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REVIEW ARTICLE

Principles of pharmacology in the eye

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The eye is a highly specialized organ that is subject to a huge range of pathology. Both local and systemic disease may affect different anatomical regions of the eye. The least invasive routes for ocular drug administration are topical (e.g. eye drops) and systemic (e.g. tablets) formulations. Barriers that subserve as protection against pathogen entry also restrict drug permeation. Topically administered drugs often display limited bioavailability due to many physical and biochemical barriers including the pre-corneal tear film, the structure and biophysiological properties of the cornea, the limited volume that can be accommodated by the cul-de-sac, the lacrimal drainage system and reflex tearing. The tissue layers of the cornea and conjunctiva are further key factors that act to restrict drug delivery. Using carriers that enhance viscosity or bind to the ocular surface increases bioavailability. Matching the pH and polarity of drug molecules to the tissue layers allows greater penetration. Drug delivery to the posterior segment is a greater challenge and, currently, the standard route is *via* intravitreal injection, notwithstanding the risks of endophthalmitis and retinal detachment with frequent injections. Intraocular implants that allow sustained drug release are at different stages of development. Novel exciting therapeutic approaches include methods for promoting transscleral delivery, sustained release devices, nanotechnology and gene therapy.

Abbreviations

AAV, adeno-associated-virus; ADAs, anti-drug antibodies; AMD, age-related macular degeneration; BAC, benzalkonium chloride; BRB, blood–retinal barrier; CMV, cytomegalovirus; CNTF, ciliary neurotrophic factor; CNV, choroidal neovascularization; DR, diabetic retinopathy; ECM, extracellular matrix; EVA, ethylene vinyl acetate; GFS, glaucoma filtration surgery; HA, hyaluronic acid; IOP, intraocular pressure; i.v.t., intravitreal; MEMS, microelectromechanical system; PAMAM, poly amidoamine; pNIPAAM, poly(N-isopropylacrylamide); PVA, polyvinyl alcohol; RP, retinitis pigmentosa; RPE, retinal pigment epithelium; RVO, retinal vein occlusion; TA, triamcinolone acetonide

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Introduction

Vision is often considered to be the most important of senses and the one that most people fear losing. Vision is often thought to be the key enabling sense for a person to work and function independently. Considerable efforts to combat vision loss continue to be made as blinding ocular diseases are more prevalent with an increasingly ageing population. All measurements for quality of life, such as disability-adjusted life-years, confirm that visual impairment is a highly ranked burden in all countries (Chiang *et al.*, 2006). The leading causes of visual impairment and irreversible blindness are posterior segment-related diseases (Pascolini and Mariotti, 2012), which include glaucoma, age-related macular degeneration (AMD), macular oedema secondary to retinal vein occlusion (RVO), cytomegalovirus (CMV) retinitis, posterior uveitis, diabetic retinopathy (DR) and retinitis pigmentosa (RP) (Thrimawithana *et al.*, 2011; del Amo *et al.*, 2015; Waite *et al.*, 2017).

Glaucoma is a term used to describe a group of conditions with an optic neuropathy, characteristic morphological changes in the optic nerve head and visual field defects. It remains a leading cause of irreversible blindness throughout the world and one of the most common neuropathies. It is estimated that 60.5 million people suffered from it in 2010 and 7 million are believed to be bilaterally blind (Quigley and Broman, 2006). The number of people with glaucoma is projected to increase to 111.8 million in 2040 (Tham *et al.*, 2014). Over the last century, in fact, the proportion of blindness attributed to glaucoma in developed countries has changed very little (10%) (Taylor, 2009). In developed countries, the main cause of blindness 100 years ago was corneal disease; fifty years ago, it was cataracts and in the present day, it is AMD. No reduction in the proportion of blindness due to glaucoma is not simply because there have been no advances in treatment. A major factor is the ageing population. As a population becomes healthier and people live longer, the incidence of this degenerative optic neuropathy that reaches a clinically detectable threshold will increase. The main treatment involves trying to slow the optic nerve degeneration. The biggest risk factor for accelerated progression is an increased level of intraocular pressure (IOP) and reducing this is the only proven therapy in everyday use.

AMD affects approximately 10 million people worldwide and is one of the leading causes of vision loss in elderly patients over 60 years of age. The prevalence of AMD accounts for 20% of all posterior segment disorders in the 65–74 years age group and 35% in older age groups (Edelhauser *et al.*, 2010) and is predicted to affect 288 million people by 2040 (del Amo *et al.*, 2017). Cataract, DR, glaucoma and AMD will increase to nearly 70 million adults by 2050 (Joseph and Venkatraman, 2017). Loss of vision will increase with the increase in the ageing population, which in turn will pose a challenge for human health and economic growth (Delplace *et al.*, 2015).

Inflammatory, angiogenic and fibrotic processes cause tissue damage and vision loss. These processes are also major contributors to the failure of current treatments for these diseases. Increased knowledge of the molecular mechanisms of blinding diseases (Rodrigues *et al.*, 2009; de Oliveira Dias *et al.*, 2011) has driven the development of therapeutic proteins including antibody-based medicines (Penedones *et al.*, 2014). Vascular endothelial growth factor (**VEGF**)-neutralizing antibodies have considerably improved the treatment of exudative macular degeneration (wet AMD) and DR. A major challenge during both preclinical and clinical development is to determine the ocular clearance rates of new medicines. Even though the anti-VEGF antibody-based medicines can be administered once a month and in some cases every other month (Stewart *et al.*, 2012), much research is focused on developing medicines that will require less frequent dosing regimens.

Another factor in the development of longer acting formulations is the cost of the drugs and overall treatment costs, which is a function of dosing frequency. The expenditure to treat blinding ophthalmic diseases is considerable, with $51.4 billion spent in the USA alone each year (2007). For example, **ranibizumab** (licensed) and bevacizumab (unlicensed) are widely used to treat AMD. Each injection of ranibizumab (Lucentis®) can cost $2000, whereas bevacizumab (Avastin®) can cost only $50 for each intravitreal (i.v.t.) dose (Raftery *et al.*, 2007). Two multi-centre randomized controlled clinical trials (IVAN and CATT) compared the use of ranibizumab and bevacizumab (Chakravarthy *et al.*, 2012; Martin *et al.*, 2012; Ahfat and Zaidi, 2013). No difference was found in visual acuity outcome during 1 and 2 year treatment periods respectively (Chakravarthy *et al.*, 2012; Martin *et al.*, 2012). Bevacizumab is normally provided as a solution in a glass vial containing 400 mg of the antibody at a concentration of 25 mg·mL^{-1}. For ocular use, bevacizumab is often transferred under aseptic conditions into ready-to-use syringes for i.v.t. injection by compounding pharmacies for local distribution. Many health systems cannot cope with the increasing demands for i.v.t. injection, so bevacizumab is widely used (Elshout *et al.*, 2014; Kwong and Mohamed, 2014).

The eye – a complex organ

The anatomy of the eye and complex physiology of the retina mean that the development of efficacious medicines is challenging. The eye is a specialized organ that enables light to be focused and processed into nerve impulses for interpretation by our brain. A huge range of pathology affects the eye, with either local triggers or manifestations of systemic disease such as diabetes mellitus. Furthermore, different parts of the eye can be affected to different degrees, with implications for drug delivery. The eye is broadly divided into two compartments called the anterior (front of the eye) and posterior (back of the eye) segment. Contact lens-related infections occur in the cornea of the anterior segment, whereas macular degeneration is in the central retina of the posterior segment. Endophthalmitis (infection within the eye) can cause blindness within a matter of hours. Optic nerve degeneration in open angle glaucoma may take many years before patients are aware that vision is compromised.

The least invasive routes for ocular drug administration are topical (e.g. eye drops) and systemic (e.g. tablets) formulations. The superficial nature of the eye enables local administration (usually eye drops), the main modality of treatment for anterior segment disease. One advantage of this is to bypass the liver, thus avoiding the need for higher systemic doses required with the oral route. The efficacy of drugs may

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also be assessed at a local level. Parameters can be measured directly, for example, the IOP in glaucoma, or through visual interpretation for signs of oedema in exudative macular degeneration.

Local applications do pose some disadvantages. Most treatments are self-administered and rely on a compliant patient, but many patients take their medication sporadically. This can be demonstrated by attaching detectors to bottles during glaucoma treatment used by patients (Kass *et al.*, 1986). The reported rate of application from patients differed significantly to the measured rate (Kass *et al.*, 1986). Consequently, even if drug delivery is optimized, efficacy may not be achieved. Topically administered drugs often display limited bioavailability (< 5%) due to many physical and biochemical barriers including the pre-corneal tear film, the structure and biophysiological properties of the cornea, the limited volume that can be accommodated by the cul-de-sac, the lacrimal drainage system, reflex tearing and the aqueous outflow within the eye (Peyman and Ganiban, 1995; Ranta and Urtti, 2006; Rodríguez Villanueva *et al.*, 2016). Newer methods of drug delivery include sustained release preparations that allow reduced frequency of drug administration (Rodríguez Villanueva *et al.*, 2016; Mehta *et al.*, 2017). Preservative-free eye drops can be achieved with a new multidose bottle with an Airless Antibacterial Dispensing System (Pfizer Inc, USA) containing a valve system and an airless pump with a silver antibacterial coil (Ghate and Edelhauser, 2008).

The patient population is also often elderly and susceptible to side effects. Despite lower doses needed than oral drugs, systemic toxicity can still be an issue. An elderly patient tends to have a lower body mass index, as well as decreased renal function. Furthermore, these patients are more likely to be on multiple medications that may interact. Even small quantities of drug absorbed systemically may have side effects, such as an increase in falls with topical β-blocker use (Glynn *et al.*, 1991). Another example is the use of atropine. A 50 μL drop of 1% atropine contains 0.5 mg of drug. Treating both eyes with one dose (1.0 mg) can cause tachycardia (Morton and Thomas, 1958).

Significant ocular barriers that exist to prevent pathogen access also hinder drug delivery (Novack and Robin, 2016; Yellepeddi and Palakurthi, 2016). Transparency is necessary for optical function and, therefore, most of the inside of the eye lacks blood vessels. This is at the expense, however, of the host defence, which is weaker compared to rest of body, and if the barriers are breached, as in endophthalmitis, there are devastating effects. However, these barriers are also key restrictors to delivering a drug to the area of need. Blinking and tear film turnover, designed to wash away foreign material and maintain a smooth clear anterior surface, also limits the residence time of a drug. Access to the deeper structures of the eye is hindered by a closely packed corneal epithelium and stroma with varying lipophilicity.

Delivery of a drug to the posterior segment has its own particular challenges. The vascular conjunctiva, sclera and choroid affect delivery, notwithstanding the tight junctions of the blood aqueous barrier of the non-pigmented ciliary body epithelium, and the endothelium of blood vessels that constitute the blood–retinal barrier (BRB). The BRB controls fluid and molecular movement between the ocular vascular beds and prevents leakage of macromolecules and harmful molecules into the retina (Kaur *et al.*, 2008). Due to the outer and inner BRB, the influx of a drug into the retina and vitreous regions is limited, requiring the systemic administration of high doses to achieve therapeutic concentrations within the eye. As a result, a drug is distributed and accumulated in all tissues in the body, causing unwanted side effects (Myles *et al.*, 2005). Therefore, i.v.t. injections are required to administer drugs directly to the posterior segment to achieve reproducible and high doses. Although i.v.t. injections are required to treat blinding conditions at the back of the eye, repeated administration poses potential complications including endophthalmitis, retinal detachment, traumatic cataract, intraocular haemorrhage and ocular infections (Jager *et al.*, 2004). Particulate contaminants that may be present in a formulation also increase the potential for harmful effects to the posterior segment (Jager *et al.*, 2004). In addition, only a little is known about vitreous dynamics, partly due to the changes in consistency and anatomy with age (Bishop *et al.*, 2004; Laude *et al.*, 2010; Awwad *et al.*, 2015).

Understanding the nature of ocular barriers and developing methods of bypassing or utilizing them together with knowledge of the fluid dynamics within the eye are keys to establishing successful ophthalmic pharmacotherapy. Experimental models act as platforms for drug development (Awwad *et al.*, 2015; Awwad *et al.*, 2017) and translation to the clinic. This review aims to examine these barriers, ways of negotiating them, and methods of pharmacotherapy employed for different types of drugs. As the pathology and pharmacology differs anatomically, we aim to discuss separately the anterior and posterior segment and look at some of the future strategies for drug development on the horizon.

Anterior segment

Barriers. The anterior segment of the eye consists of the cornea, conjunctiva, iris, ciliary body and the lens. The common chronic anterior segment diseases are cataract, glaucoma and uveitis, with cataract accounting for 51% blindness (according on a 2010 survey; Pascolini and Mariotti, 2012; Joseph and Venkatraman, 2017). The vast majority of pharmacotherapy is through topical application by the patients themselves, although other routes such as subconjunctival injection are also used. Tear film turnover is the main factor that limits topical drug residence time. Human tear volume is approximately 7 μL (Gaudana *et al.*, 2010). The volume of an eye drop of drug is up to 50 μL, and although the cul-de-sac may briefly expand to 30 μL (Nagataki and Mishima, 1980; Gaudana *et al.*, 2010), most drug is cleared by nasolacrimal drainage, and then to the systemic circulation directly after instillation. Increasing the drop size will therefore not deliver a greater amount of drug to the eye but, instead, increases the chances of systemic side effects. The optimal size of drop to prevent systemic overflow is zero, which is obviously not achievable. Droppers have been created that consistently produce smaller volumes (Van Santvliet and Ludwig, 2004; Kumar *et al.*, 2011). Simpler strategies include nasolacrimal occlusion or closing the eyelid.

Lacrimation caused by irritant drugs, certain excipients and pH deviation increase tear turnover and drug loss. Increase in tonicity can also have significant implications for

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increase in tearing. The normal tear turnover in humans is 16%·min^{-1} (Mishima *et al.*, 1966). Increased lacrimation due to drop instillation results in turnover being increased up to 80%·min^{-1}. Therefore, the half-life of a drug is only 4 min even with a normal turnover. Blinking also increases drainage as eyelid muscle contraction encourages tear flow down the nasolacrimal duct. A significant increase in the drug residence time can be achieved simply by asking patients to close their eyes after drop instillation and apply pressure nasolacrimal duct (Flach, 2008).

The tear film itself traditionally used to be thought of as a simple tri-layer structure, consisting of an oily surface layer that reduced evaporative loss, an aqueous middle layer that contained enzymes and other anti-bacterials (such as lactoferrin) and a mucin layer that provides lubrication and protection of the corneal surface. More recently, that view has altered with the discovery of numerous glycosaminoglycans (GAGs), mucopolysaccharides and other molecules responsible for tear film integrity (Tiffany, 2008). Furthermore, the tear film is dynamic as fluids are 'reshuffled' with blinking (Cher, 2012). An unstable tear film is a result of dry eye syndrome and chronic eye irritation with changes in the level of lipid, protein and mucin profiles (Yellepeddi and Palakurthi, 2016).

Understanding the tear film constituents and dynamics has led to the development of methods that increase drug residence time and bioavailability. Inserts provide sustained release of drug. Emulsions enable an increased drug load that may not be achievable in solution. Vehicles or ointments increase viscosity. Carriers of drug exist that bind to the tear film or ocular surface with greater adhesion than drug alone.

Bacterial ulceration of the cornea is a significant risk factor in people who regularly wear contact lenses. Poor hygiene combined and overnight use increases the risk of a bacterial infection (Stapleton *et al.*, 2008). Aggressive dosing is required in the initial stages of therapy, with hourly or even half hourly drops to prevent ocular spread and loss of the eye. It is not uncommon for patients to be admitted to hospital for both day and night treatment (Teo *et al.*, 2011). Ocular inserts (Table 1) are solid implants that sit in the cul-de-sac of the eyelids and slowly dissolve, releasing drug over a long period of time. The concept is constantly being explored and reported in literature (Saettone *et al.*, 1994; Kumari *et al.*, 2010; Aburahma and Mahmoud, 2011; Franca *et al.*, 2014; Everaert *et al.*, 2017; Rathod *et al.*, 2017; Sharma *et al.*, 2017). Pilocarpine, an IOP lowering treatment, was one of the first drugs to be formulated as an insert (Ocusert) (Langer, 1983). Ocusert is a cul-de-sac sustained release insert composed of two membranes of ethylene vinyl acetate (EVA) copolymer surrounding a reservoir of pilocarpine allowing drug diffusion (40 μg·h^{-1}) for over a period of a week for the treatment of glaucoma (del Amo and Urtti, 2008; Peyman and Hosseini, 2011; Barar *et al.*, 2016). An ocular insert that combined ciprofloxacin with sodium carboxymethylcellulose and polyvinyl alcohol (PVA) has shown significant promise. *In vitro* studies have shown that this insert can maintain levels within a therapeutic range for up to 48 h (D. Jain *et al.*, 2010). Good tissue compatibility has also been demonstrated in an *in vivo* rabbit model. Ocular inserts have also been clinically tested for patients with dry eye symptoms, with release of hydroxypropyl cellulose alleviating foreign body sensation significantly with blurring of vision being the most common side effect (in 8.7% patients) (Koffler *et al.*, 2010).

Suspensions are commonly used to enable application of poorly water-soluble drugs such as the steroid prednisolone acetate. The suspension requires shaking to disperse drug particles in the bottle. However, drug distribution is still not uniform. Oil-in-water (o/w) emulsions are prepared to dissolve the drug in an oil phase and disperse it in the aqueous phase with a surfactant, which has been found to improve consistency (Koffler *et al.*, 2010). Microemulsions, which are emulsions that are thermodynamically stable and form spontaneously, have also shown promise. A low concentration of surfactant maintains the drug suspension stability and may enhance penetrance by interacting with the corneal epithelial cells (Fialho and da Silva-Cunha, 2004). A recent study found that a microemulsion of **dexamethasone** had an improved anti-inflammatory effect in a rabbit uveitic model compared to a marketed formulation of dexamethasone (Kesavan *et al.*, 2013).

An alternative to formulating an emulsion for poorly water-soluble drugs is to combine it with a cyclodextrin (Kurkov and Loftsson, 2013). Cyclodextrins are oligosaccharides consisting of a hydrophilic outer surface and a hydrophobic core (Loftsson and Stefansson, 1997). They are able to form inclusion complexes with 'guest' drug molecules, which sit within the core and the hydrophilic outside aids aqueous solubility. A dynamic equilibrium exists between the cyclodextrin drug complex and disassociated drug, enabling drug release (Kek *et al.*, 2010). Cyclodextrins have been used preclinically to formulate eye drops (Loftsson and Brewster, 2011) with various drugs (Siefert and Keipert, 1997; Bary *et al.*, 2000; Cappello *et al.*, 2001; Okamoto *et al.*, 2010; Bozkir *et al.*, 2012; Loftsson *et al.*, 2012; Jóhannsdóttir *et al.*, 2015; Rodriguez-Aller *et al.*, 2015). Several cyclodextrin-based eye drops have been evaluated clinically, for example, latanoprost and dexamethasone (Gonzalez *et al.*, 2007; Tanito *et al.*, 2011; Krag and Hessellund, 2014; Shulman *et al.*, 2015), while others have been registered in Europe for clinical use, for example, chromaphenicol (Clorocil®), diclofenac (Voltaren Ophthalmic®) and indomethacin (Indocid®) (Davis and Brewster, 2004). The percentage of cyclodextrin used for drug solubilization was between 10 and 30% w·v^{-1} for most of these formulations (Kearse *et al.*, 2001).

Increasing the viscosity with a carrier increases residence time and, in theory, the drug bioavailability (Bourlais, 1995). Ointments are often made from mineral oil, which can pose a challenge for the formulation of water-based treatments. The oily base may impair drug dissolution, reducing therapeutic efficacy (McCarthy, 1975). The oil itself also interferes with tear film clarity, leading patients to complain of blurry vision or eyelashes being stuck together. Ointments still have an important therapeutic role in the setting of corneal exposure or thermal burns, where production of tear film constituents has been disrupted or where the ocular surface is damaged (Fish and Davidson, 2010).

Another method of increasing the viscosity with less tear film disruption is to use a mucoadhesive component (polymer or excipient) such as polycarbophil (Agrahari *et al.*, 2016) or carboxymethylcellulose (Reddy and Kim, 2011). These substances are long chain polymers that bind to the

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Table 1

FDA-approved drugs and those in current clinical trials for treatment of anterior segment diseases

Name and company	Drug delivery platform	Drug and excipients	Indications	Status
Acuvail™ (Allergan)	Eye drops	Solution of ketorolac tromethamine (0.45%) in carboxymethylcellulose (pH 6.8)	Reduction of pain and inflammation post cataract surgery	Approved
Azasite® (InSite Vision)	Eye drops	Solution of azithromycin (1.0%) in polycarbophil (Durasite®)	Bacterial conjunctivitis	Approved
Azasite Plus™ (InSite Vision)	Eye drops	Solution of azithromycin (1.0%)/ dexamethasone (0.1%) (ISV-502) in Durasite®	Blepharoconjunctivitis	Phase III completed
Bromsite™ (InSite Vision)	Eye drops	Solution of bromfenac sodium (0.075%)	Reduction of pain and inflammation post cataract surgery	Approved
Betoptic-S™ (Alcon)	Eye drops	Suspension of betaxolol (0.25%) in polystyrene-divinylbenzene) sulfonic acid, carbomer and BAC (0.01%)	Open angle glaucoma	Approved
Cationorm® (Santen Pharma)	Eye drops	Cationic emulsion	Dry eye	Approved
DexaSite™ (InSite Vision)	Eye drops	Solution of dexamethasone (0.1%) (ISV-305) in Durasite®	Reduction of pain and inflammation post cataract surgery; and non-bacterial blepharitis	Phase III
Durezol™ (Alcon)	Eye drops	Emulsion of difluprednate (0.05%) in castor oil, glycerine, boric acid, polysorbate 80 and sorbic acid (0.1%)	Anterior uveitis	Approved
EGP-437 (Eyegate Pharma)	Iontophoresis	Solution of dexamethasone phosphate in EyeGate® II Drug Delivery System (EGDS)	Anterior uveitis	Phase III
Indocollirio® (Bausch & Lomb)	Eye drops	Suspension of indomethacin (0.1%) and hydroxypropyl-b-cyclodextrin (IND-CD)	Mydriasis during cataract surgery or conjunctivitis	Approved
Lacrisert® (Aton Pharma)	Ocular insert	Sterile, rod-shaped HPMC insert	Dry eye	Approved
Lumigan® (Allergan)	Eye drops	Solution of bimatoprost (0.03%) in BAC (0.005%)	Glaucoma	Approved
Mydriasert (Thea Pharma)	Ocular insert	Tropricamide (0.28 mg) and phenylephrine hydrochloride (5.4 mg) in ammoniomethacrylate copolymer, glycerol and ethylcellulose	Induced pre-operative mydriasis	Approved
Ocusert® (Alza)	Ocular insert	Pilocarpine with EVA and alginic acid	Glaucoma	Approved
Prolensa™ (Bausch & Lomb)	Eye drops	Solution of bromfenac (0.07%)	Postoperative inflammation	Approved
Propine™ (Allergan)	Eye drops	Solution of dipivefrin-HCl (0.1%) with BAC (0.02%)	IOP control in open angle glaucoma	Approved
Restasis™ (Allergan)	Eye drops	Cationic emulsion of cyclosporine (0.05%) in castor oil, glycerine, polysorbate 80 and carbomer	Dry eye	Approved
TobraDex® (Alcon)	Eye drops	Suspension of tobramycin/ dexamethasone in xanthan gum	Blepharitis	Approved
Timoptic-XE™ (Aton Pharma)	Eye drops	*In situ* gel solution of timolol maleate (0.25 or 0.5%) in gellan gum, mannitol, tromethamine and BAC (0.012%)	Glaucoma	Approved

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Table 1 (Continued)

Name and company	Drug delivery platform	Drug and excipients	Indications	Status
Travatan™ (Alcon)	Eye drops	Solution of travaprost (0.004%) in BAC (0.015%)	Glaucoma	Approved
Xalatan™ (Pfizer)	Eye drops	Solution of latanoprost (0.005%) in BAC (0.015%)	Glaucoma	Approved
Zirgan™ (Bausch & Lomb)	Ophthalmic gel	Ophthalmic gel with ganciclovir (0.15%) with carbopol and BAC (0.0075%)	Acute herpetic keratitis	Approved

All drug therapies mentioned above were also cross-checked with www.clinicaltrials.gov.uk and its official company site. BAC, benzalkonim chloride; HPMC, hydroxypropylmethylcellulose.

mucous layer constituents on the ocular surface. Amongst the salts, lipids and proteins are numerous glycoproteins, such as mucin, that have charged carbohydrate groups and end in sialic acid groups. The polymer chains of mucoadhesives, which consist of both polar and non-polar groups, swell on contact with water, become entangled and adhere to the mucin molecules (Khutoryanskiy, 2010). The polymer length and the pH affect drug residence time and bioavailability. A longer chain polymer will have more viscosity, entanglement and greater adhesiveness to a certain extent. It will also restrict the ability to swell and result in less adhesion. Furthermore, there may be an increase in irritation and lacrimation. The pH is also important in determining how strongly a polymer binds as it governs whether hydrogen bond formation is able to take place (Grabovac *et al.*, 2005). For many polymers, a more acidic pH results in greater hydrogen bonding between polymer and the mucin on the surface.

Hyaluronic acid (HA) has the additional property of shear thinning unlike other mucoadhesive polymers. With movement, the viscosity of HA decreases. Therefore, with blinking, the polymer rearranges, but with an eye that is open there is greater stability (Rah, 2011). HA is commonly used for maintaining anterior chamber volume during intraocular surgery and for the treatment of dry eye. Its potential for enhancing ocular delivery is still being evaluated, although it has shown promise with drugs such as pilocarpine (Pahuja *et al.*, 2012). HA stabilizes and organizes the extracellular matrix (ECM), regulates cell adhesion and motility and mediates cell proliferation and differentiation (Shu *et al.*, 2004; Guter and Breunig, 2017). The commercially available form, sodium hyaluronate, is widely used in biomedical applications such as a scaffold material for wound healing and tissue engineering due to its biocompatible and biodegradable properties (Ha *et al.*, 2006; Baino, 2011).

Routes of drug absorption. From the tear film, there are two main routes of drug absorption to the anterior segment:

- The corneal route. The drug passes through the structural layers of the cornea to reach the anterior chamber, including the corneal epithelium, stroma and endothelium.
- The non-corneal route. Drugs pass through the conjunctiva, Tenon's tissue and sclera to reach the anterior chamber.

Corneal route. The cornea consists of three major layers with variable permeability to drug molecules. The epithelium and endothelium both consist of cells linked by tight junctions, which restrict the passage of large molecules, while the stroma is made up of closely packed collagen. It is the epithelium, however, that provides the most resistance to solute diffusion. Its influence on restricting large molecules may be demonstrated by comparing the absorption of [^{14}C]-dexamethasone with the epithelium intact to when it is debrided in an animal model (Cox *et al.*, 1972a,b). Only after epithelial debridement is [^{14}C]-dexamethasone detected in the anterior chamber of rabbits after topical administration. Intercellular spaces with pore sizes of 60 A allow the passage of small ionic and hydrophilic molecules (<350 Da) *via* the paracellular route (Lee, 1990). The transcellular route is the main mechanism of absorption of ocular drugs and other larger lipophilic molecules.

The lipophilicity of the cornea varies with the different layers. The epithelium and endothelium are relatively hydrophobic, whereas the corneal stroma is hydrophilic. Consequently, there is a parabolic relationship between the corneal permeability and the diffusion coefficient (Yoshida and Topliss, 1996). With the stroma playing a lesser role in resistance, corneal penetrance increases with hydrophobicity with a decrease in penetrance in only the most hydrophobic of compounds. In conjunction with lipophilicity, pH is another key factor in determining the permeability of the cornea to a drug (Pahuja *et al.*, 2012) There is a delicate balance therefore between aiming for a pH appropriate for corneal penetration and the physiological pH (7.4), which will result in the least tearing.

Certain drugs harness the differing lipophilicity of the cornea to their advantage for drug delivery. Latanoprost is one of the most common ocular drugs used for the treatment of glaucoma, a disease in which there is accelerated optic nerve degeneration in association with a raised IOP (Digiuni *et al.*, 2012; Pek *et al.*, 2016). Latanoprost is a prostaglandin analogue that reduces IOP by increasing the uveoscleral outflow, through targeting receptors near the drainage angle. Although not fully understood, it is believed that it may cause ciliary muscle relaxation or remodelling of the tissue matrix within the ciliary muscle.

Latanoprost is formulated as a lipophilic pro-drug ester, which is absorbed easily into the corneal epithelium. It is

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activated by esterases within the epithelium to the active hydrophilic moiety and passes through into the anterior chamber. The cornea therefore acts as a depot for the pro-drug with prolonged release after a single application. The elimination half-life in rabbit corneas has been found to be over an hour (Sjöquist *et al.*, 1998). In humans, IOP lowering begins between 2 and 4 h after application, with the peak effect reached between 8 and 12 h (Digiuni *et al.*, 2012). This effectively means a once a day treatment, which helps with patient compliance.

The non-corneal route. Less is known about the permeability of the conjunctiva, Tenon's and sclera and the associated pharmacokinetics. Most studies that have examined these different tissues report that the conjunctiva is more permeable to hydrophilic molecules than the cornea, though it is not clear that it is less permeable to hydrophobic molecules. Using a hollow glass cylinder fixed to the corneal surface to isolate cornea versus conjunctiva tissue, Ahmed and Patton (1985) found that the hydrophilic molecule inulin penetrated conjunctiva and sclera more easily than the cornea.

Other conjunctival properties that increase drug absorption include a greater surface area (17 times that of the cornea in humans; Watsky *et al.*, 1988) and a larger pore size with greater permeability than the cornea (Lawrence and Miller, 2004). However, factors that reduce absorption include mucous produced by goblet cells and the presence of lymphatics and a vasculature that enhance systemic loss of drug.

Changing the epithelium permeability directly may increase drug penetration. Surfactants are ionized substances, which can disrupt the plasma membrane of the epithelium. One surfactant in common use is the preservative benzalkonium chloride (BAC). Its ability to kill bacteria and prolong the shelf life of medicines makes it the preservative of choice in many preparations. BAC can cause membrane disruption and emulsification of the lipid layer of the tear film, where the delivery of drug through the corneal layers may be significantly enhanced. This can be seen in studies with acyclovir ointment, a common treatment for herpes simplex keratitis. Acyclovir penetrance in *ex vivo* corneas of rabbits was increased by three times with a 0.005% BAC solution and by 10 times with a 0.01% BAC solution (Majumdar *et al.*, 2008). A standard formulation is between 0.004 and 0.02%. However, the effects of BAC are toxic and even at low concentrations the presence of BAC can cause cell death. A concentration of 0.0001% is sufficient to cause apoptosis. Higher concentrations cause necrosis. It is no surprise that patients complain of ocular surface irritation when using treatments that contain BAC. Ensuring ocular medications are pathogen-free is a mandatory part of formulating an ophthalmic preparation, and much research has been performed to find other preservatives that have effective bactericidal properties but without the toxicity seen with BAC.

Iontophoresis is the application of electrical current to drive ionized substances into tissue (del Amo and Urtti, 2008). It is a method of enhancing ocular drug delivery that has been in and out of vogue over many years since Wirtz reported its use for the treatment of infective corneal ulcers with zinc salts in 1908 (Wirtz, 1908). The apparatus usually consists of a reservoir of drug connected to an electrode with the other electrode attached to the patient. It has been used with some success to enhance delivery of antibiotics such as gentamicin, steroids and antiviral treatments both through the cornea and sclera. Side effects such as tissue necrosis and patient discomfort have precluded its widespread use; however, in some circumstances, it may have a useful role (Güngör *et al.*, 2010). Recent phase I/II trials have shown promise in the treatment of dry eye and uveitis (Patane *et al.*, 2011; Cohen *et al.*, 2012; Kang-Mieler *et al.*, 2014),

Periocular injections. Drugs that are injected underneath the conjunctiva or Tenon's tissue effectively bypass the superficial tear film and conjunctival barriers. Another advantage of this route is the ability to leave a depot of drug within the potential space between tissue layers. Furthermore, more posterior placement of the drug enables delivery to the posterior segment (discussed below). Periocular injections (Ghate *et al.*, 2007) are particularly pertinent for drugs poorly soluble in water such as steroids. Sub-conjunctival, sub-Tenon's, peri-bulbar injections are commonly used in ophthalmology practice. Sub-conjunctival steroid is used for the treatment of refractory anterior uveitis. Injections of the antifibrotic agents 5-flurouracil and mitomycin C are also applied using this route to prevent scarring after trabeculectomy surgery. Sub-Tenon's and peribulbar injections are often the method of choice for administering local anaesthetic. Less commonly used is the retrobulbar injection, which is associated with the small, albeit life-threatening risk of brain stem anaesthesia.

Posterior segment

The posterior segment of the eye refers to the posterior two-thirds of the eye, including the anterior hyaloid membrane and all the structures behind it such as the vitreous, retina choroid and optic nerve. The leading causes of visual impairment and irreversible blindness are posterior segment-related diseases (Thrimawithana *et al.*, 2011). Inflammation and fibrosis are also symptoms associated with posterior segment disorders and ocular tissue damage. The uniqueness of the cellular composition and anatomical structure of the retina means that these biological processes are devastating and detrimental to vision. Inflammatory responses or hypoxic stimuli cause abnormal blood vessels to leak resulting in retinal thickening and oedema, fibrovascular proliferation and fractional retinal detachment. To preserve the retinal structure and function, it is crucial to prevent any primary vascular abnormality (Friedlander, 2007).

Only a decade ago, ocular treatments for these diseases were limited and were, at best, kept in check with laser therapy. With the advent of anti-VEGF treatment, there have been significant improvements in stabilizing and sometimes improving disease states and vision (Jain *et al.*, 2012). The delivery of anti-VEGF medicines to the posterior segment poses a particular challenge. The structures in the anterior segment serve as additional barriers to those discussed above. Furthermore, the direction of aqueous flow in the eye (ciliary body to anterior chamber angle) is against the direction of drug delivery *via* a topical route. It is estimated that drugs applied in a conventional topical way are diluted by a factor of between 250 000 and 1 000 000 by the time the vitreous is reached

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(Maurice, 2002). Instead, the only modalities of treatment in everyday use are either periocular or i.v.t. injections. Avoiding all barriers, the i.v.t. route results in the greatest bioavailability and, for large molecules, such as proteins, is the only route in current clinical practice. However, each time an injection is performed, there is a small but significant risk of a blinding complication. The risks of retinal detachment and infective endophthalmitis have been reported as 0.9 and 0.2% per injection respectively (Jager *et al.*, 2004). Frequent (4–8 weeks) anti-VEGF treatment will result in a cumulative increase. Much research has been performed to improve transscleral delivery (Amrite *et al.*, 2008), which would remove the need to breach the walls of the eye, and to develop sustained release preparations, which would reduce the frequency of repeated i.v.t. injections.

Periocular barriers. A drug must penetrate the tissue layers of the globe to reach the back of the eye at a therapeutic level. Various tissue barriers have varying tissue permeability. Unlike the cornea; however, there is blood flow through the periocular tissues, and considerable amounts of a drug are lost to the systemic circulation *via* venules and lymphatics. The Tenon's tissue and episclera are highly vascular with a network of capillary plexi. The reported rate of removal of drugs is between 5 and 80% (Edelhauser *et al.*, 2010). This has implications not just for reaching the therapeutic concentration but also for preventing systemic toxicity.

Molecules that evade clearance by the subconjunctival circulation reach the sclera, the outer coating of the globe. The sclera is continuous with the cornea at the limbus and has a larger surface area. The sclera is hydrated, mainly composed of collagen fibres and proteoglycans embedded in an ECM, but with few protein binding sites. Its permeability is comparable to the corneal stroma, and aqueous intercellular media carry molecules through pores between the collagen fibres. Molecular radius appears to play a bigger role in determining drug permeability than lipophilicity (Prausnitz and Noonan, 1998), and molecules of linear shape, for example, dextrans, are less able to permeate than globular proteins (Geroski and Edelhauser, 2001). Electrical charge also contributes to permeation. Positively charged molecules penetrate the sclera poorly, believed to be due to their binding to the negatively charged proteoglycan matrix (Kim *et al.*, 2007).

The choroid is below the sclera and is a vascular network whose high flow rate results in considerable drug loss. The main function of the choroid is to supply the retina with nutrients and oxygen. Due to the retina being the most metabolically active tissue in the body (per unit mass), the choroid has a correspondingly high blood flow through it, again being the highest (per unit mass) in the body (Scheinman *et al.*, 2011). The choroid contains an outer layer of large vessels, a middle layer of small vessels and an innermost capillary layer, the choriocapillaris. The choriocapillaris is fenestrated, which enhances metabolic exchange, but this highly dynamic equilibrium also contributes to drug loss.

The retinal pigment epithelium (RPE) is a single-layered structure that acts as the metabolic interface between the photoreceptors and choroid. It forms the outer BRB, with tight junctions between cells preventing the passage of large molecules and cells across it. The inner BRB is formed by the endothelium of retinal arterioles, which are also linked with tight junctions. Both the choroid and the RPE are pigmented and rich in melanin. Melanin binds to free radicals and other molecules by electrostatic and Van der Waals forces, or simple charge transfer (Larsson, 1993). Melanin-bound drug can form a reservoir that is then gradually released to the surrounding cells and, as a result, prolongs drug release (Urtti, 2006; Gaudana *et al.*, 2010; Manzanares *et al.*, 2016). The binding of drugs to melanin is especially important in transscleral drug delivery. The unbound drug is the driving force in drug permeation, and the bound drug acts as a reservoir (Ranta and Urtti, 2006). Significant retention of lipophilic compounds occurs within the Bruch's membrane and the choroid presumably due to the high levels of melanin (Cheruvu and Kompella, 2006). However, the choroid-RPE is even more impermeable to hydrophilic molecules, being up to 20 times less permeable to hydrophilic β-blockers than lipophilic ones (Pitkanen, 2005). There does appear to be regional and species-specific differences within the choroid-RPE (Durairaj *et al.*, 2012). The peripheral choroid-RPE contains more melanin than the central choroid-RPE. In humans, unlike monkeys or rabbits, there is also evidence that the sclera may contain more melanin than the central choroid-RPE (Durairaj *et al.*, 2012). These differences should be considered when using animal models for drug development as it can cause changes in the drug's pharmacokinetic and clearance profiles.

Injection routes of administration

Sub-Tenon injections. Sub-Tenon's injection of certain drugs has been shown to be effective for posterior segment disease. Inflammation is a key part of the pathophysiology of macular oedema following cataract surgery (Irvine Gass syndrome), diabetic macular oedema and posterior uveitis. The steroid **triamcinolone acetonide** (TA) has been used as treatment for these conditions with variable success. Most commonly, it is administered as an i.v.t. injection but this route of administration is associated with the additional specific complications of cataract and raised IOP. Sub-Tenon's administration may carry a lower risk of complications. No difference in visual acuity outcomes for uveitic macular oedema was noted between i.v.t. and sub-Tenon administration of TA (Choudhry and Ghosh, 2007). There is also evidence that sub-Tenon injection leads to a significant increase in the levels of steroid in vitreous fluid. Patients that received sub-Tenon's TA (40 $mg{\cdot}mL^{-1}$) preoperatively before macular hole surgery were found to have vitreous levels maintained between 17 and 31 $ng{\cdot}mL^{-1}$ for 2 months (Kovacs *et al.*, 2012). Systemic toxicity may be an issue with sub-Tenon's steroid injection. In this study, plasma cortisol levels were also altered, which has important implications for patients with poor glucose control such as diabetics.

Other transscleral delivery systems are at different stages of development. Implants have been designed with an impervious casing for sub-Tenon's placement and openings directed towards the sclera (Pontes De Carvalho, 2006). Clinical trials evaluating efficacy are currently underway for the treatment of retinoblastoma using this method of delivery for the chemotherapeutic agents, carboplatin and topotecan Intravitreal (i.v.t.) injections

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The delivery of most treatments for the posterior segment must be by i.v.t. injection due to transscleral barriers. This is particularly pertinent for the delivery of large molecules such as anti-VEGF antibodies, although periocular delivery and even topical delivery continue to be explored (Chen *et al.*, 2011). Due to the invasive nature and the associated risks of i.v.t. injections, there is much effort to develop longer lasting therapies, which require a reduced dosing frequency.

Understanding the pharmacokinetics of drugs in the vitreous fluid in humans is a significant challenge. The vitreous of humans is of a different consistency to that of other animals. It also undergoes considerable changes with age (Sebag, 1989). As people grow older, the vitreous loses viscosity, with lakes of fluid appearing within it (Bishop, 2000). Collagen fibril links break down in some parts of the vitreous, and fibrils aggregate in other parts (Bishop, 2000; Bishop *et al.*, 2004). At the weaker points, the attachments can fall away and float around inside the eye. This lack of uniformity will affect drug distribution but may preferentially result for example in anti-VEGF treatment being located over the macula.

The administration of humanized protein therapeutics (e.g. antibodies) intravitreally results in proteins diffusing more slowly in the vitreous than low molecular weight (MW) molecules. Therapeutic protein clearance times tend to be much longer than that of low MW compounds (days rather than hours) (Hayreh, 1966; Kwak and Amico, 1992; Cunha-Vaz, 1997; Pitkanen, 2005; Urtti, 2006; Laude *et al.*, 2010; Thrimawithana *et al.*, 2011; Haghjou *et al.*, 2013). An issue with therapeutic proteins is that the use of animals to evaluate pharmacokinetics will result in the development anti-drug antibodies (ADAs). The presence of ADAs will result in the rapid clearance of the candidate protein and can also cause acute hypersensitivity or infusion reactions. ADAs can also bind to the active region of the therapeutic protein, such as the receptor-binding site, to neutralize the drug. Therefore, it is difficult to determine the efficacy of a drug. ADAs unpredictively change the pharmacokinetic properties, biological effects and toxicity profile of a drug (Brinch *et al.*, 2009; Tamilvanan *et al.*, 2010; Brinks *et al.*, 2011; Vugmeyster *et al.*, 2012; Wang *et al.*, 2012). ADAs are an intractable problem in the growth of protein therapeutics, especially for the development of new formulations with a prolonged duration of action.

Another factor whose contribution to drug distribution in the vitreous is difficult to quantify is eye movements. Saccades can result in sudden globe rotations of up to $900°{\cdot}s^{-1}$, approximately half the speed of an old washing machine on a spin cycle. These abrupt changes shake up the vitreous and affect drug distribution. As the posterior segment is not a perfect sphere, with the lens causing a concave indentation, vortices are created with eye movements (Stocchino *et al.*, 2007; Repetto *et al.*, 2010).

Drug distribution and clearance is difficult to estimate in the new *in vitro* models recently developed (Repetto *et al.*, 2005; Awwad *et al.*, 2013, 2015, 2017; Fogli *et al.*, 2014; Patel *et al.*, 2015). Therapeutic proteins do not readily permeate the retina and thus clear *via* aqueous outflow through the anterior chamber into the conjunctiva. A two-compartment model of the eye that mimics aqueous outflow mimics the human clearance times of therapeutic proteins (Awwad *et al.*, 2015).

Some controversy exists as regards the frequency of the treatment regimen for certain pathologies. Exudative macular degeneration is a pathology that involves blood vessel growth and leakage from the choroid underneath the macula [choroidal neovascularization (CNV)]. Disruption of the retinal layers results in loss of visual function. A major factor for new blood vessel growth is VEGF that is released in association with oxidative stress. Anti-VEGF antibodies have revolutionized treatment outcomes, with patients showing stabilization or improvement of vision together with a reduction in leakage. What is still unclear is the frequency and time span of drug administration. Both the ANCHOR and MARINA trials examined visual outcomes after monthly dosing of ranibizumab, a humanized monoclonal antibody fragment antigen-binding (Brown *et al.*, 2006; Rosenfeld *et al.*, 2006). Other trials such as the PRONTO or SUSTAIN trial have shown significant benefit from monthly injections of ranibizumab for 3 months only, then injections as required (Lalwani *et al.*, 2009; Holz *et al.*, 2011). Comparative data between injections-as-required and monthly injections found little difference in outcome at 1 year in the CATT trial. The CATT and IVAN trials also compared the use of ranibizumab and bevacizumab, which is used unlicensed. Bevacizumab is a full IgG1 antibody registered for systemic use in cancer, but its use has been shown by the CATT (CATT Research Group *et al.*, 2011) and IVAN (Chakravarthy *et al.*, 2012) trials to be broadly similar to that of ranibizumab. Issues arise because bevacizumab has not been formulated for ocular use (Liu *et al.*, 2011a; Ng *et al.*, 2012; Palmer *et al.*, 2013). Presentation of bevacizumab is in a vial containing $25\ mg{\cdot}mL^{-1}$ of antibody, and the dose was established simply by what was in a 50 mL injection. However, costs are dramatically reduced compared to ranibizumab, so bevacizumab is also widely used to treat conditions related to vascularization (e.g. AMD).

Intravitreal (i.v.t.) implants. A strategy to prolong drug action that has long been used clinically for low MW molecules is the i.v.t. injection of a drug suspension of a poorly soluble drug. Although not yet possible with therapeutic proteins, this approach works with poorly soluble steroids. TA, as discussed earlier, consists of a suspension of steroid particles. An injection of 4.0 mg of TA has been shown to maintain elevated levels of steroid up to 3 months in non-vitrectomized eyes (Mason *et al.*, 2004). The 4.0 mg dose is too large of a mass to dissolve in the eye, so TA dissolution occurs slowly over time with aqueous flow (Jermak *et al.*, 2007; Yilmaz *et al.*, 2011; Zacharias *et al.*, 2013).

A growing field of research is the use of implants for the treatment of posterior segment disease (Figure 1, Table 2). These allow sustained release of drug, removing the need for multiple injections. Taking advantage of the privileged immune state of the inside the eye, many implants have been used successfully without provoking the significant inflammatory reaction that would normally be found with implants used elsewhere in the body (Ratner, 2002). Chronic diseases or those with a relapsing remitting course are particularly appropriate for sustained release implant therapy. The implants may be formulated from biodegradable or non-biodegradable substances (Choonara *et al.*, 2010; Lee *et al.*, 2010; Thrimawithana *et al.*, 2011; Christoforidis *et al.*, 2012; Gilger

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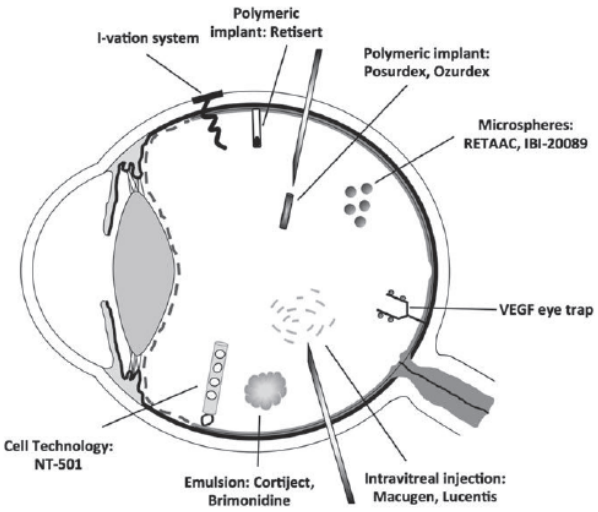

Figure 1
Some of the drug delivery technologies indicated for the treatment of posterior segment diseases

et al., 2013; Cima *et al.*, 2014; Kang-Mieler *et al.*, 2014; Thakur *et al.*, 2014; Bisht *et al.*, 2017; Prata *et al.*, 2017).

In immunocompromised patients, such as those with leukaemia or acquired immune deficiency syndrome, the opportunistic infection CMV can cause retinal destruction (Pollard, 1980). Patients may remain asymptomatic until advanced stages of retinal necrosis and haemorrhage. The advent of highly active antiretroviral therapy has reduced the incidence of patients developing CMV retinitis; however, individuals with specific immune deficiency may still develop retinitis when infected with CMV. Effective treatment of CMV retinitis not only reduces the risk of vision loss but is also associated with a decrease in mortality. Standard therapy may include ganciclovir (Pollard, 1996; Martin *et al.*, 1999), foscarnet (Berthe *et al.*, 1994; López-Cortés *et al.*, 2001) and cidofovir (Ljungman *et al.*, 2001; Biron, 2006).

The ganciclovir slow release implant (Vitrasert) was one of the first approved implants for i.v.t. application (Sepahvandi *et al.*, 2016). It releases ganciclovir at 1 $mcg{\cdot}h^{-1}$ from a PVA/EVA polymer-based system. In a clinical trial, it was found to be twice as effective at slowing retinitis progression as treatment with intravenous ganciclovir (Musch *et al.*, 1997). One of the pitfalls of implant treatment to one eye

Table 2
FDA-approved drugs and those in current clinical trials for treatment of posterior segment diseases

Name and company	Drug delivery platform	Drug and excipients	Indications and ongoing trials	Status
Brimonidine (Allergan)	i.v.t. implant	Brimonidine (0.4 mg) with PLGA	Dry AMD with GA	Phase II
Cortiject®/NOVA63035 (Novagali Pharma)	i.v.t.	Dexamethasone emulsion (preservative and solvent-free)	DME	Phase I/II
Durasert™ (pSivida Corp)	i.v.t. implant	Latanoprost with EVA/PVA	Ocular hypertension and glaucoma	Phase I/II
I-vation® (Surmodics Inc)	Reservoir i.v.t. implant	TA (0.925 mg) with PMMA/EVA	DME	Phase IIb
IBI-20089 (Icon Biosciences Inc)	i.v.t.	TA (6.0 mg or 13.8 mg) and benzyl benzoate with lucentis (0.5 mg)	AMD CNV	Phase I Phase II
Iluvien™ (Alimera Sciences, Inc.)	25-gauge i.v.t. implant	FA (0.19 mg) with polyimide/PVA	DME AMD with GA Posterior uveitis	Approved Phase II Phase III
NT-501/Renexus (Neurotech Pharma)	i.v.t. implant	CNTF with PET and ARPE-19 cells	Glaucoma RP	Phase I
NT-503 (Neurotech Pharma)	i.v.t. implant i.v.t. injection	Anti-VEGF receptor protein	Recurrent CNV with wet AMD	Phase I Phase II
NT-506 (Neurotech Pharma)	i.v.t. implant	Anti-**PDGF**/Anti-VEGF	Wet AMD	Preclinical
Ozurdex® (Allergan)	i.v.t. implant	Dexamethasone (0.7 mg) with PLGA	DME, CRVO, uveitis	Approved
Posurdex (Allergan)	i.v.t. implant	Dexamethasone	Posterior uveitis DME	Phase III Phase III
Retisert® (Bausch & Lomb)	Reservoir i.v.t. implant	FA (0.59 mg) with silicone/PVA	Non-infectious uveitis	Approved
Vitrasert® (pSivida Corp)	Reservoir i.v.t. implant	Ganciclovir with EVA/ PVA	CMV retinitis	Approved

All drug therapies mentioned above were also cross-checked with www.clinicaltrials.gov.uk and its official company site. BRVO, branch retinal vein occlusion; CNTF, ciliary neutrophic factor; CRVO, central retinal vein occlusion; EVA, ethylene-vinyl acetate; FA, fluocinolone acetonide; GA, geographical atrophy; PET, polyethylene terephthalate; PLGA, poly(lactide-co-glycolide); PMMA, nn(methyl methacrylate); PVA, poly(vinyl alcohol).

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only, however, is that is that the other eye is not protected, and, in the implant-treated group of patients, the time to second eye involvement was significantly shorter. Combination therapy with both implant and oral valganciclovir (oral version of ganciclovir) is now advocated for sight threatening retinitis (Jabs, 2008). Controversy exists as to whether implants should be removed from the eye after the drug has been released, as its removal is associated with a risk of retinal detachment, haemorrhage and infection.

Treatment of macular oedema has also been successfully achieved with steroid implants. Macular oedema results from the leakage of fluid from vessels within the retina, which disrupts the retinal architecture, and causes vision loss. It is seen in association with uveitis, RVO and diabetic maculopathy. The i.v.t. injection of the steroid TA has been shown to be beneficial in reducing oedema; however, recurrence is a common feature of all diseases. Work on a dexamethasone pellet coated in PVA/EVA showed promise using a uveitic model of rabbits (Cheng *et al.*, 1995). The Ozurdex® implant has recently been approved by the Food and Drug Administration (FDA) for the treatment of macular oedema in association with non-infectious uveitis and RVO (Mehta *et al.*, 2015; Barar *et al.*, 2016). It consists of 0.7 mg of dexamethasone integrated into a poly(lactic–co-glycolic acid) polymer. Pharmacokinetic studies revealed that the steroid continues to be released into the vitreous over a period of 4 months (Chang-Lin and Attar, 2011). The polymer has the added advantage of being biodegradable, requiring no surgical removal. Other implants are at different stages of development (Table 2).

Future directions

Prolonging the duration of drug action to targets while maintaining drug stability and specificity are key goals of current research. Novel ways of solubilizing drugs by conjugation with carriers, gene therapy and implants that are refillable or able to be directed remotely to the area of treatment are concepts in the process of development.

Dendrimers

Nanotechnology has been used to increase the drug residence time. Dendrimers are nano-sized, three-dimensional structures with a central core from which polymeric branches emerge (Patri *et al.*, 2002). The hydrophobic drug is encapsulated in the internal cavity or complexed to the dendrimer. Its tree-like structure consists of a high surface density of functional groups, which make it possible to attach a wide variety of conjugates. Dendrimers can aid in controlled drug release due to their small size, high chemical versatility, drug loading capacity and ease of preparation (Yavuz *et al.*, 2015; Rodríguez Villanueva *et al.*, 2016). The poly amidoamine (PAMAM) dendrimers include functional carboxylic, hydroxyl and amine groups, and these increase with generation number.

Dendrimer drug complexes have shown potential as an application for prolonging treatment of CNV. A dendrimer complex of anti-VEGF antisense oligonucleotide was found to inhibit CNV in a laser-induced rodent model when injected into the vitreous fluid. The effective lifespan of the dendrimer-bound oligonucleotide was reported to be between 4 and 6 months, with effective protection against nucleases whilst also facilitating its delivery to the target site (Marano *et al.*, 2005).

In a rabbit scarring model after glaucoma filtration surgery (GFS), dendrimer conjugates (dendrimer-glucosamine as an immunomodulator and dendrimer glucosamine 6-sulphate as an anti-angiogenic molecule) were found to be efficacious as an anti-scarring agent. Histopathological examination of the eyes showed minimal scar tissue formation and no inflammatory or neoangiogenic response, and the success of the GFS was increased from 30 to 80% (Shaunak *et al.*, 2004).

PAMAM dendrimers have also been found to be compatible with topical ocular dosage forms in terms of physicochemical properties (pH, osmolality and viscosity) (Vandamme and Brobeck, 2005). Topical formulations of drugs such as pilocarpine nitrate (Vandamme and Brobeck, 2005), carteolol (Spataro *et al.*, 2010) and dexamethasone (Yavuz *et al.*, 2015) have been reported. The puerarin-PAMAM dendrimer complex increased the corneal residence time of the drug thereby improving the efficacy and decreasing the frequency of dosing (Yao *et al.*, 2010). This system also improved the availability of puerarin and enhanced the half-life of the drug in the aqueous humour of rabbits from 0.48 to 1.30 h (Wang *et al.*, 2011).

Hydrogels

Hydrogels have received considerable interest in recent years due to their ability to prolong efficacy whilst being biocompatible and biodegradable. Hydrogels are an alternative to particulate-associated formulations and have been used for the delivery of large MW molecules (Mitragotri *et al.*, 2014). They are polymeric materials that do not dissolve in water under physiological conditions and swell considerably in aqueous medium. Many hydrogel systems that have been described that do not require organic solvents during preparation (Stile *et al.*, 1999; Vermonden *et al.*, 2012; Shi *et al.*, 2013). Hydrogels are networks of polymer main chains covalently linked together. This is known as cross-linking, and sometimes, the polymer cross links can be strong non-covalent interactions. The cross-linking of polymer chains prevents complete dissolution of the polymer in a good solvent. Hence, hydrogels made of hydrophilic polymers can absorb water into their network structure and swell. The high water content property of hydrogels makes them biocompatible and is being examined for their application for tissue regeneration. However, the high water content of hydrogels is a challenge for developing extended drug release. Hydrogels can be made with polymers that can undergo a phase transition in response to different external conditions such as pH, temperature, electric currents and ionic strength (Simões *et al.*, 2012). Such hydrogels can be made to 'collapse' to entrap a drug after administration.

One thermosensitive material that shows gel–sol transition is poly(N-isopropylacrylamide) (pNIPAAM) (Yıldız *et al.*, 2002; Silva and Oliveira, 2007; Li *et al.*, 2008; Drapala *et al.*, 2011; Klouda, 2015). It has been used in many drug delivery and tissue engineering studies (Li *et al.*, 2008), with no signs of retinal toxicity (Turturro *et al.*, 2011). PNIPAAM has a transition temperature of 32°C, and cross-linking the hydrogel with poly(ethylene glycol) diacrylate improves the drug release profile by forming homogenous pores

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(Zhang *et al.*, 2009). This characteristic has been exploited to encapsulate and release protein for ocular delivery to the posterior segment.

Reservoir-type devices

Another approach being explored recently is the use of refillable reservoir-type devices. When the existing drug depletes, the reservoir can be refilled with more drug. A microelectromechanical system (MEMS) drug delivery device consisting of a pump, drug reservoir and transscleral cannula showed promise in *ex vivo* porcine eyes (Li *et al.*, 2008). Another MEMS device described in the literature is formed of layers, by using lithography and master moulds made up of silicon wafers or acrylic. A hard-core base plate made up of polyetherether ketone is used to control the penetration depth of the needle and prevent the puncture of the entire device by the force of the needle. It is surgically implanted with the reservoir placed beneath the conjunctiva, with the cannula inserted through the sclera and drug dispensing tip, terminating in either the anterior or posterior segment depending on the site of treatment required. When mechanically actuated by force applied by the patient's finger, a pressure gradient occurs and the drug flows into the transscleral cannula. The one-way valve at the end of the cannula opens and results in the release of a specific dose of the medication. This device was tested *in vivo* on rabbits using phenylephrine as a model drug (Lo *et al.*, 2009). The Port Delivery System (ForSight VISION4, Inc.) is a refillable drug delivery device currently in phase 1 for preliminary safety for neovascular AMD (www.clinicaltrials.gov: NCT01186432) (Pearce *et al.*, 2015).

Selective i.v.t. drug delivery using a microrobotic device is a novel invention. Controlled electromagnetically, it acts as an i.v.t. implant with a drug reservoir (Weidle *et al.*, 2013). These microrobots can be placed in the lower vessel arcade without obstructing vision. They are non-biodegradable and are made biocompatible by coating them with polypyrrole. It was demonstrated *in vitro* and *ex vivo* that the movement of this device could be wirelessly controlled in the vitreous. The group proposed an algorithm taking into account the complex optics of the eye that helps to localize the device based on 3-D structure and allows a precise calculation of the gradients and forces acting on the device.

Stem cell technology

Stem cells have been explored as a potential path for the treatment of ocular diseases (Ramsden *et al.*, 2013; Jeon and Oh, 2015; Rao *et al.*, 2017). Stem cells have the ability to grow into many different cell types, that is, specialized cells can arise from their differentiation (Vellonen *et al.*, 2014). Stem cells are critical for the renewal and repair of tissue in the body. Pluripotent stem cells (embryonic stem cells and induced pluripotent stem cells) are a rich source for the regeneration of damaged organs and tissues (Higuchi *et al.*, 2017). Methods have been reported for the differentiation of human pluripotent stem cells (Gao *et al.*, 2013; Burridge *et al.*, 2014; Pagliuca *et al.*, 2014; Rezania *et al.*, 2014; Bao *et al.*, 2015). A key challenge for the clinical use of stem cells is to optimize cell placement and the local host environment so that cell survival, differentiation and integration leads to the long-term restoration of tissue function (Limb and Daniels, 2008). One of the major barriers to successful stem cell implantation and integration is to control the local inflammatory and fibrotic responses to the transplantation.

Stem cell treatments for corneal injuries are available clinically (Daniels *et al.*, 2006; Daniels *et al.*, 2007). Recent progress in retinal stem cell research has been reported for the treatment of dry AMD. Although no treatment is available for the management of dry AMD, vitamins and anti-oxidants have been used to slow its progression. Stem cells could possibly help treat the severe retinal degeneration involved in the condition to restore sight (Jeon and Oh, 2015). Several trials are currently underway to treat AMD and retinitis pigmentosa (RP) using human embryonic, fetal and umbilical cord tissue-derived stem cells and bone marrow-derived stem cells (Ramsden *et al.*, 2013).

Even though stem cell transplantation for retinal diseases has made considerable progress in the last decade, many issues still need to be resolved before stem cell therapies can be routinely used in the clinic (Rao *et al.*, 2017). Biological risk and technical difficulties associated with the differentiation and culture procedures still need to be resolved (Jeon and Oh, 2015). As our understanding of the retinal regeneration phenomena increases, the likelihood of using cell therapies to regenerate retinal ocular tissues will increase.

Gene therapy

Gene products have been reported to slow or prevent neovascularization (Garoon and Stout, 2016). Gene therapies (Rowe-Rendleman *et al.*, 2014) require direct cellular delivery (del Amo *et al.*, 2017) using a carrier system (Schön *et al.*, 2015), where a key challenge is to release the gene drug from the carrier within the cell (Karimi *et al.*, 2016). Furthermore, this often requires the gene drug to enter the nucleus. Vector loaded suspensions can be administered *via* a sub-retinal injection by pars plana vitrectomy with retinotomy and injection into the subretinal space with a 41-gauge cannula (Garoon and Stout, 2016). For the gene therapy to be successful, there needs to be no toxicity or immunogenicity after the delivery of highly efficient genes to specific targeted cells (Bloquel *et al.*, 2006). Both viral and non-viral vectors are available. Examples of viral vectors include adenoviruses, adeno-associated-virus (AAV), retroviruses and lentivirus vectors (Chaum and Hatton, 2002; Borrás, 2003; Mohan *et al.*, 2005; Garoon and Stout, 2016). Adenoviruses are the most commonly used vector for targeting a variety of chronic ocular diseases. Some of the AAV-based systems in clinical trials require a sub-retinal injection (Bainbridge *et al.*, 2015; Weleber *et al.*, 2016). The administration of adenoviruses allows for stable, long-term transgene expression in photoreceptors, RPE cells, ganglion cells and Müller cells (Liu *et al.*, 2011b). Viral vectors have been reported to display potential risks, such as mutagenesis and immunogenicity, which hinders the future development of these systems (Bloquel *et al.*, 2006). An alternative solution is non-viral vectors, which display no toxicity and do not induce significant ocular inflammation (Bloquel *et al.*, 2006). Reviews dedicated to gene therapy for ocular drug delivery can be found elsewhere (Bainbridge *et al.*, 2006; Bloquel *et al.*, 2006; Boye *et al.*, 2013; Garoon and Stout, 2016; Oliveira *et al.*, 2017).

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Neuroprotective agents

Ciliary neurotrophic factor (CNTF) is a cytokine belonging to the **IL-6** family. CNTF promotes neurotransmitter synthesis, neurite outgrowth and photoreceptor survival (Liu *et al.*, 2011b). CNTF has been shown to be protective against ganglion cell death in models of oxidative stress and experimental glaucoma (Lipinski *et al.*, 2015). Studies with recombinant adeno-associated virus-mediated gene therapy showed sustained expression of CNTF with the protection of photoreceptors (Liang *et al.*, 2001; Bok *et al.*, 2002; Schlichtenbrede *et al.*, 2003). NT-501 (Neurotech Pharmaceuticals) is an implantable cell technology comprising encapsulated human RPE cells (ARPE-19) genetically modified to secrete CNTF over an extended period of time for the treatment of glaucoma and retinitis pigmentosa. In a clinical trial, NT-501 was found to reduce photoreceptor degradation in patients with retinitis pigmentosa and improve their objective visual acuity (Sieving *et al.*, 2006). The implant consists of a scaffold of six strands of polyethylene terephthalate yarn and is sutured onto the sclera through a titanium loop (Thrimawithana *et al.*, 2011). A semipermeable membrane allows the inward and outward diffusion of CNTF and protects the host immune system from being attacked. NT-501 represents a unique platform for the delivery of protein therapeutics (Sieving *et al.*, 2006). NT-501 has completed phase II clinical trial for patients with atrophic macular degeneration (NCT00447954), and the implant was reported to be well tolerated (Girmens *et al.*, 2012).

Conclusions

The eye is unique as a therapeutic target in the body. Whilst exhibiting a vast range of pathologies, both local and systemic, its superficial nature enables effective local therapy. The privileged state of the immune system inside the eye means that there is a greater tolerance of non-host materials, although the risk of infection significantly increases each time the walls are breached. Ocular therapeutic development is therefore geared towards improving the bioavailability of topical and periocular treatments, while efficacious sustained release implants are the cornerstone of development for therapies of the posterior segment.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (Alexander *et al.*, 2015).

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Conflict of interest

The authors declare no conflicts of interest.

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