinal distribution with the AUC of choroid-RPE/plasma ratios to be 24 and 0.7 in pigmented and non-pigmented rats, respectively [40].

These preclinical results shed light on the possibility of applying oral melanin-binding drugs to treat neovascular AMD and other retinal disorders. However, this approach faces some biological challenges. As melanin is present in a membrane-bound structure called melanosome in the eye, drug molecules theoretically must first get into the cell membrane and melanosome before interacting with melanin [41]. This can limit its application on large molecules such as proteins and nucleotides due to their insufficient transmembrane permeability. Besides, the drug molecule must be able to release from melanin and melanosome thereafter and maintain their intact biological activities when released from pigmented cells. This might be difficult for basic drugs which may bound more firmly and even get ionized as melanosome has an acidic environment. Importantly, the accumulation of drug molecules in the retina should not cause undesired effects as that seen in the cases of Chloroquine, Gentamycin, Chlorpromazine, and Thioridazine [42–44]. Therefore, compounds designed for the melanin-binding approach must have a balanced melanin-binding ability and releasing profile.

RPE and the endocytosis approach

Endocytosis is a fundamental function of cells that internalizes extracellular materials by membrane deformation [45]. There are 4 major types of endocytosis, i.e., phagocytosis, macropinocytosis, clathrin-mediated endocytosis, and caveolin-mediated endocytosis as illustrated in Fig. 2. These activities also take part in maintaining the normal functions of the eye [46]. Among different ocular tissues, the RPE has the most active endocytosis activity [47]. The apical side of the RPE forms numerous membrane processes that extend to the photoreceptor layer and engulfs the shedding discs of photoreceptor cells [48]. This dynamic endocytosis activity plays vital role in maintaining visual function [49]. Certain drug molecules may utilize the endocytosis activity of RPE to achieve better retinal exposure and retention time [50].

Endocytosis was found to be mediated by some surface receptors, such as the CD44 receptor, flotillin, and scavenger receptor [51, 52]. The endocytosis activity of the retina might be a potential non-invasive approach to deliver drug

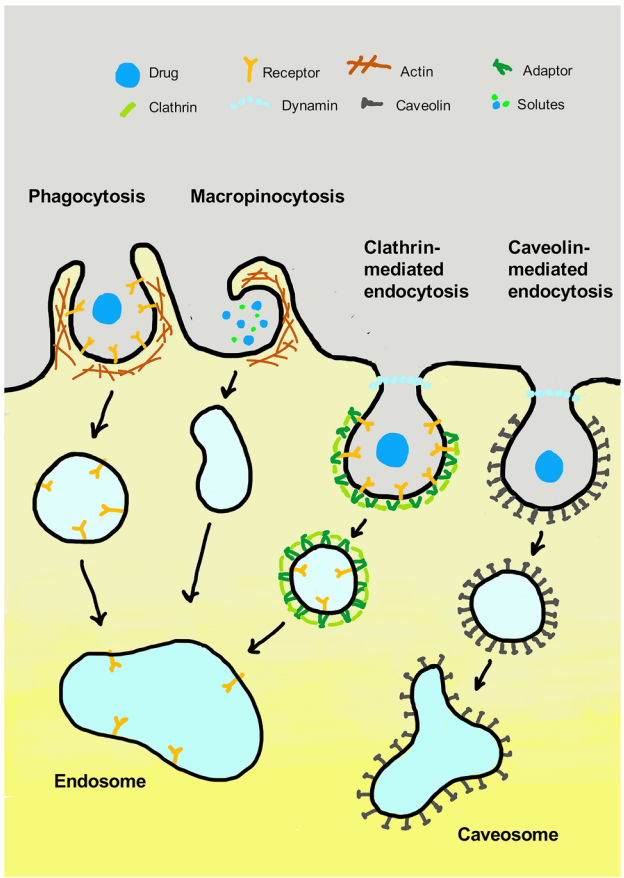

Fig. 2 Schematic diagram of different endocytosis modes of cells. Phagocytosis is a receptor-dependent uptake assisted by actin filaments. Macropinocytosis is the non-selective engulfment of a bulk of solutions containing drugs. The form of clathrin-mediated endocytosis and caveolin-mediated endocytosis relies on clathrin and caveolin-mediated cell membrane invagination to take up molecules, respectively. These activities can help drugs, especially macromolecular drugs cross the inner BRB and outer BRB

molecules by oral, intravenous, and topical administration. For example, hyaluronan-modified liponanoparticles administrated into the rat vitreous body were specifically enriched in RPE cells through the interaction of hyaluronan and CD44 receptors on the cell surface. Approximately 75% of these injected nanoparticles were retained in the choroid/PRE 7 days after the injection. In contrast, nanoparticles without hyaluronan modification did not penetrate so well and were presented at the surface layers of the retina [52]. Rapamycin, a mammalian target of rapamycin (mTOR) inhibitor, was packed in poly (lactic-*co*-glycolic) acid (PLGA) nanocarriers that were integrated with macrophage membrane-derived vesicles. This approach led to a 5.6-fold elevation of drug concentration in the choroid compared to unmodified nanocarriers in mice [53]. Similarly, rapamycin packed in synthetic high-density lipoprotein nanoparticles showed a 125-fold increase in drug aqueous concentration and reached the RPE cells in a rat AMD model by intravitreal injection [54]. Wei et al. compared the anti-tumor efficiency of topotecan carried in normal nanoparticles and folic acid-conjugated nanoparticles and found that folic acid facilitated endocytosis activities by human retinoblastoma cells, which showed 58% apoptosis efficiency compared to only 18% in normal nanoparticles [55].

With the development of technologies, many technical hurdles of nanomedicine manufacturing have been lifted, and numerous novel formulations of nanomedicines for retinal diseases are generated and investigated in the preclinical stage [56]. A clinical trial of a topical formulation of dexamethasone γ-cyclodextrin nanoparticle showed significant improvement of vision and macular thickness in patients with diabetic macular edema, which achieved similar efficacy to subtenon triamcinolone injection [57]. With decades of investigation and clinical data on nanomedicines in many other fields, the endocytosis-targeted approach is a promising drug delivery tool for retinal diseases in the future. More explorations of surface ligands to enhance the specificity and efficiency of retinal drug delivery will speed up the clinical translation of the technology. In addition, the charge of drug molecules, size of drug molecules, temperature, and density of surface ligands are other factors to be optimized to achieve a satisfactory pharmacokinetic property [58, 59].

Transient opening of the BRB

To increase the entry of circulating drugs into the retina, various means have been explored to temporarily "open" the BRB. For example, some physical methods have been tested to transiently disrupt the BRB. A focused ultrasound sonication in combination with a microbubble ultrasound contrast agent was explored preclinically. The 1-ms ultrasound bursts at the frequency of 1 Hz and with pressure amplitudes ranging from 0.81 to 1.10 MPa for 1 min resulted in a transient opening of BRB for 3 h [60]. The application temporarily widened the tight junctions and stimulated active transport, thus enhancing drug delivery. The effect generally lasted for several hours [61], but may cause some undesired adverse effects on the eyes such as petechiae and retinitis especially when high-power ultrasound was applied [60]. To avoid potential damage to retinal vessels, a low-energy ultrasound with pressure amplitudes ranging from 0.36 to 0.84 MPa was used to facilitate the delivery of intravenous AAV vectors to retinal microglia cells. However, off-target expression in peripheral tissues was also observed [62].

The hyperthermia effect induced by the magnetic field has been explored in animals as well. In this approach, nanoparticles are linked with transferrin on their surface and therefore can adhere to the vascular endothelial cells

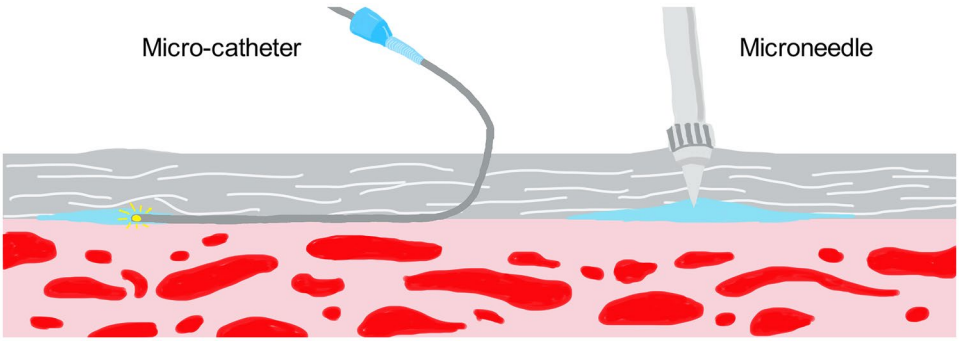

Fig. 3 Schematic drawing of suprachoroidal space injection with a microcatheter and a microneedle. The suprachoroidal space is a potential space between the choroid (pink) and the sclera (gray)

after injection into the blood. They induce a vibration under the magnetic field that increases the local temperature. The application of the magnetic field generated a very mild temperature elevation of 1–2 °C, which faded away in less than 1 μm from the surface of nanoparticles, indicating a very focused effect with good safety. The procedure temporarily increased the retinal exposure of sodium fluorescein by about 1.2-folds and Evans blue dye by about 8-folds, which lasted for less than 2 h [63].

Besides physical methods, biological means such as small interfering RNA (siRNA) were also explored. Suppression of a tight junction protein claudin-5 by siRNA resulted in the temporary opening of the iBRB that enabled the penetration of molecules with sizes up to 1 kDa. The effect started 24 h after the delivery of siRNA and lasted for up to 36 h [64]. No serious adverse events or significant disturbance of transcriptional patterns of neuronal tissues were noted in test animals [65]. In addition, a doxycycline-inducible gene encoding short hairpin RNA (shRNA) carried by viral vectors also demonstrated a temporal but controllable suppression of claudin-5, which enabled the entrance of low-molecular compounds up to 1 kDa. The time of BRB opening could be controlled by administration of doxycycline, and the effect theoretically lasted for no more than 30 h after withdrawal of doxycycline due to the short half-life of claudin-5 [66]. Another transmembrane molecule of endothelial cells, Unc5B, has also been identified as a possible target of BRB opening. Knockout of Unc5B in mice resulted in embryonic lethality, and conditional knockout in endothelial cells of adult mice resulted in capillary leakage in the brain [67]. Turning off Unc5B, either by gene knockout or antibody neutralization to Unc5B, caused an effective opening of the BBB [68]. Intravenous injection of Unc5B antibody resulted in a transient opening of the BBB for several hours before a full return to the normal sealing state. Interestingly, molecules with sizes of 10 kDa and 40 kDa could penetrate from the blood into the brain tissue, but larger molecules such as 70 kDa dextran, IgG, and fibrinogen could not leak into the brain. This spatiotemporal opening of the BBB appeared very attractive for drug delivery across the BRB.

These explorations suggest that transient breakdown of the BRB might be useful in eye drug delivery for patients. Up to now, however, all related researches are conducted on rodents, and no human data are available. Therefore, we can only speculate that safety should be the top concern for such approaches, particularly considering the chronic nature of retinal diseases such as nAMD and DR, which require long-term treatment. Any repeated disturbance of BRB may cause some problems, such as an increase of pathogens or toxins into the retina. How to precisely control the temporary "opening" of the BRB and how often one can safely open the BRB need more research and innovations.

Suprachoroidal space as a reservoir with extensive retinal exposure

The suprachoroidal space is the gap between the choroid and sclera [69]. It is not visible under physical conditions as the two tissues are closely packed. But it is expandable under external forces and can serve as a reservoir for drug delivery. In rabbits, the space can be expanded to a thickness of 2.8 mm and hold as much as 150 μL of fluid [70]. With the development of compatible instruments, the suprachoroidal space becomes readily accessible as a new drug delivery site. Drugs delivered into this narrow space by different approaches can instantly distribute to the choroid and retina as illustrated in Fig. 3.

The microcatheter by Ellex Medical is a device designed for suprachoroidal space drug delivery and approved by FDA [69]. It requires a surgical dissection of the sclera to implant the catheter into the suprachoroidal space and adjust its location [71]. In a clinical trial, patients with severe subfoveal hard exudates and retinal vasculopathy received Bevacizumab and Triamcinolone by suprachoroidal space injection. A significant reduction of macular thickness and complete resolution of hard exudates was observed at 12 months [72]. Another cohort study demonstrated that this device and operation were generally well-tolerated without serious adverse events [73].

The recent development of microneedles makes it possible to inject drugs into the suprachoroidal space in a less

invasive way [74], although the insertion depth and injection pressure need to be accurately controlled. With the advancement of fabrication technology and 3D printing techniques, novel microneedles enable reliable injection even in small animal eyes [75]. Due to its easy operation and low invasiveness, microneedle injection can be used in the outpatient setting. Currently, several clinical trials are using suprachoroidal space injection including triamcinolone acetonide and cell therapy as summarized in Table 1. The phase III PEACHTREE trial of a suprachoroidal microneedle injection of triamcinolone acetonide is particularly interesting. It achieved at least a 15-letter improvement in BCVA than patients in the control arm in 24 weeks and demonstrated a good safety profile [76]. The product XIPERE™ has become the first FDA-approved microneedle-based therapy for suprachoroidal space injection in 2021.

Preclinical data demonstrated that about 15% amount of the injected drugs could reach the posterior region of the eye in this way [77]. As the suprachoroidal space wraps the whole posterior eye, a wider area of retinal drug distribution was expected and confirmed in animal studies. In one monkey study, the expression of adeno-associated virus (AAV) vectors with reporter genes was injected into the suprachoroidal space or the subretinal space. It was found that the suprachoroidal injection led to a large area of reporter gene expression, while subretinal injection gave an intensive focal expression [78]. Another monkey study demonstrated that the expression area of AAV8 vectors with green fluorescein protein (GFP) was 23% after a single suprachoroidal space injection, and 42% after 2 injections [79].

Suprachoroidal space injection also provides fast and high drug exposure to the outer retina and choroid. Drugs injected in the space are initially present in the narrow depot, which then gradually penetrate the adjacent retina, with very low exposure in the vitreous body [80, 81]. The peak concentration of fluorescein sodium injected into the rat suprachoroidal space was 36-fold and 25-fold higher in the choroid/retina compared to the posterior subconjunctival and intravitreal injections, respectively [82]. The retinal exposure of Axitinib after suprachoroidal injection was 11-folds higher than that of intravitreal injection [83]. Reversely, suprachoroidal injection led to low exposure to other ocular tissues. In a rabbit model, suprachoroidal injection of triamcinolone acetonide suspension by microneedles resulted in 460-, 34-, and 22-fold lower exposure in the lens, iris-ciliary body, and vitreous humor, and negligible exposure in the aqueous humor compared to intravitreal injection [84].

There are some challenges in the application of suprachoroidal injection. As the space is very narrow, the resistance to the injection is strong, and the volume capacity is limited. Some innovative methods have been tested to enlarge the space and increase the delivery capacity, such as swelling hydrogels [85], collagenase breakdown [86], high-density particle emulsions [87], and iontophoresis [77]. Another bottleneck is the short retention time of drug molecules in the space, as molecules are excreted rapidly in the area. Different excretion pathways have been discussed, including pressure-driven reflux, trans-scleral leakage, diffusion, clearance by blood circulation, and convection [88–90]. It is found that both Bevacizumab and Ketorolac injected into the suprachoroidal space have significantly shorter retention time in the eye compared to intravitreal injection [91, 92]. Formulations with sustained release might be more appropriate for those drugs designed for suprachoroidal space delivery. For example, solid particles with sizes of 20 nm to 10 μm were particularly resistant to clearance in the suprachoroidal space and could remain locally for months [93]. In short, the suprachoroidal space as a newly explored drug delivery site has great advantages for faster delivery, specificity to the fundus, and wider posterior area affected.

Subretinal space as a reservoir for direct drug/gene/cell delivery to PRE and photoreceptors

The subretinal space is also a potential gap normally located between the RPE and the photoreceptor layer. As the two neighboring tissues are easily separated under moderate external forces, a relatively large volume of materials can be placed into the space for the treatment of hereditary or degenerative retinal disorders [94]. This delivery route bypasses the BRB and directly loads materials between the PRE and photoreceptors, which often gives good bioavailability [95, 96]. Numerous clinical studies with subretinal delivery are ongoing as listed in Table 2.

Gene therapy adopts a viral vector, most commonly AAV, to express its carried gene in cells that replace dysfunctional genes of certain inherited fundus diseases. The gene expression by AAV vectors often has long-term effects, ranging from months to years in patients' eyes. This method is also applied as an alternative approach to long-term exposure of biological drugs for the treatment of chronic fundus diseases, such as nAMD. Currently, more than 50 clinical trials investigating gene therapy for retinal and optic nerve diseases have been registered [97]. RGX-314 is an anti-neovascular gene therapy that is carried by AAV8, and transfected ocular cells could continuously express the protein for at least 6 months. nAMD patients receiving subretinal injections of RGX-314 had sustained visual improvement and were injection free during the time [98]. Another anti-neovascular gene therapy, RetinoStat®, was constructed with recombinant equine infectious anemia virus (EIAV) and expresses endostatin and angiostatin. In the phase I trial, 21 patients with nAMD who responded

Table 1 Summary of recent clinical trials of suprachoroidal space delivery

Agent	Indication	Phase	Patient number	Follow-up period	Main outcomes	Adverse events	Reference
Triamcinolone acetonide suspension	Macular edema secondary to noninfectious uveitis	II	22	2 months	Significant reduction of macular thickness and improvement of VA	Procedure-related AEs such as conjunctival hemorrhage, eye pain, and macular edema, each reported in 3 patients	[157]
Triamcinolone acetonide suspension	Macular edema secondary to noninfectious uveitis	III	160	24 weeks	Significant reduction of macular thickness and improvement of VA	Comparable rates of increased IOP and cataract with the control group. No serious AEs occurred	[76]
Triamcinolone acetonide (SC) + aflibercept (IVT)	Macular edema due to retinal vein occlusion	II	46	3 months	Combination therapy had superior control of macular edema and reduced the need for additional therapy	1 and 4 patients in the combination therapy group had progression of cataract and increase of IOP, which were not reported in IVT group	[158]
Triamcinolone acetonide	Resistant DME	I	24	3 months	Significant improvement of VA and reduction of macular thickness	1 case of controllable elevation of IOP was reported	[159]
Adipose tissue-derived mesenchymal stem cell	Dry AMD and Stargardt's macular dystrophy	II	8	6 months	All patients had improved VA, visual field, and mf-ERG recordings	No serious systemic or ocular AEs reported	[160]
Umbilical cord-derived mesenchymal stem cell	Retinitis pigmentosa	III	82	6 months	Significant improvement of VA, visual field, and mf-ERG recordings	No serious systemic or ocular AEs reported	[161]
Triamcinolone acetonide	Branch retinal vein occlusion	I	30	12 months	Significant improvement of VA and reduction of macular thickness	No serious systemic or ocular AEs reported	[162]
Triamcinolone acetonide	Diabetic macular edema	II	32	6 months	Comparable effect with IVT	Comparable events of cataract progression and IOP elevation with IVT	[163]
Triamcinolone acetonide injectable suspension	Macular edema associated with non-infectious uveitis	III	53	48 weeks	50% of patients did not require additional treatment for up to 9 months after last injection	No serious systemic or ocular AEs reported	[164]

IOP intraocular pressure, *VA* visual acuity, *AEs* adverse events, *SC* suprachoroidal injection, *IVT* intravitreal injection, *mf-ERG* multifocal electroretinogram, *DME* diabetic macular edema

Table 2 Summary of recent clinical trials with subretinal space delivery for gene and cell therapy

	Agent	Indication	Phase	Patient number	Follow-up period	Main outcomes	Adverse events	Refs
Gene therapy								
	Knock-in of CNGA3 via AAV	Achromatopsia	I	9	12 months	Improvement of VA and contrast sensitivity	No AEs related to injection	[165]
	Knock-in of hRPE65v2 via AAV	RPE65-mediated inherited retinal dystrophy	III	29	12 months	Improvement of functional vision	Only mild ocular AEs (increase of IOP, cataract, retinal tear, etc.) were reported	[166]
	Knock-in of RPGR via AAV	X-linked retinitis pigmentosa	I/II	18	6 months	Improvement of visual function and night vision	Intraocular inflammation at higher doses, which required additional corticosteroids	[167]
	Knock-in of sFLT-1 via AAV	Wet AMD	I/IIa	37	3 years	No significant difference of VA change or central point thickness change between control and therapy groups	Transient choroiditis reported in 1 patient of the control group. Other severe AEs were not related to therapy	[117]
	Knock-in of ABCA4 via AAV	Stargardt dystrophy	I/IIa	22	3 years	No significant change of VA. 27% of treated eyes showed exacerbation of retinal pigment epithelium atrophy	1 case of chronic ocular hypertension reported	[116]
	Knock-in of CHM via AAV	Choroideremia	I/II	15	2 years	No significant differences in light-adapted sensitivity	Acute fovea thinning and macular hole in 2 patients	[115]
	Knock-in of REP1 via AAV	Advanced choroideremia	II	6	2 years	Sustained improvement and maintenance of VA in high doses	No serious AEs occurred	[168]
Cell therapy								
	RPE derived from ESC	AMD	I	2	12 months	Great improvement of visual acuity	No AEs related to injection was reported. Both patients had worsening of diabetics related to oral Prednisone	[169]
	RPE derived from ESC	AMD and Stargardt's macular dystrophy	I/II	18	A median of 22 months	Improvement in visual acuity and Vision-related quality of life in most individuals	No evidence of adverse proliferation, rejection, or serious adverse events	[170]
	RPE derived from ESC	Advanced Stargardt disease	I/II	12	12 months	Borderline improvement in visual acuity and no significant change of quality of life	No uncontrolled proliferation or inflammation. Retinal thinning and decrease of contrast sensitivity in 1 patient	[171]
	Cells derived from human umbilical tissue	Geographic atrophy secondary to AMD	I/IIa	35	12 months	Significant improvement of VA	No immune rejection or tumor formation. Retinal detachment (17%) and retinal perforation (37%) were reported	[172]

Table 2 (continued)

Agent	Indication	Phase	Patient number	Follow-up period	Main outcomes	Adverse events	Refs
Cells derived from human umbilical tissue	Geographic atrophy secondary to AMD	IIb	21	12 months	No apparent effect in the improvement of VA or area of atrophy	Mild ocular AEs, including conjunctival or retinal hemorrhage and vitreous floaters were reported	[173]
RPE derived from ESC	Stargardt disease	I	3	3 years	The VA stayed stable or improved, which was superior to the natural course	No serious AEs occurred	[174]

AAV adeno-associated virus, *VA* visual acuity, *AE* adverse event, *ESC* embryonic stem cell, *AMD* age-related macular degeneration

poorly to conventional anti-VEGF therapy received a single dose of subretinal RetinoStat®. The expressed proteins remained detectable in the aqueous humor during the 12-month observation period [99]. FDA approved one subretinal gene therapy in 2017, the AAV2-mediated voretigene neparvovec-rzyl (Luxturna®), for the treatment of inherited RPE64 mutation-associated retinal diseases [100].

The physiological environment of the subretinal space also favors cell survival and differentiation [101]. Early experimental studies demonstrated that human fetal retinal cells injected into the rat subretinal space could differentiate into rods and cones with inner and outer segments, synaptic terminals, bipolar cells, and amacrine cells with typical conventional synapses in corresponding regions [102]. Lately, experimental and clinical data showed that embryonic stem cells, mesenchymal stem cells, or induced pluripotent stem cells (iPSC) given into the subretinal space could rescue certain physiological functions [94]. For example, RPE cells derived from human embryonic stem cells and injected into the subretinal space of a porcine model could partially restore the anatomy and function of host photoreceptors [103]. More data confirm that cell therapy has great potential to treat currently incurable retinal degenerative diseases that cause poor vision and blindness [104]. A recent report of 5-year follow-up results of patients with early-stage Stargardt disease receiving subretinal injections of human embryonic stem cell-derived retinal pigment epithelial cells indicated that the injection resulted in durable effect of visual and functional improvement and was well tolerated in the long term [105].

The subretinal space is an immune-privileged site that is able to tolerate the introduction of antigens without eliciting an inflammatory immune response [106]. Cells can often survive for an extended period of time without rejection occurring, and immunosuppression may not be needed for subretinal therapy, especially for embryonic stem cells and mesenchymal stem cells [107]. A postmortem pathology examination of a patient who had advanced geographic atrophy secondary to dry AMD and who received subretinal injections of RPE derived from allogeneic human embryonic stem cells with mismatched human leukocyte antigen (HLA) class I molecules revealed no detectable intraocular or serologic immune response [108]. However, some reports suggested that subretinal injection of iPSCs could trigger local immune responses [109]. Activation of resident microglial cells and local innate immune responses may also reduce the survival rate of donor cells [107, 110]. Some studies applied immunosuppressants for a certain period in such cases [111]. Besides, some studies of subretinal gene therapy reported a higher incidence of inflammation compared to intravitreal injection, potentially due to damage associated with the injection procedure [78, 112]. Subretinal gene therapy may

also lead to the formation of intraretinal hyper-reflective foci, an infrequent but severe inflammatory response that can lead to irreversible visual loss. But the mechanisms are poorly understood [113]. A better understanding and mitigation of immune risk factors that induce host immunity is the key to achieving better clinical results [114].

Compared with other intraocular injections, subretinal injection is harsher. It directly places the materials between the RPE and the photoreceptors, which leads to a certain degree of retinal detachment. The volume of delivered material therefore should be limited to minimize the severity of the detachment. Hemorrhage is another risk that can lead to further detachment. Some severe ocular adverse events reported in previous clinical trials, including macular holes, choroiditis, retinal tear, and fovea thinning, tend to be associated with the injection procedure [115–117]. These risks may limit its application in drug delivery which could be delivered more conveniently by intravitreal or periocular injections. As seen in Table 2, subretinal injection is commonly applied for gene therapy and cell therapy, which are not candidates for conventional drug delivery approaches. A recent first-in-human clinical trial of robot-assisted subretinal drug delivery of plasminogen activator was conducted on 6 patients with acute subfoveal hemorrhage. The procedure demonstrated a better operative precision and minor retinal microtrauma compared to the conventional subretinal injection method [118].

The vitreous humor as a reservoir for sustained drug delivery

The human vitreous humor is a large pool in the eye with a volume of 4–5 mL in adults [119]. In the gelatinous vitreous body, there is a fibrous structure of collagens and glycosaminoglycans which forms a loose network with a predominant anterior–posterior orientation, particularly in young adults [120, 121]. The average pore size of vitreous fibers is 550 nm as estimated [122]. Small molecular drugs can diffuse freely while large biologic drugs diffuse slower, and therefore, biologics often have longer intravitreal half-lives [122, 123] as clinical data showed that the intravitreal half-life of Ranibizumab (48 kDa) and Bevacizumab (149 kDa) is about 3–5 days, much longer than small molecular drugs that generally last for several hours [124–126]. The hyaluronic acid and heparin sulfate contents in the vitreous body play a major role in the electrostatic interactions with drugs [127]. Experiments show that large particles with anionic surface modifications of carboxyl groups have significantly higher mobility in the vitreous compared to those with cationic modifications of amine groups [128, 129]. Therefore, drugs with larger molecular weight and cationic modification theoretically have a longer retention time in the vitreous.

Adding a large conjugate to the drug molecule has been one strategy to increase the drug retention time in the vitreous humor. KSI-301 is a humanized anti-VEGF antibody conjugated to an optically clear phosphorylcholine polymer (800 kDa), which forms a huge drug molecule (950 kDa). The huge molecule has a half-life of 10 days in the rabbit vitreous humor [130, 131]. This extended retention time in the eye supported the design of longer dosing intervals. The first phase II/III clinical trial in nAMD patients designed dosing intervals of 12, 16, or 20 weeks compared to 8 weeks for standard Aflibercept therapy (NCT04049266). The study unfortunately did not show comparable visual improvement as that of Aflibercept. The biotech Kodiak Sciences believes that the failure may be related to the long dose intervals. The company is conducting more clinical trials including nAMD, diabetic macular edema, and retinal vein occlusion. And the dosing intervals cover 8 to 20 weeks (https://ir.kodiak.com/static-files/62db4322-c626-4d39-bf44-2a4cec62f5c0). These new trial results will help to further understand the relationship between dosing intervals, pharmacokinetics, and efficacy.

Intravitreal implants are designed to extend the drug release time in the eye. The first approved implant, Vitrasert®, released Ganciclovir in the vitreous humor for more than 7 months at a rate of 1 μg/h. It effectively controlled the progression of cytomegalovirus retinitis in acquired immune deficiency syndrome (AIDS) patients for up to 8 months. But this non-degradable implant requires the surgical procedure for its implantation and removal [132, 133]. The first biodegradable implant Ozurdex® contains 700 mg of Dexamethasone and can maintain Dexamethasone in the vitreous humor for 6 months [134]. This tiny implant (0.46 mm in diameter) can be injected into the vitreous cavity and therefore is convenient to give to patients during their outpatient visits [135]. Another injectable intravitreal implant Iluvien has a core containing 0.19 mg of fluocinolone acetonide and a polyimide outer shell to control the rate of drug release. The device achieved zero-order release for up to 3 years after a burst release in the first few weeks [136]. A small molecular tyrosine kinase inhibitor, Axitinib, packed in a hydrogel implant was tested in a phase I trial on patients with nAMD. The implant demonstrated good tolerability and sustained effect for up to 13 months in many subjects [137].

For large molecular drugs, controlling their release rates from implants is more challenging. The implant needs to have pores of appropriate size to allow a certain amount of the large molecules to leak out of the matrix [138]. In addition, loss of protein activities in the long term is also a challenge. An intravitreal implant containing Ranibizumab used a sealed nanoporous membrane with a desired diameter of 10 mm, which could continuously release the drug for more than 12 weeks in animal eyes without obvious immune reaction [139]. Many attempts have been made to

improve protein stability and durability. Currently, intravitreal implants have enabled sustained release of anti-VEGF biologics for more than 6 months in animal eyes by using techniques such as ion pairing, hydrophobic encapsulation, solid-state nanoparticles, liposomes, and hollow cylinders. Fatemeh et al. proposed the ion pairing method to maintain protein activities of intravitreal Ranibizumab implants. The PLGA-based implant of Ranibizumab could achieve first-order drug release kinetics and release 90% of the drug within 1 month [140]. By encapsulating solid-state protein microparticles into hydrogel implants, the protein activity of anti-VEGF biologics was found to be maintained for several months in vivo and demonstrated good tolerability in monkey eyes [141, 142]. The intravitreal Aflibercept concentration remained above the therapeutic level for more than 3 months in African green monkeys [143]. The liposomal formulation is another tested option for sustained release. By trapping Bevacizumab-loaded liposomes in hydrogels and drug depot implant, the drug could achieve extend release for 8 months in rabbit eyes [144]. An intravitreal protein reservoir is becoming a more popular choice due to easier control of protein release and larger loading capacity. A hollow hyaluronic acid intravitreal cylinder with the outer radius size of 1 mm containing antigen-binding fragments was tested to achieve sustained drug release at 4 μg per day for more than 4 months [145]. A polycaprolactone reservoir-based thin film intravitreal device was designed for sustained protein release and has been tested on non-human primates. The device could be adjusted to achieve zero-order release for up to 6 months and was found to be well-tolerated in monkey eyes [146].

The port delivery system (PDS) is a novel invention that can refill drug solutions. This tubular device has two ends: the inner end is inserted into the vitreous cavity for drug release, and the outer end is sutured onto the sclera to reload the drug. In the phase III Archway study, Ranibizumab delivered by the port delivery system with a refill interval of every 24 weeks had equivalent efficacy to that of monthly intravitreal injections of 0.5 mg Ranibizumab in patients with nAMD. However, the PDS group had higher ocular adverse events (19.0%) compared to the intravitreal injection group (6.0%), which were considered to be associated with the implantation procedure [147]. The device was approved by FDA recently as the first refillable intravitreal implant.

With the development of material science, more smart intravitreal drug delivery devices are being explored. On-demand drug delivery devices or smart drug delivery devices have a sensor to detect external stimuli, such as light, pH, magnetic field, electric currents, pressure, or drug concentration, which can trigger the drug release [148–151]. A light-sensitive on-demand implant made of photosensitive nanoparticles could respond to laser light and trigger the release of drug molecules from degraded nanoparticles [152]. Some potential smart implants could even respond to internal signals, such as intraocular pressure and inflammation [153]. One smart intravitreal implant carrying Indomethacin and Ciprofloxacin by chitosan-containing nanoparticles could react to hydroxyl radicals and boost drug release under inflammatory conditions. MicroPump Drug Delivery System is the first ocular on-demand system tested in patients' eyes (Fig. 4). The intravitreal implant has a 60 μL drug reservoir with an electrode that can induce the electrolysis of water. When the pressure in the drug reservoir exceeds the threshold, drug molecules are released from the reservoir and enter the vitreous cavity. The device can be remotely controlled by a handheld telemetry system and reliably control drug release for over 100 times, corresponding to 8 years of lifetime if drugs are given monthly. A preclinical study with 11 dogs was followed for 1 year with an acceptable profile [154]. A pilot clinical study was conducted on 11 patients with DME and followed for 90 days. All patients showed a normal healing process after surgical implantation without serious adverse effects. The device delivered Ranibizumab at a programmed dosage in 7 patients, but at a lower dosage in 4 patients who received additional intravitreal injections [155].

In short, the vitreous humor is an important drug reservoir in the middle of the eye. Its large volume and loose structure provide a large space to maintain biological drugs or place implant devices in the center of the eye. Its relatively static nature allows immunogenic drugs or implants to survive a long time without immunological problems. Its physiological nature permits the use of external signals such as light to trigger drug delivery in future smart systems.

Considerations about the innovative drug delivery systems and clinical translation

Quite a few novel drug delivery means, including microneedles for suprachoroidal injection (XIPERE™), subretinal gene therapy (Luxturna®), intravitreal implants (Vitrasert®, Ozurdex®, Iluvien®, and Vitrasert®), and the port delivery system have received approval and readily become available weapons for retinal specialists. In recent decades, another revolutionary breakthrough for fundus diseases is the approval and wide application of anti-VEGF biological drugs and their biosimilars, which have become first-line therapy for many proliferative retinal diseases. A lot of work has been done to combine the advantage of novel drug delivery methods with biological drugs. However, the delivery of biological agents to the retina is far more challenging than small molecular drugs, and many unmet challenges need further exploration. Topical delivery and systemic delivery, which have been extensively explored for small molecular drugs, are generally ineffective for the delivery of large biological agents due to the corneal epithelial barrier. For long-term drug release,

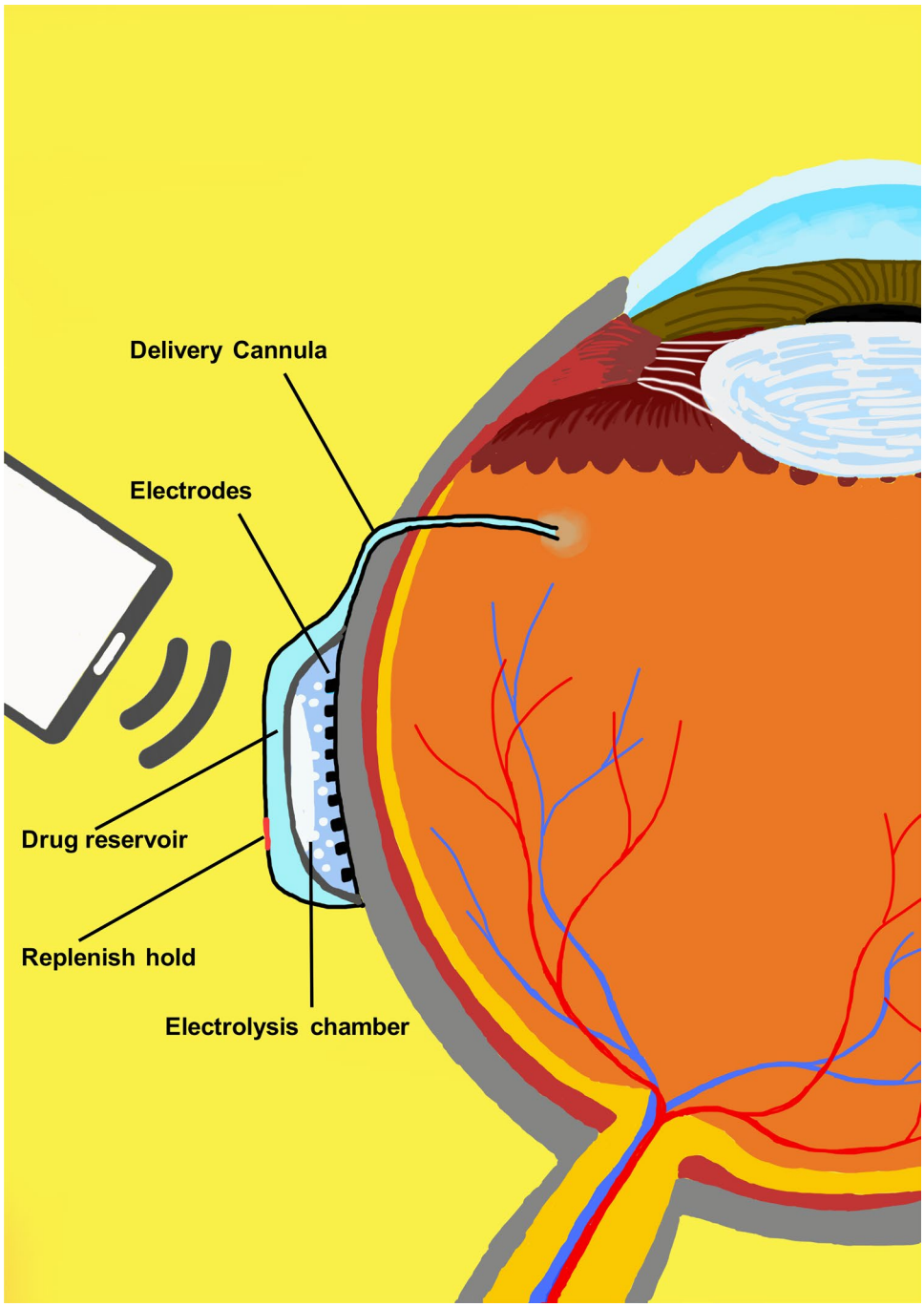

Fig. 4 Schematic drawing of drug delivery by MicroPump Drug Delivery System. The on-demand drug delivery system consists of drug reservoir, replenish hole, electrodes, hydrolysis chamber, and cannula to the vitreous cavity. It is sutured on the scleral wall. The drug reservoir connects with the electrode which can induce electrolysis of water into hydrogen and oxygen. When the pressure drug reservoir exceeds the threshold of the check valve, drug molecule is released from the reservoir and enters the vitreous cavity through the cannula. The device is remotely controlled by a handheld telemetry system

a controlled release system is simply not enough, and efforts should be taken to maintain drug activity in the long term. In addition, common intravitreal solid implants lack sufficient drug loading capacity and generally have shorter drug release time compared to small molecular drugs. Another great trouble for biological agents is the potential drug toxicity and immunological response, which is not sufficiently understood and can hardly be predicted. Recently, cluster cases of retinal vasculitis after intravitreal injection of brolucizumab indicate a closer look at drug safety issues of intraocular biological agents [156]. Some invasive approaches, including subretinal injection, suprachoroidal injection, and implantation of intravitreal devices, may further boost immunological response and tissue damage due to the exposure of ocular antigens during the operation. More clinical data are needed to guide their translation.

Systemic drug delivery to the retina by transporter-targeted prodrug approach, melanin-binding approach, endocytosis approach, and transient opening of the BRB are appealing ways of noninvasive retinal drug delivery, which are generally

Table 3 Comparison of different retinal drug delivery methods

Methods	Administration	Merits	Suitability	Limitations and challenges	Safety concerns	Current phases and available products
Transporter-targeted prodrugs	Systemic Repetitive Frequently	Systemic administration Possible for repetitive use for chronic diseases	Small molecular drugs	May not be suitable for large molecules Degradation before it enters the eye	Exposure to other organs with high expression of the transporters	Preclinical testing in vivo No approved products
Melanin-binding drugs	Systemic Repetitive Frequently	Systemic administration High exposure to RPE area Possible for repetitive use for chronic diseases	Small molecular drugs with melanin-binding ability. Often hydrophobic and basic molecules	May not be suitable for large molecules Drug molecules must be able to get into melanosome Drug molecules must be able to release from melanosome and maintain the biological activity	Exposure to other organs containing melanin Potential retinal toxicity due to high local exposure	Preclinical testing in vivo No approved products
Endocytosis approach	Systemic Repetitive Frequently	Systemic administration Can have high delivery efficiency and sustained release with the help of nanocarriers	Small molecular drugs	May not be suitable for large molecules May have insufficient retinal specificity	Exposure to other organs with active endocytosis Potential toxicity of degraded nano-materials	Preclinical testing in vivo No approved products
Transient opening of BRB	Systemic Infrequently	Systemic administration	Small molecular drugs Large molecules up to 1 kDa	Potential retinal damage if conditions not properly controlled May not suitable for frequent use in chronic diseases	Risk of exposure to pathogens Impairment of BRB	Preclinical testing in vivo No approved products
Suprachoroidal space injection	Locally Invasive Infrequently	Wider exposure area in the fundus as drugs can move along the space Fast choroid exposure of injected drug/gene/cell	Small molecular drugs, large molecules, genes, cells	Technical requirement (needle, control of the injection pressure) High resistance and limited volume capacity due to the connection of the tissue and narrowness of the space Often have short exposure time for soluble drugs due to fast excretion How frequently one can use the injection is unclear yet	Injection procedure-related lesions Potential immunological responses	Preclinical testing in vivo Clinical trials Product: XIPERE™
Subretinal injection	Locally Invasive Infrequently	Fast and focused exposure to the retina and RPE Better quantitative control of injected materials Gene expression can extend to the inner retina Low immunological response due to immune privileged state of retina	Genes, cells, and transplants	May require surgery process The volume should be limited due to development of retinal detachment Often not use for drug delivery, particular small molecules	Injection related reversible and non-reversible lesions Risk for IOP elevation	Preclinical testing in vivo Clinical trials Product: Luxturna®

Table 3 (continued)

Methods	Administration	Merits	Suitability	Limitations and challenges	Safety concerns	Current phases and available products
Intravitreal injection	Locally Invasive Infrequently	Easy to perform Drug stays in the vitreous for days and often have effect for 1–3 months Can change to other drugs during treatment	Small molecules Large molecules	The concentration of drug molecules can decrease fast Immunological response and high systemic exposure	Have low risk of procedure-related infection and/or lesion	Preclinical testing in vivo Clinical trials Products: intravitreal antibiotics, steroids, and biological drugs
Intravitreal devices	Locally Invasive Occasionally	Sustained and controlled drug release Less invasive as they have long term effect Some are refillable (for PDS) or even biodegradable	Small molecules Large molecules	Not for fast degraded molecules Consistent drug release	Immunological response Procedure-related damage	Preclinical testing in vivo Clinical trials Products: Vitrasert®, Ozurdex®, Iluvien®, and Vitrasert®, PDS

suitable only for small molecular drugs. However, the majority of new advancements stay in the preclinical stage. The greatest challenge is to achieve high retinal specificity and avoid untargeted delivery to other tissues and degradation by hepatic enzymes and the reticuloendothelial system. Many drug delivery methods have shared mechanisms with other systems, such as the transient opening of BRB with the BBB and the melanin-binding approach with other pigmented tissues. More efforts should be done to achieve targeted drug delivery to the retina and explore drug toxicity to corresponding organs. On the other hand, enrichment and deposition of drugs in the retina may also cause retinal toxicity, as seen in the case of many melanin-binding drugs. Currently, many proof-of-concept studies only focus on short-term efficacy and explore drug pharmacokinetic properties to demonstrate the efficiency of retinal delivery. For chronic ocular diseases, the safety of the eye and other organs in the long-term application of systemic drugs to the retina needs to be further studied, especially for the invasive approach of transient opening of BRB.

Summary

Multiple ocular structures have barrier functions that physically protect the retina from being exposed to foreign molecules including drugs. Therefore, currently available drugs are delivered by invasive methods into the eye. Innovative drug delivery systems have been continuously explored to meet clinical needs. In this review, we reviewed anatomical and physiological features of ocular barriers, their physiologic roles, and their application as gates for drugs or as depots for biologics, genes, and cells (summarized in Table 3). The innovative work on transporter-targeted prodrugs, melanin-binding drugs, cell endocytosis, and transient opening of BRB has been explored as retinal drug delivery tools, particularly for small-molecular drugs. The different ocular spaces such as suprachoroidal space, subretinal space, and intravitreal cavity have their features as intraocular depots for retinal biologics, genes, or cell therapy. This insightful review help readers understand different approaches to overcome the challenges of efficient drug delivery to the retina based on the unique anatomical and physiological features of the ocular barriers.

Abbreviations AAV: Adeno-associated virus; ABC: ATP-binding cassette; AIDS: Acquired immune deficiency syndrome; AMD: Age-related macular degeneration; AUC: Area under the curve; BRB: Blood-retinal barrier; BBB: Blood-brain barrier; iBRB: Inner blood-retinal barrier; oBRB: Outer blood-retinal barrier; CNS: Central nervous system; CNV: Choroidal neovascularization; DR: Diabetic retinopathy; FDA: Food and Drug Administration; GFP: Green fluorescein protein; HLA: Human leukocyte antigen; iPSC: Induced pluripotent stem cells; LAT1: L-type amino acid transporter 1;

mTOR: Mammalian target of rapamycin; PDGF: Platelet-derived growth factor; PDS: Port delivery system; PLGA: Poly (lactic-*co*-glycolic) acid; RPE: Retinal pigment epithelium; SLC: Solute carrier; siRNA: Small interfering RNA; shRNA: Short hairpin RNA; VEGF: Vascular endothelial growth factor

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