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Teaser Sustained release formulations administered via the periocular route could provide a more effective approach to treating posterior segment diseases compared with topical drug administration.

Depot formulations to sustain periocular drug delivery to the posterior eye segment

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The periocular space is a promising alternative route for the delivery of drugs to the posterior eye segment, especially when treating conditions in the outer ocular layers. In this review, we discuss the different periocular routes as well as the physiological barriers and elimination mechanisms limiting drug bioavailability at the back of the eye. We then highlight various types of depot formulations, including particulate delivery systems, semisolid formulations, and implants, used to increase the contact time with the ocular tissues. With the additional advantage of sustaining drug release, such depot formulations could enhance periocular drug delivery to the posterior eye segment.

Introduction

Many of the common diseases affecting the posterior eye segment can lead to irreversible visual impairment. Posterior eye diseases accounting for most cases of vision loss worldwide include age-related macular degeneration (AMD), diabetic retinopathy, glaucoma, and retinitis pigmentosa. These conditions are most prevalent in the older population and, because of a globally aging population, the number of patients affected is expected to grow rapidly [1]. Posterior eye diseases are often multifactorial in origin and are typically chronic; thus, successful therapy requires continuous delivery of effective drug doses to the target site. Given its isolated location, one might assume that the eye is an easily accessible organ for drug administration. However, the eye is equipped with numerous physiological and anatomical barriers that substantially restrict drugs from reaching tissues in the posterior segment.

Drug delivery to the back of the eye can be achieved via topical, systemic, intravitreal, and periocular routes. Topical administration is non-invasive and, thus, is the most common method used; however, it results in negligible drug bioavailability at the back of the eye [2]. This is primarily because of the short contact time of the formulation with the ocular surface resulting from extensive and rapid precorneal loss as a result of nasolacrimal drainage and tear turnover. Moreover, drug absorption into the conjunctival blood flow can decrease drug access to the posterior tissues [3]. Systemic administration is another commonly used route,

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although efficient delivery to the ocular target site is limited by the blood–retinal barrier (BRB), which restricts the movement of molecules from the systemic circulation into the retina. As a result, the systemic route requires large doses of drug to be administered to achieve therapeutic concentrations in the eye, which can lead to adverse effects elsewhere in the body [4]. Currently, the most effective means to deliver drugs to the posterior eye segment is via direct intravitreal administration, which involves injection or insertion of drug formulations and devices into the vitreous humor [5]. However, this procedure is invasive and can be accompanied by various complications, including hemorrhage, endophthalmitis, and retinal detachment [6]. Furthermore, most low-molecular-weight drugs injected as a solution are rapidly cleared from the vitreous, which increases the need for frequent injections to maintain therapeutic effectiveness.

The periocular route is an alternative approach to deliver drugs to the posterior eye segment. This route involves administering drugs as a depot into tissues and spaces surrounding the eye, from where they can slowly permeate through the sclera and into other posterior segment tissues. The periocular route is considerably less invasive than intravitreal injection and can provide local delivery of even high-molecular-weight substances to the back of the eye. Compared with the cornea, the human sclera has a larger surface area (~16.3 cm^2) and is more porous, which generally facilitates transport of drugs across the tissue [7,8].

Structure of the sclera

The sclera is a white, viscoelastic connective tissue comprising irregularly arranged dense collagen fibers. This arrangement gives the sclera its opacity and elasticity, allowing it to have a key role in maintaining the shape of the eyeball. The sclera also offers support and protection for the inner parts of the eye during variations in intraocular pressure and eye movements, which would otherwise adversely affect the visual process [9]. Collagen type I is the predominant protein comprising the scleral extracellular matrix and accounts for 95% of the total scleral collagen. The remaining 5% comprises collagen types III, V, VI, and VII. Proteoglycans are the most abundant molecules trapped within the collagen fibrils. They have a strong negative charge and are highly hydrophilic [10].

Structurally, the sclera can be divided into three layers: the episclera; the scleral stroma (also known as sclera proper); and the lamina fusca (Fig. 1, top inlay) [9]. The episclera, the outermost region of the tissue, is a densely vascularized, thin layer of connective tissue comprising collagen fibers, melanocytes, and a few macrophages. The episclera has a large supply of blood vessels as well as a few myelinated and unmyelinated nerve fibers [10]. The scleral stroma, which comprises most of the tissue, contains irregularly arranged collagen bundles, elastic fibers, fibroblasts, proteoglycans, and glycoproteins. The scleral stroma does not contain any blood capillaries and derives its nutrition from the episcleral and choroidal vascular networks. The lamina fusca, the

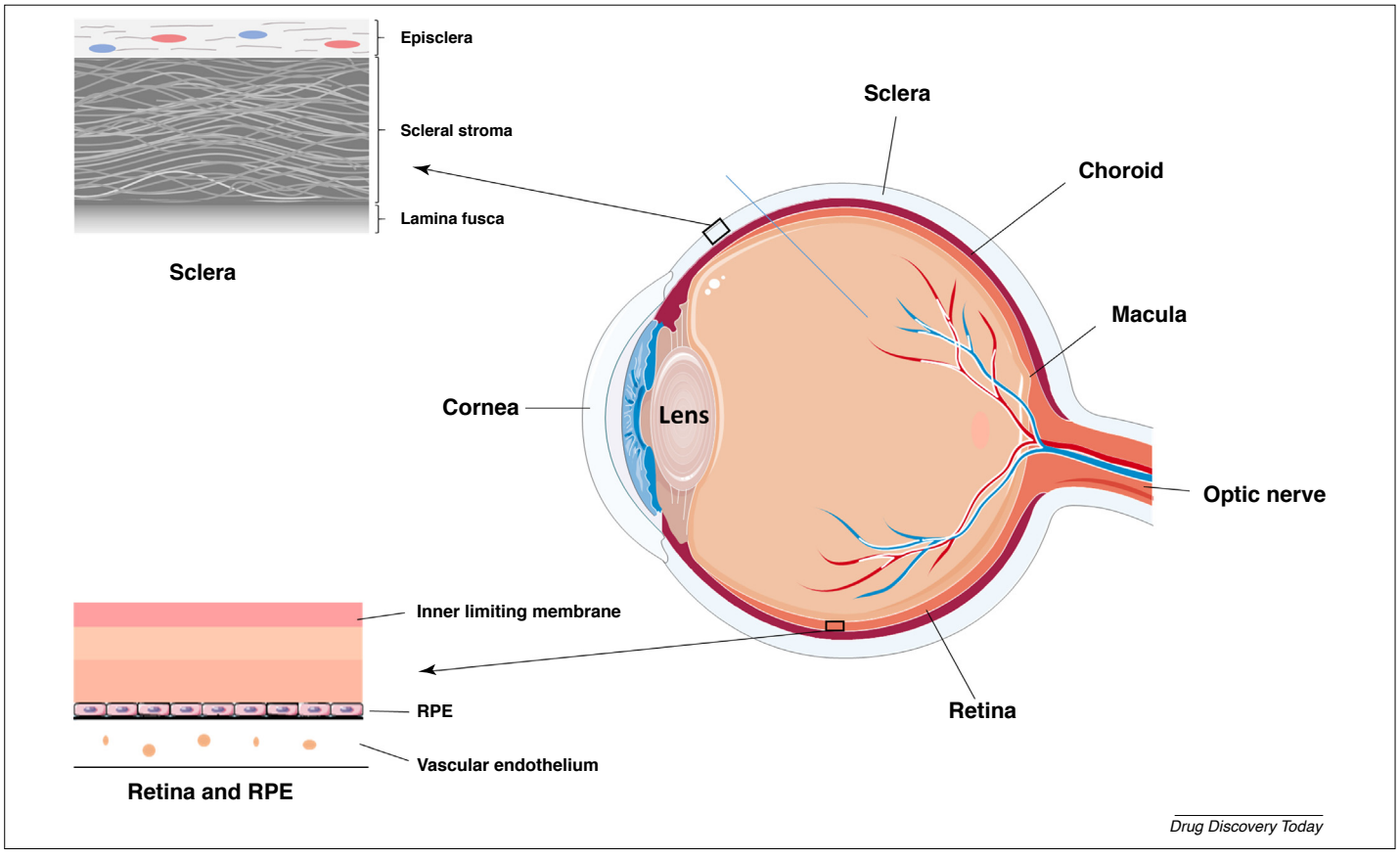

FIGURE 1

Important anatomical structures of the eye and detailed schematic of the static barriers affecting periocular drug delivery. Abbreviation: RPE, retinal pigment epithelium.

thin innermost scleral layer, is characterized by the presence of pigmented cells or melanocytes that migrate mostly from the choroid, and also comprises loosely arranged collagen bundles. The lamina fusca is separated from the choroid by a thin suprachoroidal space [10]. Finally, a thin membrane comprising compact collagen bundles and known as Tenon's capsule, surrounds the eye globe from the optic nerve to the corneal limbus. It is separated from the episclera by the periscleral lymph space, which forms a smooth inner surface for ocular motility [11].

Periocular drug delivery routes

In periocular delivery, drugs are placed into the spaces immediately surrounding the eye. Given the proximity to the sclera, drugs given via this route can penetrate the ocular tissues and into the vitreous 20–30 min after administration [12]. The periocular route combines high efficacy and relatively low patient discomfort. It is widely used for anesthesia during ocular surgery, although it can also be used for the delivery of other therapeutics. Delivery via this route can be divided into subconjunctival, sub-Tenon, retrobulbar, peribulbar, or posterior juxtascleral approaches (Fig. 2, Table 1).

Subconjunctival

The subconjunctival route can be used to deliver drugs to both the anterior and posterior segments of the eye. To utilize this route, the formulation is placed underneath the conjunctiva, which allows drug molecules to diffuse directly through the sclera. This route overcomes the conjunctival epithelial tight junctions, which can limit permeation of hydrophilic molecules when applied topically [13]; however, other conjunctival and episcleral flow mechanisms still play a major role. To determine the suitability of this route to deliver drugs to the retina, a clinical study compared the concentration of dexamethasone in subretinal fluid after subconjunctival injection (2.5 mg dexamethasone disodium phosphate) to that after peribulbar injection (5 mg dexamethasone disodium phosphate) and oral administration (7.5 mg dexamethasone). Results showed that patients receiving the subconjunctival injection exhibited the highest subretinal dexamethasone concentrations (359 ng/ml), despite the overall lower dose given, compared with the peribulbar or oral routes, which resulted in subretinal dexamethasone concentrations of 82.2 ng/ml and 12.3 ng/ml, respectively [14].

Sub-Tenon

Sub-Tenon delivery involves injection of the formulation into the sub-Tenon space. For this procedure, the eyelids are typically retracted with a lid speculum and with the patient directed to look in the position that best exposes the capsule. The conjunctiva and capsule are incised and a blunt-tipped cannula is advanced into the sub-Tenon space before the drug solution is injected in a controlled manner. This route is commonly used to administer local anesthesia during ocular surgery because the risk profile is lower than when using sharp needles or cannulas to enter the peribulbar compartment [15].

Retrobulbar

For this route, the drug is injected into the retrobulbar space, which is located within the muscle cone, inside the four rectus muscles and their intramuscular septa. A blunt needle that penetrates no further than 1.5 cm behind the globe is usually used to avoid optic nerve injury [8]. A comparison study between retrobulbar and sub-Tenon anesthesia in patients undergoing cataract surgery showed no significant difference between the two in terms of onset and effectiveness of akinesia [16].

Peribulbar

The peribulbar approach has fewer complications than those associated with retrobulbar injections, although it is more likely to result in intraocular pressure elevation compared with the retrobulbar route. With this approach, the drug is injected external to the four rectus muscles and their intramuscular septa [8].

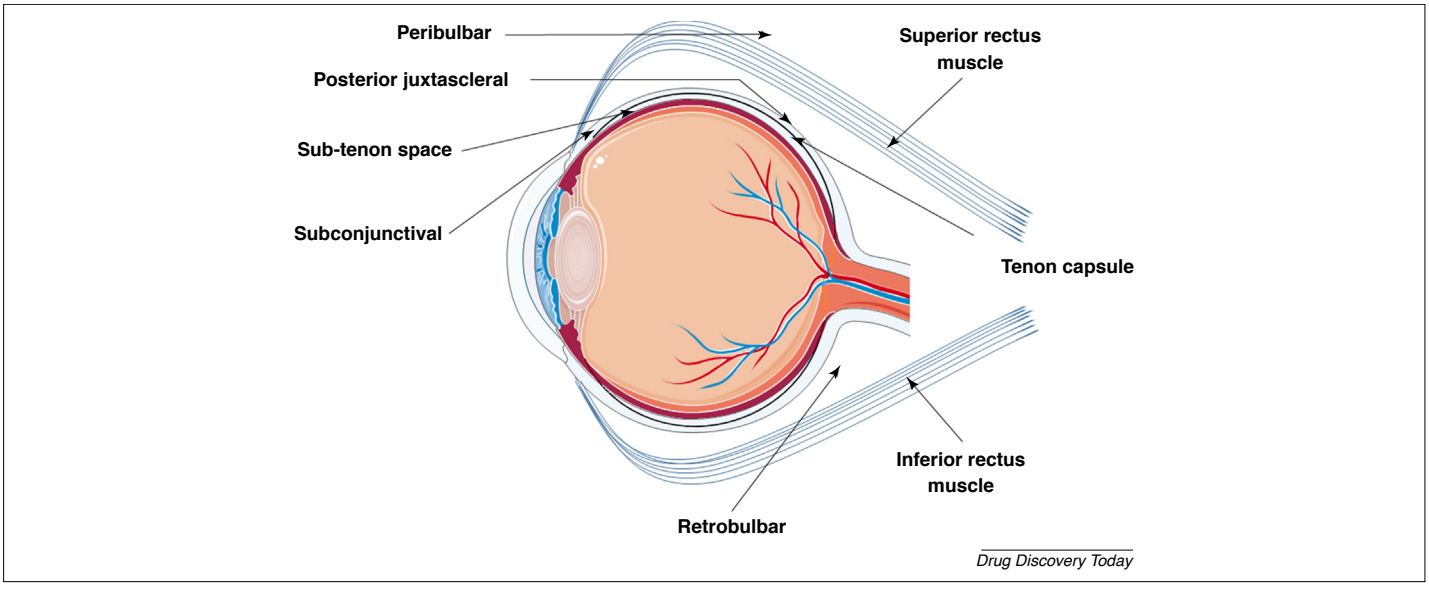

FIGURE 2

Schematic of the ocular structures highlighting the various periocular drug delivery routes.

TABLE 1

Summary of different periocular routes[a]

Periocular route	Needle specification			Current usage	Advantages	Limitations
	Gauge	Volume	Length			
Subconjunctival	25–30	up to 0.5 ml	30 mm	Administration of various drugs; e.g., antibiotics and corticosteroids	Can also be used for drug delivery to anterior eye segment	Subconjunctival hemorrhage; can be avoided with use of cannula needle
Subtenon	26	up to 4 ml	25 mm	Commonly used for anesthesia during ocular surgery, but also for delivery of other drugs, such as long-acting steroids	Drug is in contact with sclera for a long time because of its avascular nature	Possibly rapid removal of drug by choroidal circulation; hemorrhage, which can be avoided by use of cannula needle
Retrobulbar	25 or 27	up to 5 ml	38.1 mm	Commonly used for anesthesia during ocular surgery	Minimal to no effect on intraocular pressure	Most painful due to blunt needle use; places optic nerve at risk of injury; this can be avoided by ensuring that needle does not penetrate >15 mm behind globe
Peribulbar	25	8–10 ml	31.7 mm	Less popular for routine anesthesia but still used for complicated ocular surgery	Safer than retrobulbar route; allows placement of higher drug volumes	Less effective than retrobulbar injections in anesthetizing globe; care should be taken to avoid perforation of globe
Posterior juxtascleral	56° curved needle	0.5 ml	N/A	Delivery of anecortave acetate suspension	Well tolerated; targets macula; longer duration of drug action	Requires surgical expertise; more complex than other periocular routes because curved needle is used

[a] Based on Refs [4,8,19].

Peribulbar anesthesia is regarded as being less efficacious than the retrobulbar route, which could be clinically significant when the objective is to provide anesthesia and/or akinesia.

Posterior juxtascleral

This route is the most recently established periocular approach and was developed by Alcon Laboratories to deliver drug formulations to the macular region [17]. It was specifically designed to deliver anecortave acetate for the treatment of subfoveal neovascularization in patients with AMD. The custom-designed cannula used for the anecortave study was curved to 56°, with the length of the cannula designed to position the drug directly behind the macula. This delivery route has not been linked to any globe injury and is well tolerated [18].

Factors affecting scleral permeation

Although the sclera is highly permeable with a relatively large surface area in contrast to the cornea, transscleral permeation is influenced by various factors. These include physicochemical properties of the drug, such as molecular size, charge, and lipophilicity, along with scleral properties, such as thickness and hydration.

At physiological pH, the scleral matrix has an overall negative charge [20]. This can result in charge-based interactions between the permeant and the sclera and possible electrostatic repulsion of negatively charged drugs further into the eye [21], whereas positively charged drugs can bind and get retained close to the surface. This was previously confirmed in bovine and porcine sclera, where negatively charged solutes showed higher permeability than those with a positive charge [22], although a more recent study by Ranta *et al*. [23] using computational modeling showed that the highly negative charge of oligonucleotides can also slow their scleral permeation. The lipophilicity of drug molecules can also influence their scleral permeation. A study looking at the correlation between drug lipophilicity and human scleral permeation showed that molecules with higher lipophilicity exhibited stronger binding to the sclera and, thus, a longer transport time across the tissue [21]. However, this might be insignificant when the drug is incorporated into a depot formulation, where lipophilicity has shown little effect on steady-state tissue permeability. Being a highly porous tissue, scleral permeability also depends on the permeant molecular weight and radius, although this effect is less pronounced than in membranes with tight junctions, such as the retinal pigment epithelium (RPE). Generally, studies looking at transscleral delivery of different-sized molecules have shown that the molecular radius is a better predictor of scleral permeation than is molecular weight or lipophilicity [24,25]. Especially for larger (>100 kDa) hydrophilic molecules, the radius is the predominant factor affecting their transport across the sclera [26–28]. As such, when comparing the transscleral permeation of proteins and polysaccharides with comparable molecular weights, polysaccharides with flexible structures showed higher permeability compared with rigid proteins [25].

Another important factor is the non-uniform thickness of the human sclera, averaging ~500 μm at the limbus and ~1000 μm at the optic nerve [7], which can also be affected by age, gender, species, and various diseases. For example, porcine sclera is approximately twice as thick as human sclera and, as a result, has a

threefold lower permeability coefficient for both low- and high-molecular-weight compounds [29]. The scleral thickness also differs between genders, with females generally having a thinner sclera than males [7], highlighting the importance of taking these factors into account when performing preclinical drug permeation studies. Finally, different diseases can also affect the scleral structure or physiology; however, there is currently insufficient literature data investigating the effect of different disease states on scleral drug permeation.

Barriers to periocular drug delivery

The primary pathway for drug transport across the sclera is by passive diffusion. However, a large portion of the drug molecules might also traverse to the conjunctival, episcleral, or choroidal vessels and reach the systemic circulation. Within these different pathways, the drug can encounter several barriers before reaching posterior segment tissues. These transscleral barriers can be divided into static, dynamic, and metabolic barriers, as briefly outlined below. For detailed information on barrier analysis with regards to drug pharmacokinetics using a computational model, we refer the reader to the excellent article by Ranta *et al.* [23].

Static barriers

Static barriers are formed by ocular tissues, such as sclera, choroid, Bruch's membrane, and RPE, and must be traversed by drug molecules to reach the retina (Fig. 1). However, when the choroid is the target site, as in the case of choroiditis or choroidal neovascularization (CNV), drugs do not have to cross Bruch's membrane or the RPE, suggesting that periocular delivery could be particularly advantageous in these conditions. After drugs permeate across the sclera, the next static barrier encountered is the choroid and Bruch's membrane [8]. The choroid is a highly vascularized tissue that provides a blood supply to the outer retina, with drug permeability within the choroid similar to that of the sclera [30]. With aging, the choroidal thickness decreases from 193.5 to 83.5 μm, whereas Bruch's membrane simultaneously increases in thickness from 2.0 to 4.7 μm [31,32]. This can alter drug permeation dynamics, as shown by Hillenkamp *et al.* [33], who showed a linear decrease in the diffusion of taurine from 162.7 to 105.9 nmol/h across Bruch's membrane–choroid in patients aged between 10 and 90 years ($P < 0.05$). However, compared with other static barriers, differences in permeation across the Bruch's–choroid complex with age might be insignificant. The RPE is the final permeation-limiting static barrier, having tight junctions between cells that restrict paracellular movement of drugs to the inner retina and, thus, forming a tight physical barrier (Fig. 1, bottom inlay). The barrier formed by RPE cells, commonly referred to as the outer BRB, is significant because it limits the amount of systemic and periocularly administered drugs reaching the retina [3,8].

Dynamic barriers

Dynamic barriers refer to clearance mechanisms that reduce the drug concentration in the ocular tissues. These include choroidal blood flow, conjunctival, and episcleral clearance, as well as transporters and efflux pumps present on the RPE.

Choroidal blood flow

The choroid has high blood perfusion and, therefore, is considered by some as the main barrier to drug permeability into the posterior eye segment [8], although studies by Robinson *et al.* [34] showed that, even after eliminating choroidal blood flow in rabbits, a sub-Tenon injection of triamcinolone acetonide resulted in undetectable drug levels in the vitreous, suggesting that other barriers also play a major role. After periocular injection, most of a drug dose is already removed by conjunctival and episcleral flow factors; thus, only a relatively small fraction reaches the choroid. While lymphatic clearance from the choroid has also been suggested as a possible elimination mechanism, it might not be as significant as clearance by the choroidal blood flow, as demonstrated by Kim *et al.* [35].

Conjunctival and/or episcleral clearance

Several studies have demonstrated that drugs in the conjunctival and episcleral tissues are cleared via blood and lymphatic vessels [36,37]. When sub-Tenon injections of 10 mg triamcinolone acetonide were given to rabbits with an incised 'conjunctival window', higher levels of drug were found in the vitreous than in rabbits without the incision [34]. A study by De Carvalho *et al.* [38] compared retinal sodium fluorescein levels after periocular solution injection and from a unidirectional episcleral silicone explant. Retinal fluorescein concentrations determined by the area under the concentration time curve were found to be 144.4 μg h/ml after administration of the silicone explant containing 5 mg fluorescein, and 3.9 μg h/ml after periocular injection of a 5 mg fluorescein solution. The lower amounts of dye detected in the retina after periocular injection suggest that conjunctival and/or episcleral clearance reduces intraocular drug concentrations and is a more significant barrier compared with choroidal blood flow [34].

RPE transporters

The RPE is characterized by the expression of various transporter proteins because of its major role in solute and ion transport into the subretinal space [39]. These transporters include ion and amino acid transporters as well as drug efflux pumps, all of which might decrease drug penetration into the retina by expelling solutes towards the choroid [40–42], although the physical barrier properties of the RPE, as discussed earlier, have a more significant role. At physiological pH, various drugs exist as anions or cations and, thus, are potential substrates of ion transporters [22,43]. Organic cation transporters have a physiological role in carrying endogenous amines; however, they also transport organic cationic drugs, such as antihistamines, vitamins, and sympathomimetics [43]. Zhang *et al.* [44] also showed that brimonidine, an antiglaucoma drug, is a substrate for organic cation transporters; however, its transport depends on physiological conditions, such as pH and temperature. Amino acid transporters for glutamate, taurine, and many other amino acids have also been found in the human RPE [45,46].

Efflux pumps are also thought to have a significant role in drug permeation across the RPE [43]. The two main efflux transporters are P-glycoproteins (P-gp) and multidrug resistance-associated proteins (MRP), both of which belong to the ATP-binding cassette superfamily [47,48]. P-gp displays a broad substrate specificity, generally eliminating large neutral or cationic compounds [49]. P-gp is expressed on the apical as well as the basolateral membrane of RPE cells [50] and, therefore, could be a significant factor behind the inability of periocularly administered P-gp substrates to achieve therapeutic concentrations in the retina. Examples of drugs that are recognized as P-gp substrates include antibiotics

(ofloxacin and erythromycin) and steroids (dexamethasone and hydrocortisone) [40]. The second major type of drug efflux pumps includes the MRP family, of which MRP1, MRP4, and MRP5 have been found to be expressed in human RPE cells [51]. They are able to transport structurally diverse lipophilic anionic compounds, such as anticancer drugs (6-mercaptopurine) and β-lactam antibiotics (penicillin G) [52,53]. However, information about the expression and function of transporters in the RPE and their involvement as transport barriers remains limited [39,43]; thus, the extent of their role in ocular drug delivery to the posterior eye segment could be greater than currently thought.

Metabolic barriers

Metabolic barriers refer to drug degradation by enzymes, such as cytochrome P450 (CYP450), before reaching the target site [22,54]. In murine and bovine ocular tissues, the highest expression of CYP450 enzymes was found in the RPE [55]. As such, drugs penetrating from the sclera could be metabolized before reaching the inner retina. Lysosomal enzymes also have a broad spectrum of action and, therefore, could lead to the degradation of drugs that passively enter cells [56]. Finally, various other enzyme systems have been found in ocular tissues of animals, all of which could have a significant effect on ocular drug bioavailability [57]; however, further studies are required to confirm this.

Melanin

The presence of melanin in the choroid and RPE can represent another barrier to drug permeation. Melanin has a protective role by binding free radicals, but in turn can also trap drug molecules by simple charge transfer or electrostatic and van der Waals forces [58]. The binding of drugs to melanin depends on their physicochemical properties. Leblanc *et al.* [59] showed that all weakly basic and lipophilic drugs bind to melanin to some extent. This is significant because it includes ~40% of all drugs [59]. Melanin binding alters the availability of free drug at the target site, lowering its therapeutic activity [60,61]. The melanin content significantly varies between species [62]. In addition, human eyes display a full range of pigmentation, which should be taken into consideration during preclinical and clinical studies.

Strategies to enhance periocular delivery

Periocular delivery achieves higher drug levels in the posterior segment compared with topical or systemic routes. However, rapid drug clearance from the site of administration remains an issue. Several approaches have been used to enhance the bioavailability of drugs administered via the periocular route (Table 2). Depot formulations, such as particulate delivery systems, semisolid formulations, and implants, can increase the contact time with the ocular tissue while sustaining the release of the contained drug [63]. This overcomes the rapid clearance of administered drug via the periocular lymphatic system and reduces the dosing frequency.

Particulate delivery systems

Micro- and nanoparticles are particulate delivery systems in which the active drug can be entrapped, encapsulated, absorbed, adsorbed, or attached. If the particle diameter is greater than or equal to 1 μm, they are known as microparticles, whereas if the diameter is less than 1 μm they are referred to as nanoparticles. Both micro- and nanoparticles have been widely used with varying levels of success to sustain periocular drug delivery. Kompella *et al.* [65] compared the release of budesonide from DL-polylactide (PLA) nano- (345 nm, 65% entrapment efficient) and microparticles (3.6 μm, 99% entrapment efficiency) in rats with the budesonide

TABLE 2

Representative list of depot formulations designed for periocular and transscleral delivery

Route	Formulation approach	Drug molecule	Target disease	Experiment/target site	Refs
Subconjunctival	PLGA microspheres	Betamethasone	Diabetic macular edema	Clinical trials/retina	[64]
	PLA nano- and microparticles	Budesonide	Diabetic macular edema	*In vivo* rat/retina	[65]
	PLGA microparticles	Celecoxib	Diabetic macular edema	*In vivo* rat/retina	[66]
	Poly(amidoamine) (PAMAM) dendrimers	Carboplatin	Retinoblastoma	*In vivo* mice/retina	[67]
	Collagen matrix gel	Cisplatin	Retinoblastoma	*In vivo* rabbit/retina	[68]
	ReGel™ (PLGA and PEG triblock copolymer hydrogel)	Ovalbumin-Alexa 647 (fluorescent model macromolecules)	Choroidal and retinal diseases	*In vivo* rat/choroid and retina	[69]
	Poly(NIPAAm-co-Dex-lactate-HEMA) hydrogel	Insulin	Diabetic retinopathy	*In vivo* rat/retina	[70]
Periocular	PLGA microspheres	EYE001 (anti-VEGF aptamer)	CNV	*In vitro* rabbit/sclera	[64]
Subtenon	Carrageenan/ methylcellulose hydrogel	Connexin43 AsODN	AMD	*In vivo* rat/retina	[71]
	Pore forming capsular system made of liquid silicone rubber	Dexamethasone sodium phosphate	Intraocular inflammation	*In vivo* rabbit/vitreous, choroid and retina	[72]
	PAMAM dendrimers	Carboplatin	Retinoblastoma	*In vivo* rat/retina	[73]
Posterior-juxtascleral	Depot suspension	Anecortave acetate	AMD	Clinical trials/retina	[17,19]

TABLE 2 (*Continued*)

Route	Formulation approach	Drug molecule	Target disease	Experiment/target site	Refs
Transscleral	PLGA scleral implant plug	Fluconazole	Fungal endophthalmitis	*In vivo* pigmented rabbit/vitreous	[74]
	PLA scleral implant plug	Ganciclovir	Cytomegalovirus retinitis	*In vivo* pigmented rabbit/retina	[75]
	PLA intrascleral implant	Betamethasone phosphate	Intraocular inflammation	*In vivo* pigmented rabbit/choroid and retina	[76]
	Ethylene vinyl acetate (EVA) intrascleral implant	Betamethasone phosphate	Intraocular inflammation	*In vivo* pigmented rabbit/choroid and retina	[77]
	EVA episcleral implant	Betamethasone and 6-carboxy fluorescein diacetate (fluorescent model drug)	Unspecified	*In vivo* non-pigmented rabbit/vitreous, choroid and retina	[78]
	Poly(methylidene malonate) scleral disc	Triamcinolone acetate	Vitreoretinal disorders	*In vivo* rabbit/vitreous	[79]
	PLA intrascleral implant	Triamcinolone acetate	Chorioretinal disorders	*In vivo* nonpigmented rabbit/vitreous, choroid and retina	[80]
	Photopolymerized tri (ethyleneglycol) dimethacrylate (TEGDM) and polyethylene glycol dimethacrylate (PEGDM) device	FITC dextran and recombinant human brain-derived neurotrophic factor (BDNF) collagen microparticles	Unspecified	*In vivo* rabbit/retina	[81]
	Vasohibin-1 and vasohibin-1-FITC-dextran	CNV	*In vivo* rat/retina		[82]
	Geranylgeranylaceton	AMD	*In vivo* rat/retina		[83]
	Photopolymerized TEGDM and PEGDM device	Fluorescein, rhodamine B, and DAPI (fluorescent model drugs)	Unspecified	*In vivo* rat/retina	[84]
	Edaravone and unoprostone isopropyl	CNV and AMD	*In vivo* rat/retina		[85]
	Unoprostone	Retinitis pigmentosa	*In vivo* rabbit/retina		[86]
	Unoprostone	Retinitis pigmentosa	*In vivo* monkey/retina		[87]
	Polycaprolactone episcleral film	Triamcinolone acetonide	Chorioretinal disorders	*In vivo* nonpigmented rabbit/choroid and retina	[88]
	Hollow microneedles with in situ gelling thermoresponsive poloxamer implant	Fluorescein sodium (fluorescent model drug)	Unspecified	*Ex vivo* rabbit/sclera	[89]
	Hollow microneedles with PLA nano- and microspheres	Sulforhodamine B or Nile red (model dyes)	Unspecified	*Ex vivo* human cadaver/sclera	[90]
	PVP-coated microneedles	Sulforhodamine B and fluorescein-bovine serum albumin (fluorescent model drugs)	Unspecified	*Ex vivo* human cadaver/sclera	[91]
	PVP-based dissolving microneedles	Fluorescein sodium and fluorescein isothiocyanate dextran (FITC-dextran) (fluorescent model drugs)	Unspecified	*Ex vivo* pig/sclera	[92]

TABLE 2 (*Continued*)

Route	Formulation approach	Drug molecule	Target disease	Experiment/target site	Refs
Transscleral/ Suprachoroidal	Clearside™ Biomedical proprietary microneedles	Triamcinolone acetonide	Acute posterior uveitis	Clinical trials as well as *in vivo* domestic weanling pig/ choroid and retina	[93]
	Metal microneedles and borosilicate hollow microneedles	Fluorescein sodium, FITC-dextran, fluorescently tagged bevacizumab and FluoSpheres® (fluorescent model drugs)	Unspecified	*In vivo* nonpigmented rabbit /choroid and retina	[94]
	Borosilicate hollow microneedles	FluoSpheres®, sulforhodamine B (fluorescent model drugs) and barium sulfate	Unspecified	*Ex vivo* whole rabbit, pig, and human eyes/choroid and retina	[95]
	Polycaprolactone dimethacrylate and hydroxyethyl methacrylate *in situ*-forming gel	Fluorescently tagged bevacizumab	CNV	*Ex vivo* rabbit eyes and *in vivo* mice/choroid and retina	[96]

particle suspensions (50 μg and 75 μg for nano- and microparticles, respectively) injected into the subconjunctival space. Results showed a burst release from nanoparticles at day 1, with a cumulative drug release of almost 25%, which was not seen with microparticles. However, after 2 weeks, the concentration of budesonide delivered by microparticles was higher because of the decline in drug release from nanoparticles. A similar pattern was reported by Amrite *et al.* [97], whereby larger particles (200 nm) were retained at the injection site for longer, resulting in greater drug levels in the periocular space compared with smaller particles (20 nm). This could be explained by the rapid clearance of smaller particles by the lymphatic system. Diabetic rats subconjunctivally injected with 75μl of a celecoxib-PLGA microparticle suspension containing 75 μg of celecoxib exhibited sustained retinal drug levels for 14 days, reducing diabetes-induced retinal oxidative stress over this time period. Moreover, drug levels were sustained in other ocular tissues, indicating that the subconjunctival route can be used for the treatment of other ocular disorders [66]. Finally, Andres-Guerrero *et al.* [98] synthesized novel polysteramide microspheres (15 μm) loaded with dexamethasone as the test drug. Microspheres were biodegradable and cytotoxicity studies of microsphere dispersions in the range of 0.001–2 mg/ml and 0.1–2 mg/ml showed good *in vitro* tolerance in mouse macrophages and ARPE-19 cells, respectively, with ocular pharmacokinetic modeling predicting an *in vivo* drug release for up to 3 months in rabbit eyes.

Dendrimers are highly branched, symmetrical polymeric molecules. Their synthesis can be regulated to precisely manipulate molecular weight and chemical composition [99]. This renders dendrimers highly efficient drug carriers [100]. A subconjunctival injection of carboplatin-loaded dendrimer nanoparticles (258 nm) was explored in the treatment of retinoblastoma. Results showed that mice treated with a single 30 μl injection of either 10 or 37.5 mg/ml carboplatin nanoparticles had a significantly smaller mean tumor burden than mice treated with 10 mg/ml conventional carboplatin aqueous solution. Furthermore, mice treated with the high-dose carboplatin nanoparticles also showed a significantly smaller tumor mass than the conventional treatment. After subconjunctival injection, none of the mice showed clinically significant complications and there was no toxicity to the retina or any other ocular tissues [67]. Another study investigated hydrophobic polymethylmethacrylate (PMMA) dendrimers as a nanocarrier for carboplatin. The study compared intravitreal drug concentrations after periocular injection of commercially available carboplatin and novel carboplatin-loaded PMMA nanoparticles. The drug was administered to rats via the sub-Tenon route and the duration of the study was 42 days. Results showed that the intravitreal carboplatin concentration from nanoparticles was significantly higher than that from the conventional formulation on all days because of the sustained release effect [73].

Another form of particulate delivery systems are depot suspensions. When injected, they slowly release the drug over a prolonged period. As mentioned early, a novel posterior juxtascleral depot suspension of anecortave acetate (Retaane®) was developed by Alcon Laboratories. Preclinical studies in rabbits and monkeys showed adequate choroidal and retinal drug levels. Moreover, in patients with AMD, therapeutic concentrations of the drug were achieved in the macular region of choroid and retina for up to 6 months [17]. However, this method of drug delivery is relatively complex, because the conjunctiva and Tenon's capsule have to be dissected before insertion of the cannula, thus special training might be required for the administering ophthalmologist. Nevertheless, during clinical studies, the depot suspension was administered to more than 3000 patients and was found to be safe and well tolerated, with no damage to the optic nerve or posterior ciliary arteries [19].

Semisolid formulations

Hydrogels are an alternative formulation approach to enhance periocular drug delivery. They are cross-linked polymeric networks that have remarkable hydrophilicity, flexibility, and elasticity [101], rendering them suitable materials for drug delivery. Gilbert *et al.* [68] incorporated cisplatin into a collagen matrix-based gel for subconjunctival administration, with *in vivo* studies performed in Dutch-Belted rabbits. The gel formulation achieved significantly higher drug concentrations in the sclera, vitreous, choroid, and retina than a subconjunctivally injected cisplatin solution. The collagen matrix remained attached to the sclera for up to 2 weeks;

however, further optimization might be needed to maintain steady-state drug release.

More sophisticated stimuli-responsive hydrogel systems have also been utilized for periocular drug delivery. Such *in situ* gelling systems are based on polymeric solutions or semisolids that undergo sol-to-gel transition in response to a stimulus, such as a change in ion concentration, pH, or temperature, as well as to UV exposure [102]. *In situ* gelling systems are often used for the delivery of macromolecules, such as proteins and peptides, to prolong their residence time and, thus, enhance their bioavailability. A biodegradable thermosensitive gel (ReGel^{TM}) comprising poly(lactic-co-glycolic acid) (PLGA) and polyethylene glycol (PEG), administered to rats through the subconjunctival route, was shown to provide sustained release of ovalbumin to the choroid and retina for up to 14 days [69]. Rats were injected with 20 μl of ReGel containing 2 mg/ml of fluorescently tagged ovalbumin (a total of 40 μg ovalbumin) with the formulation being free flowing below 15 °C and forming a gel at body temperature.

Misra *et al*. [103] developed subconjunctival insulin hydrogels to prevent retinal degeneration in diabetic rats. Hydrogels were synthesized by UV photopolymerization of *N*-isopropylacrylamide monomer (NIPAAm) and a dextran macromer containing multiple hydrolytically degradable oligolactate-(2-hydroxyetheyl methacrylate) units (Dex-lactate-HEMA) with insulin loaded into the hydrogel during synthesis. Insulin was detected in the retina by confocal microscopy for 7 days. There was also a significant reduction in DNA fragmentation of diabetic retinas, implying that these hydrogels have the potential for sustained drug delivery to the back of the eye. Another *in situ* gel example includes the ion-dependent sol-to-gel polymeric system developed by Thrimawithana *et al*. [71] for the periocular delivery of connexin43 antisense oligonucleotides (AsODN). The formulation comprised two polymers: iota carrageenan, which forms soft elastic gels in contact with calcium ions; and methylcellulose, which undergoes gelation at elevated temperatures. The AsODN formulation was injected via the sub-Tenon route with *in vivo* data showing a significant reduction in choroidal connexin43 levels 24 h after treatment in a light-induced damage rat model compared with the control [104]. This suggests this polymeric system as a potential depot for bioactive delivery via the periocular route.

Implants

Implants are another formulation strategy that is being widely researched for sustained ocular delivery, although mainly via the intravitreal route. Although injectable formulations are easier to administer, implants can be recovered more easily in instances of complications. Intrascleral implants are surgically placed into the sclera and have the advantage of avoiding penetration across the entire scleral barrier while also limiting elimination via episcleral clearance. Implants can be biodegradable or nonbiodegradable, both of which can achieve sustained delivery. Whereas nonbiodegradable implants can provide more controlled drug release for longer periods, they are usually larger in size and, thus, more invasive, especially when considering removal after drug depletion.

A biodegradable intrascleral implant developed by Okabe *et al*. [76] was able to achieve therapeutic drug concentrations in rabbit choroid and retina for up to 8 weeks. The implant was 0.5 mm thick with a diameter of 4 mm and was fabricated from PLA containing 25% w/w betamethasone phosphate. A scleral pocket with a depth of 100 μm was surgically formed, allowing the implant to be placed intrasclerally. A periocular capsular delivery system intended for the sub-Tenon route was recently investigated by Huang *et al*. [72]. The system was fabricated from silicon into a V-shaped capsule and filled with 0.5 ml dexamethasone sodium phosphate (5 mg/ml). The periocular capsule was surgically implanted in the Tenon's sac of rabbits, where it showed promising preliminary results of sustained dexamethasone sodium phosphate release for 56 days.

Kawashima *et al*. [81] designed a scalable controlled release device for transscleral drug delivery of proteins. The device comprised a reservoir made from TEGDM and a controlled-release membrane fabricated by photopolymerizing a mixture of PEGDM and collagen microparticles, with drug release kinetics controlled by varying the density of the membrane. Photopolymerized TEGDM is impermeable to macromolecules, which forces a unidirectional drug flow through the porous membrane into the sclera. The device was initially tested in rabbits using fluorescent dyes, where zero-order release was observed. It was subsequently loaded with vasohibin-1 and tested in a laser-induced CNV rat model [82]. After 2 weeks of treatment, there was a significant reduction in lesion size comparable to treatment with vasohibin-1 given via intravitreal injection. A modified device was recently tested for long-term pharmacokinetics and safety of unoprostone in monkeys [87]. After 1 year of implantation, no changes in retinal function, intraocular pressure, or retinal histology were observed. With the physicochemical and biological characterization of the device for clinical use already reported, the next steps include the evaluation of the unoprostone-loaded device in patients with retinitis pigmentosa [105].

Microneedles

Microneedles are one of the newer approaches being researched for periocular drug delivery, with delivery of drug suspensions precisely into the suprachoroidal space using hollow microneedles, such as with the Clearside^{TM} Biomedical proprietary microneedles, discussed elsewhere [93,106,107]. The size of microneedles ranges within the micron-scale range, which makes them less invasive and potentially more precise than hypodermic needles. Various types of microneedle have been studied for ophthalmic delivery, such as coated, hollow, and dissolving microneedles [108]. Coated microneedles are solid microneedles coated with the drug [91]. The coating procedure can thereby affect the particle size of the coated drug, which in turn affects drug release [109] and could result in a lack of reproducibility [91]. Hollow microneedles, mainly made of silicon, metal, and glass, generally have a similar structure to hypodermic needles except that they are shorter [110]. The drug solution is infused into the microchannels formed in the tissue. Drawbacks of silicon-made hollow microneedles include brittleness and difficulty of drug infusion into the tissue [92]. Dissolving microneedles are usually made of polymers or polysaccharides, and the encapsulated drug is released during the dissolution of the microneedles [111].

Thakur *et al*. [92] used rapidly dissolving microneedles to enhance the ocular delivery of macromolecules via the intrascleral route. Microneedles were fabricated from polyvinylpyrroli-

done (PVP) of various molecular weights, with dimensions of 800 μm in height and 300 μm in base diameter. Test molecules were fluorescein sodium and FITC-dextran. *In vitro* studies were performed on rabbit scleral tissue and a significant enhancement in macromolecule permeation was observed compared with topical application of an aqueous macromolecule solution. However, no significant difference was seen in the permeation of the small fluorescein molecule. Biocompatibility studies of PVP on ARPE-19 cells suggested the polymer to be safe when used at concentrations <2 mg/ml. Thus, PVP dissolving microneedles could have the potential to enhance transscleral drug delivery of macromolecules.

Concluding remarks and future perspectives

Periocular drug delivery to the posterior eye segment offers clear advantages over topical and systemic routes in terms of therapeutic bioavailability. It is also comparatively less invasive than intravitreal injection, although retinal drug bioavailability remains low, potentially limiting this route to the treatment of conditions effecting the outer layers of the eye. Depot formulations retain the drug formulation and, thus, enhance drug bioavailability, with preclinical and clinical studies having shown promising results.

Future studies on posterior segment drug delivery through periocular routes should focus on improving drug bioavailability while maintaining a drug steady state for prolonged periods. This could be achieved by performing more in-depth research, particularly on metabolic and dynamic barriers, to gain a better understanding of their limitations. Another factor that must not be overlooked is the impact of various ocular diseases on ocular physiology and how this might affect drug permeation and distribution. Considering all of these factors will help to develop more successful periocular formulations in the future.

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