

OCULAR DRUG DELIVERY

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CHAPTER OBJECTIVES

Upon completing this chapter, the reader should be able to

- ▶ Describe the anatomy and physiology of the eye.
- ▶ Understand the importance of various routes of drug administration to the eye.
- ▶ Describe various barriers to ocular drug delivery and constraints with conventional ocular therapy.
- ▶ Determine the ideal physicochemical properties for ocular drug candidates.
- ▶ Acquire sound knowledge of various approaches to increase ocular drug absorption.
- ▶ Understand the utility, recent progress, and specific development issues relating to dendrimers, cyclodextrins, nanoparticles, liposomes, nanomicelles, microneedles, implants, *in situ* gelling systems, and contact lens in ocular drug delivery.
- ▶ Explore key advances in the application of nanotechnology for gene delivery to the eye.

CHAPTER OUTLINE

Introduction

Anatomy of the Eye

Routes of Ocular Drug Administration

Role of Efflux and Influx Transporters in the Eye

Prodrug Strategies for Increasing Ocular Drug Absorption

Colloidal Dosage Forms for Enhancing Ocular Drug Absorption

- Dendrimers

- Cyclodextrins

- Nanoparticles

- Liposomes

- Nanomicelles

- Microneedles

Implants

- Nonbiodegradable Implants

- Biodegradable Implants

Contact Lens

In Situ Gelling Systems

Gene Delivery

- Adenovirus Vectors

- AAV Vectors

- LV Vectors

- Nonviral Vectors

Acknowledgment

Summary

Review Questions

References

INTRODUCTION

Drug delivery to the eye has been one of the most challenging tasks to pharmaceutical scientists. The unique anatomy and physiology of the eye renders it a highly protected organ, and the unique structure restricts drug entry at the target site of action. Drug delivery to the eye can be generally classified into anterior and posterior segments. Conventional delivery systems, including eye drops, suspensions, and ointments, cannot be considered optimal; however, more than 90% of the marketed ophthalmic formulations indicated for the treatment of debilitating vision-threatening ophthalmic disorders are in the form of eye drops. These formulations primarily target diseases of the

front of the eye (anterior segment). Most topically instilled drugs do not offer adequate bioavailability due to the wash off of the drugs from the eye by various mechanisms (lacrimation, tear dilution, and tear turnover). In addition, the human cornea composed of epithelium, substantia propria, and endothelium hinders drug entry; consequently, less than 5% of administered drug enters the eye.

Alternative approaches are continuously sought to facilitate significant drug absorption into the eye. Currently, the treatment of disorders of the back of the eye (posterior segment) still remains a formidable task for the ocular pharmacologists and physicians. The tight junctions of blood–retinal barrier (BRB) limit the entry of systemically administered drugs into the retina. High vitreal drug concentrations are required for the treatment of posterior segment diseases. This can be accomplished only with local administration (intravitreal [IVT] injections/implants and periocular injections). Periocular injections are associated with fairly high patient compliance relative to IVT injections. Nevertheless, structural variations of each layer of ocular tissue can pose a significant barrier upon drug administration by any route (i.e., topical, systemic, and periocular). To date, remarkable changes have been observed in the field of ophthalmic drug delivery.

This chapter offers great insight into anatomical and physiological features of the eye and various routes of ocular drug administration. Furthermore, this chapter emphasizes the role of various ocular transporters, prodrug strategies, colloidal dosage forms, implants, contact lens, *in situ* gelling systems, and other recent developments in drug delivery strategies, including gene therapy.

ANATOMY OF THE EYE

The eye is an isolated, highly complex, and specialized organ for photoreception. A complex anatomy and physiology renders it a highly protected organ [1]. Generally, the eye can be divided into two segments: anterior and posterior. The anterior segment of the eye is composed of cornea, conjunctiva, iris, ciliary body, aqueous humor, and lens, whereas the posterior segment includes sclera, choroid, retina, and vitreous body (**Figure 10-1**). The front part of the eye is bound by a transparent cornea and a minute part of the sclera. The cornea and sclera join together through the limbus. Cornea is devoid of blood vessels and receives nourishment and oxygen supply from the aqueous humor and tear film, whereas the corneal periphery receives nourishment from the limbal capillaries. The human cornea measures approximately 12 mm in diameter and 520 μm in thickness. It is composed of six layers: the epithelium, Bowman's membrane, stroma, Dua's layer, Descemet's membrane, and endothelium (**Figure 10-2**) [2]:

- **Epithelium:** The corneal epithelium is a stratified, squamous, nonkeratinized layer approximately 50 μm in thickness. It serves as an outer protective barrier comprising five to six cell layers, including two to three layers of flattened superficial cells, wing cells, and a single layer of columnar basal cells separated by a 10- to 20-nm intercellular space. Desmosome-attached cells can communicate by gap junctions through which small molecules permeate. The superficial epithelial cell layers are sealed by tight junctions called zonulae occludens that prevent the permeation of compounds with low lipophilicity across the cornea. Thus, the corneal epithelium is a rate-limiting barrier and hinders the permeation of hydrophilic drugs and macromolecules.
- **Bowman's membrane:** This acellular thin basement layer is made up of collagen fibrils. It is not considered as a rate-limiting barrier.

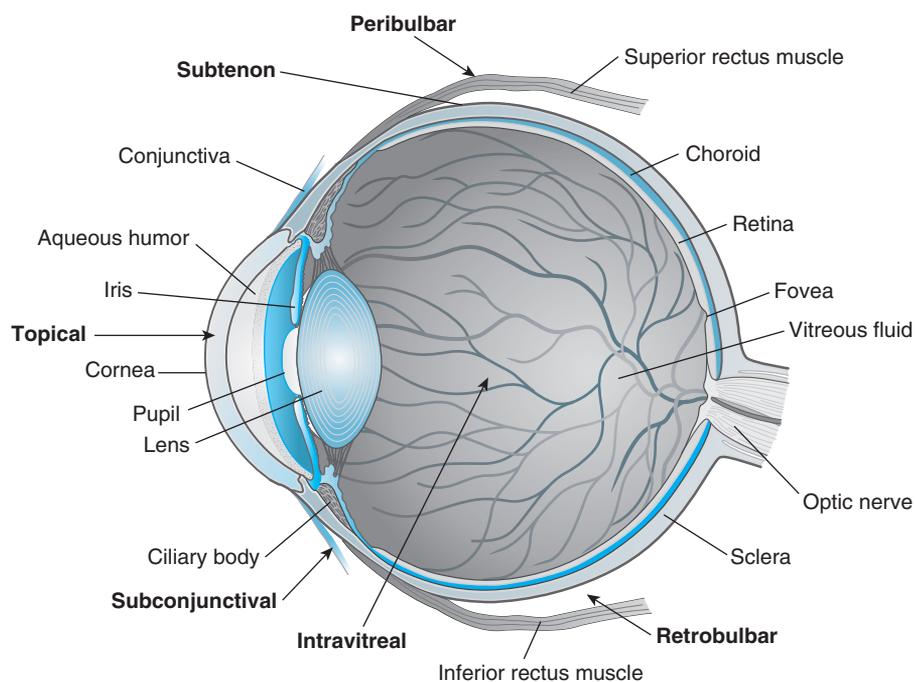


FIGURE 10-1 Anatomy of the eye and various routes of ocular drug administration.

- **Stroma:** Constituting about 90% of the cornea, this layer is abundant in hydrated collagen. Because of its hydrophilic nature, the stroma offers minimal or low resistance to diffusion of highly hydrophilic drugs.
- **Dua's layer:** It is a newly identified, well-defined, acellular, strong layer in the pre-Descemet's cornea. Its functional role is yet to be determined [3].
- **Descemet's membrane:** It is a thin homogeneous layer sandwiched between the stroma and the endothelium.
- **Endothelium:** It is a single-layered squamous epithelium posterior to the corneal surface. The stroma and Descemet's membrane cover the inner endothelial cells, which contain macula adherens. The endothelial cells do not act as a barrier to permeant molecules due to lack of tight junctions.

Conjunctiva is a vascularized mucous membrane lining the inner surface of eyelids and the anterior surface of sclera up to the limbus. It facilitates lubrication in the eye by generating mucus and helps with tear film adhesion. It offers less resistance to drug permeation relative to cornea. Iris, the most anterior portion of the uveal tract, is the pigmented portion of the eye consisting of pigmented epithelial cells and circular muscles (constrictor iridial sphincter muscles). The opening in the middle of the iris is called the pupil. The iris sphincter and dilator muscles aid in tuning the pupil size, which regulates the entry of light into the eye. The ciliary body, a ring-shaped muscle attached to the iris, is produced by ciliary muscles and the ciliary processes. The aqueous humor, a fluid present in the anterior segment, is secreted by the ciliary processes into the posterior segment at the rate of 2 to 2.5 $\mu\text{L}/\text{min}$. It supplies most nutrition and oxygen to avascular tissues (lens and cornea). It flows continuously from the posterior to the anterior through the pupil and leaves the eye via trabecular meshwork and Schlemm's canal. Such continuous flow maintains the intraocular pressure (IOP). The lens is a crystalline and flexible structure enclosed in a capsule. It is suspended from the ciliary muscles by

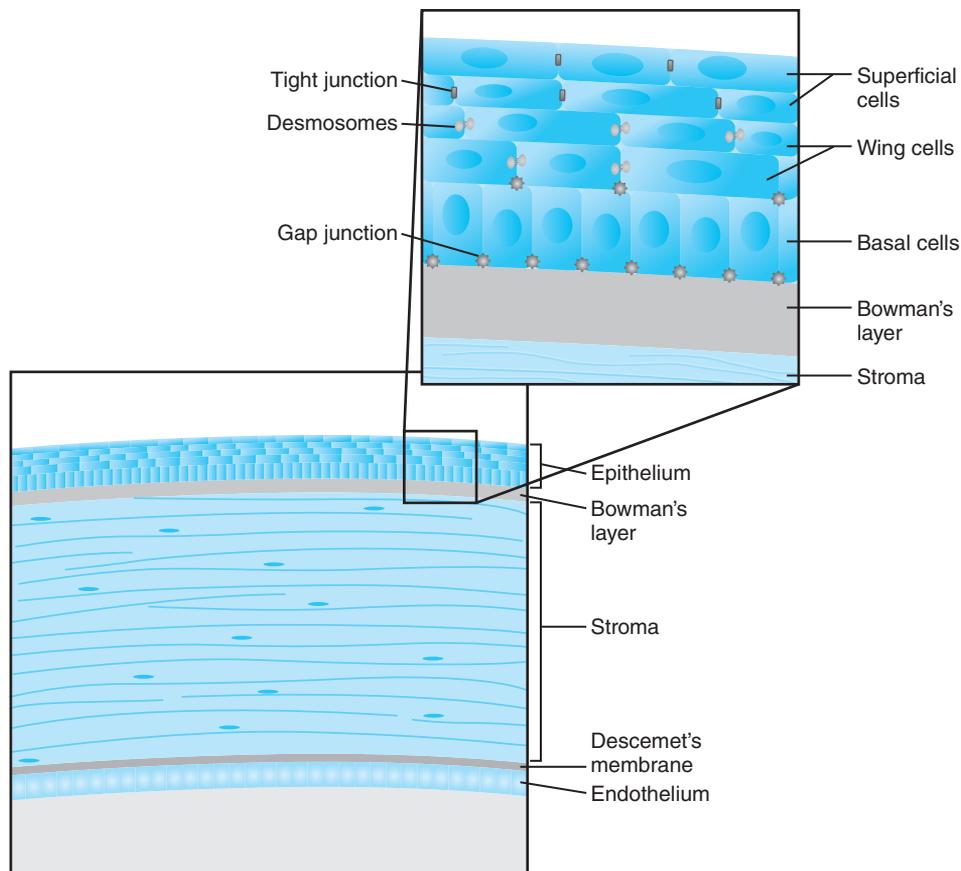


FIGURE 10-2 Cornea and its cellular organizations depicting various barriers to drug transport. The outer superficial epithelial cells possess tight junctions and exhibit the tightest monolayer. The wing and basal cells exhibit gap junctions. The stroma, Dua's layer, and Descemet's membrane covers the inner endothelial cells, display macula adherens, and are more permeable.

very thin fibers called the zonules. It is very important for vision and offers protection to the retina from ultraviolet radiation in conjunction with ciliary muscles.

The posterior segment comprises the retina, vitreous humor, choroid, sclera, and the optic nerve. The retina is a multilayered sensory, light-sensitive tissue that lines the back of the eye. It consists of a neural layer, pigment epithelium, and millions of photoreceptors (rods and cones) that capture and subsequently convert light rays into electrical impulses. Such impulses are transferred by the optic nerve to brain where images are formed. The vitreous humor is a jelly-like substance between the retina and lens. This hydrogel matrix consists of hyaluronic acid, proteoglycans, and collagen fibrils. Separated from the anterior segment by hyaloid membrane, the vitreous is joined to the retina via ligaments. Choroid is a highly vascularized tissue located between the retina and sclera. Its major function is to provide nourishment to the photoreceptor cells in the retina. Sclera is the whitish outermost layer, surrounding the globe, and is called the "white of the eye." It is composed of collagen bundles, mucopolysaccharides, and elastic fibers. This tissue acts as a principal shield to protect the intraocular contents. The scleral tissue is about 10 times more permeable than the cornea and at least half as permeable as the conjunctiva. Hence, permeants can diffuse and enter the posterior segment through the transscleral route.

ROUTES OF OCULAR DRUG ADMINISTRATION

Ophthalmic diseases are primarily treated conventionally by medications administered via either the topical or systemic route. Topical application remains the most preferred route due to ease of administration, low cost, and patient compliance. It is generally useful in the treatment of anterior segment disorders [4]. Drug delivery to the posterior segment still is a major challenge to pharmaceutical scientists. Anatomical and physiological barriers obstruct drug entry into posterior ocular tissues such as retina and choroid. After topical instillation, a large fraction (about 90%) of the applied dose is lost due to nasolacrimal drainage, tear dilution, and tear turnover, leading to poor ocular bioavailability. Less than 5% of the administered dose reaches the aqueous humor after topical administration [5]. Frequent dosing is required, which ultimately results in patient discomfort and inconvenience. Two major absorption routes have been proposed for drugs instilled via topical route: corneal route (cornea–aqueous humor–intraocular tissues) and noncorneal route (conjunctiva–sclera–choroid/retinal pigment epithelium [RPE]). The preferred mode of absorption depends on the physicochemical properties of the permeant [6, 7].

Conventional topical formulations require frequent large doses to produce therapeutic amounts in the back of the eye. Therefore, oral delivery alone [8–10] or in combination with topical delivery [11] has also been investigated because the oral route is considered as noninvasive and high in patient compliance, especially for chronic retinal disorders. High doses are required to achieve significant amounts in the retina. Such high doses, however, lead to systemic adverse effects, and safety and toxicity become a major concern. Oral administration is not predominant and may be highly beneficial only if the drug possesses high oral bioavailability. Nevertheless, molecules in systemic circulation should be able to cross the blood–aqueous barrier (BAB) and BRB after oral administration.

Systemic administration is often preferred for the treatment of posterior segment disorders. A major disadvantage with this route, however, is that it only allows 1% to 5% of administered drug into the vitreous chamber. After systemic administration, the availability of drug is restricted by the BAB and BRB, which are the major barriers for anterior segment and posterior segment ocular drug delivery, respectively. The BAB consists of two distinct cell layers: the endothelium of the iris/ciliary blood vessels and the nonpigmented ciliary epithelium. Both layers prevent drug entry into the intraocular tissues, including aqueous humor, because of the presence of tight junctions [12]. In a similar manner, BRB prevents drug entry from blood into the posterior segment. BRB is composed of two types: retinal capillary endothelial cells (inner BRB) and RPE cells (outer BRB) (Figure 10-3). RPE is a monolayer of highly specialized cells sandwiched between neural retina and the choroid. It selectively transports molecules between photoreceptors and choriocapillaris [13]. Tight junctions of RPE, however, also restrict intercellular drug transport.

Drug entry into the posterior ocular tissues is mainly governed by the BRB. It is selectively permeable to highly hydrophobic drug molecules. To maintain high drug concentrations, frequent dosing is necessary, which often leads to systemic adverse effects [14]. Drawbacks such as lack of adequate ocular bioavailability and failure to deliver therapeutic drug concentration to the retina led ophthalmic scientists to explore alternative administration routes.

Over the past decade, IVT injections have drawn significant attention to scientists, researchers, and physicians. This method involves injection of the drug solution directly into the vitreous via the pars plana using a 30-G needle. This route provides higher drug concentrations in the vitreous and retina. Unlike other routes, the drug can be directly

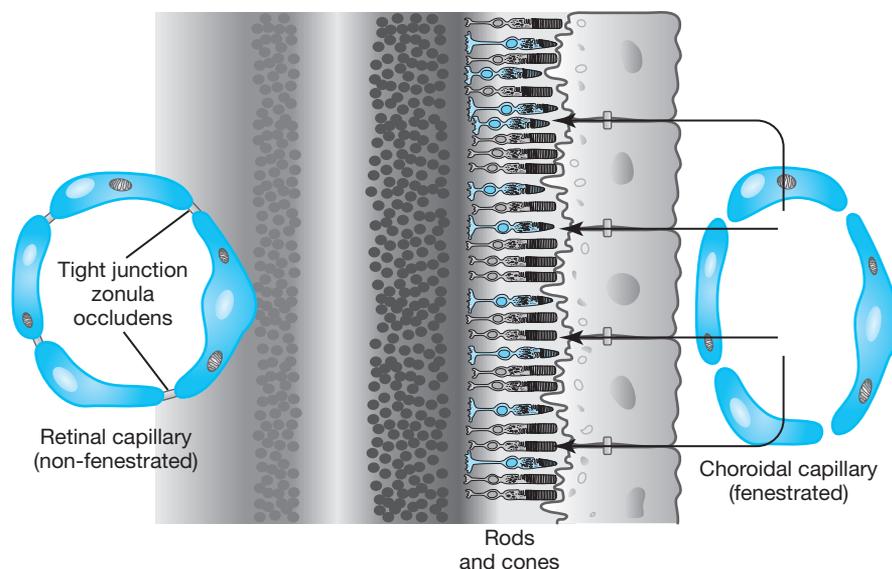


FIGURE 10-3 Retinal cellular architecture: RPE cells, and retinal capillary endothelial (RCE) cells represent the outer and inner retinal barriers, respectively. RPE and RCE represent major drug transport barriers. The outer layer of RPE possesses tight junctions (zonula occludens). Inner RCE expresses tight junctions and are usually nonfenestrated as opposed to choroidal capillary endothelial cells.

injected into the vitreous cavity; however, drug distribution is not uniform. Although small molecules can rapidly diffuse throughout the vitreous fluid, the distribution of macromolecules is restricted or limited. After IVT administration, drug elimination depends on the molecular weight of the compound as well as the pathophysiological condition [15]. For example, macromolecules, which are linear and globular-shaped compounds (especially protein and peptide drugs) with molecular weights between 40 and 70 kDa, tend to cause longer retention in the vitreous humor [16]. The half-life of the drug in the vitreous fluid is also a major determinant of therapeutic efficacy. Elimination of a drug after IVT administration may occur via the anterior or posterior route. The anterior route of elimination involves drug diffusion to the aqueous from the vitreous humor via zonular spaces followed by elimination through aqueous turnover and uveal blood flow. On the other hand, the posterior route of elimination involves drug transport across the BRB, which necessitates optimal passive permeability or active transport. Consequently, drug molecules with higher molecular weight and hydrophilicity tend to be retained in the vitreous humor for longer periods due to longer half-lives of the compounds [13]. Although IVT administration is advantageous in achieving larger drug concentrations in the retina, frequent administration is often associated with complications such as endophthalmitis, retinal detachment, and IVT hemorrhages, leading to poor patient outcome [17].

Periocular administration has also been considered as an efficient route for drug delivery to the posterior segment. Periocular refers to the periphery of the eye or the region surrounding the eye. This route includes peribulbar, posterior juxtascleral, retrobulbar, subtenon, and subconjunctival routes (Figure 10-1). Drugs administered by the periocular route can reach the posterior segment of the eye by three different pathways: transscleral pathway, systemic circulation through the choroid, and anterior pathway through the tear film, cornea, aqueous humor, and vitreous humor [18].

Subconjunctival injection involves the introduction of an active ingredient beneath the conjunctiva. Conjunctival epithelium serves as a rate-limiting barrier for the

permeability of water-soluble compounds. As a result, the transscleral pathway bypasses the cornea–conjunctiva barrier. Various dynamic, static, and metabolic barriers, however, impede drug entry to the back of the eye. Several publications reported rapid drug elimination via these pathways after subconjunctival administration [19–21]. Therefore, most of the administered dose drains into the systemic circulation, leading to poor ocular bioavailability. However, molecules that escape conjunctival vasculature may pass through sclera and choroid and ultimately reach the neural retina and photoreceptor cells. Sclera offers less resistance to drug transport and is more permeable than the cornea [22]. Unlike corneal and conjunctival tissues, scleral permeability is independent of lipophilicity/hydrophobicity but is dependent on the molecular radius [13, 23]. On the other hand, high choroidal blood flow can reduce substantial fractions of the dose reaching the neural retina. Moreover, BRB also hinders drug availability to the photoreceptor cells. Although the periocular route is considered suitable for sustained-release drug delivery systems, several anterior segment complications, such as increased IOP, cataract, hyphema, strabismus, and corneal decompensation, have been observed [24, 25].

Subtenon injection usually involves injection into the tenon's capsule located around the upper portion of the eye and into the belly of the superior rectus muscle. The tenon's capsule is a fibrous membrane that covers the globe from the corneal margin to the optic nerve. A blunt-tipped cannula needle is generally inserted into the tenon's capsule after a surgical incision into the subtenon's space. This technique is widely used during anesthesia for ocular surgery because the cannula approach reduces sharp-needle complications [22].

Retrolbulbar injections usually involve the injection in the conical compartment within the rectus muscles and intramuscular septa. These injections provide higher local drug concentrations with negligible influence on IOP. The peribulbar route involves the injection in the extracellular spaces of the rectus muscles and their intramuscular septa. Although drug administration through the peribulbar route is safer, it is less effective than the retrolbulbar route. Posterior juxtasceral injection using a blunt-tipped curved cannula delivers the drug directly onto the outer surface of sclera. This route may allow sustained delivery to the macula. Retrolbulbar injections are considered the most efficient among all periocular routes but are associated with serious complications, such as retrolbulbar hemorrhage, globe perforation, and respiratory arrest.

ROLE OF EFFLUX AND INFLUX TRANSPORTERS IN THE EYE

One conventional approach to enhance ocular bioavailability is to modify the parent drug chemically to achieve desired solubility and lipophilicity with minimal toxicity. A more coherent approach, however, would be a transporter-targeted ligand modification of the drug. Transporter-targeted delivery has turned out to be a powerful strategy to deliver drugs to target ocular tissues because of the ability of the transporter to translocate the cargo inside the cell as well as into intracellular organelles at a higher rate. Transporters are membrane-associated proteins that are actively involved in the translocation of nutrients across cellular membranes. Of particular interest in ocular drug delivery are efflux and influx transporters [6, 26, 27]. Efflux transporters belong to the ATP binding cassette superfamily, whereas influx transporters belong to the solute carrier (SLC) superfamily of proteins. Efflux transporters carry molecules out of cell membrane and cytoplasm, thereby resulting in low bioavailability. Major efflux proteins identified on various ocular tissues include P-glycoprotein, multidrug resistance protein (MRP),

and breast cancer resistance protein. P-glycoprotein extrudes lipophilic compounds from both normal and malignant cells and is involved in the emergence of drug resistance. Functional and molecular aspects of P-glycoprotein have been characterized on various ocular cell lines and tissues, such as the cornea [28–30], conjunctiva [31, 32], and RPE [33–35]. MRP also works in a similar manner but effluxes organic anions and conjugated compounds. Three of nine isoforms of the MRP family have been identified on ocular tissues. MRP1 is primarily expressed on rabbit conjunctival epithelial cells [36] and RPE [37], whereas MRP2 and MRP5 have been identified on corneal epithelium [38, 39]. The molecular presence of breast cancer resistance protein was also reported on the corneal epithelium [40]. Breast cancer resistance protein-mediated transport occurs in conjunction with nonpolar substrates in the lipid bilayer and can function as a drug flippase, transferring drugs from the inner to the outer portion of the membrane. Expression levels and patterns of these transporter proteins in cells may differ based on its origin and culture conditions.

On the other hand, influx transporters are involved in transporting xenobiotics and essential nutrients, such as amino acids, vitamins, glucose, lactate, and nucleobases, into cells. Influx transporters are often targets for prodrug delivery because these derivatives can improve absorption of poorly diffusing parent drugs. The prodrug is not a good substrate for efflux transporters. In addition, physicochemical properties of the active (i.e., solubility and stability) can be enhanced. Influx transporters usually targeted for ophthalmic drug delivery are amino acid, vitamins, and peptide transporters. These proteins facilitate physiological roles of transporting various amino acids, vitamins, and nutrients into ocular tissues. Amino acid transporters belonging to the SLC1, SLC6, and SLC7 gene families were detected in ocular tissues, mainly on corneal epithelium and RPE cells. Five high-affinity glutamate transporters (EAAT1–EAAT5) and two neutral amino acid transporters (ASCT1 and ASCT2) compose the SLC1 family. ASCT1 (SLC1A4) was detected on rabbit cornea and in rabbit primary corneal epithelial cells [41], whereas ASCT2 was expressed in retinal Müller cells [42]. Alanine and serine translocated by ASCT1 and 2, respectively. B^(0,+), a neutral and cationic amino acid transporter with broad substrate specificity, has been identified on cornea [43] and conjunctiva [44]. This system is associated with arginine transport across cornea and conjunctiva. Also, a sodium-independent large neutral amino acid transporter, LAT1, has been detected on human and rabbit corneas [45], whereas expression of LAT2 was confirmed on ARPE-19 and hTERT-RPE cells [46, 47]. These carriers translocate phenylalanine into ocular tissues.

Gene products from the SLC gene families, such as sodium-dependent multivitamin transporter (SMVT), derived from *SLC5A6* gene, are responsible for translocation and absorption of vitamins such as biotin, pantothenate, and lipoate. SMVT specifically carries biotin as directed by the Na⁺ gradient [48, 49]. Functional and molecular aspects of biotin uptake via SMVT have been delineated on cornea and retina of both human-derived [50] and rabbit-derived cells [51, 52]. SMVT has been explored by designing targeted lipid based drug conjugates that can significantly enhance absorption of a parent drug [48]. Peptide transporters have been extensively studied for ocular drug delivery. Oligopeptide transporter has been identified on rabbit cornea [53]. Both PEPT1 and PEPT2 have been detected on the newly introduced clonetics human corneal epithelium (cHCE) cell line and on human cornea [54, 55]. A proton-coupled dipeptide transport system on conjunctival epithelial cells can mediate the uptake of dipeptide L-carnosine [56]. Furthermore, peptide transporter expression was also studied in retinal Müller cells [57] and neural retina [58].

In addition to amino acid, vitamin, and peptide transporters, several others, including organic cation/anion [55, 59], monocarboxylate [60], folate [61], ascorbate [62], and

nucleoside transporters [63], have been characterized on various ocular tissues. Overall, influx and efflux transporters play vital roles in the absorption of vital nutrients and drugs. These proteins also deal with the elimination of waste and harmful xenobiotics.

PRODRUG STRATEGIES FOR INCREASING OCULAR DRUG ABSORPTION

Prodrugs are bioreversible derivatives of drug molecules that are enzymatically or chemically transformed *in vivo* to release the active parent drug, which can then elicit the desired pharmacodynamic response [6, 64]. Prodrugs have been primarily designed to improve physicochemical, biopharmaceutical, and pharmacokinetic properties of pharmacologically active ophthalmic drugs. In particular, most prodrugs are designed to increase solubility, improve drug shelf life, or stability both chemically and metabolically so these conjugates can reach their physiological target, minimize the side effects, and aid in formulation [65]. In addition to facilitating improved therapeutic efficacy, prodrug strategy can cause evasion of efflux pumps. Lipophilic prodrugs of acyclovir (ACV) and ganciclovir (GCV) caused enhancement of drug transport across corneal epithelium [66–68]. Apparent permeability of the valerate ester prodrug of GCV across cornea is about six times higher than the parent drug GCV [67]. Pilocarpine and some natural prostaglandins also demonstrated enhanced corneal and scleral permeation after lipophilic prodrug derivatization [69, 70].

Malik et al. [71] investigated transscleral retinal delivery of celecoxib, an anti-inflammatory and anti-vascular endothelial growth factor (VEGF) agent, which is poorly water soluble and, moreover, binds readily to melanin pigment in choroid-RPE. These researchers developed three hydrophilic amide prodrugs of celecoxib: celecoxib succinamic acid (CSA), celecoxib maleamic acid (CMA), and celecoxib acetamide (CAA). These prodrugs have been developed to improve solubility of celecoxib, reduce pigment binding, and enhance retinal delivery. Aqueous solubilities of CSA, CMA, and CAA were 300-, 182-, and 76-fold higher than celecoxib, respectively. *In vitro* transport studies across isolated bovine sclera and sclera-choroid-RPE demonstrated eight-fold higher transport for CSA than celecoxib. The rank order for cumulative percent transport across bovine sclera was CSA > CMA > CAA ~ celecoxib and across bovine sclera-choroid-RPE was CSA > CMA ~ CAA ~ celecoxib (Figure 10-4). *In vivo* delivery in pigmented brown Norway rats showed concentrations of total celecoxib (free + prodrug) were significantly higher in the CSA group compared with the celecoxib group for all posterior eye tissues except choroid-RPE and periocular tissues [71].

A crystalline lipid prodrug, octadecyloxyethyl-cyclic-cidofovir, was developed for the treatment of cytomegalovirus retinitis. Intraocular pharmacokinetics of octadecyloxyethyl-cyclic-cidofovir after IVT injection was evaluated in rabbits. IVT injection of ^{14}C -octadecyloxyethyl-cyclic-cidofovir displayed biphasic drug elimination. The initial phase lasted from the time of injection to day 21, whereas the second phase was observed from day 21 to day 63. Noncompartment analysis demonstrated a half-life of 5.5 days for the first phase and a terminal half-life of 25 days. The maximum concentration (C_{max}) values were calculated as $130.43 \pm 24.42 \mu\text{g/mL}$, $45.22 \pm 10.98 \mu\text{g/mL}$, and $16.10 \pm 1.66 \mu\text{g/mL}$ in the vitreous, retina, and choroid, respectively. This prodrug generated more drug exposure to the retina than the vitreous [72].

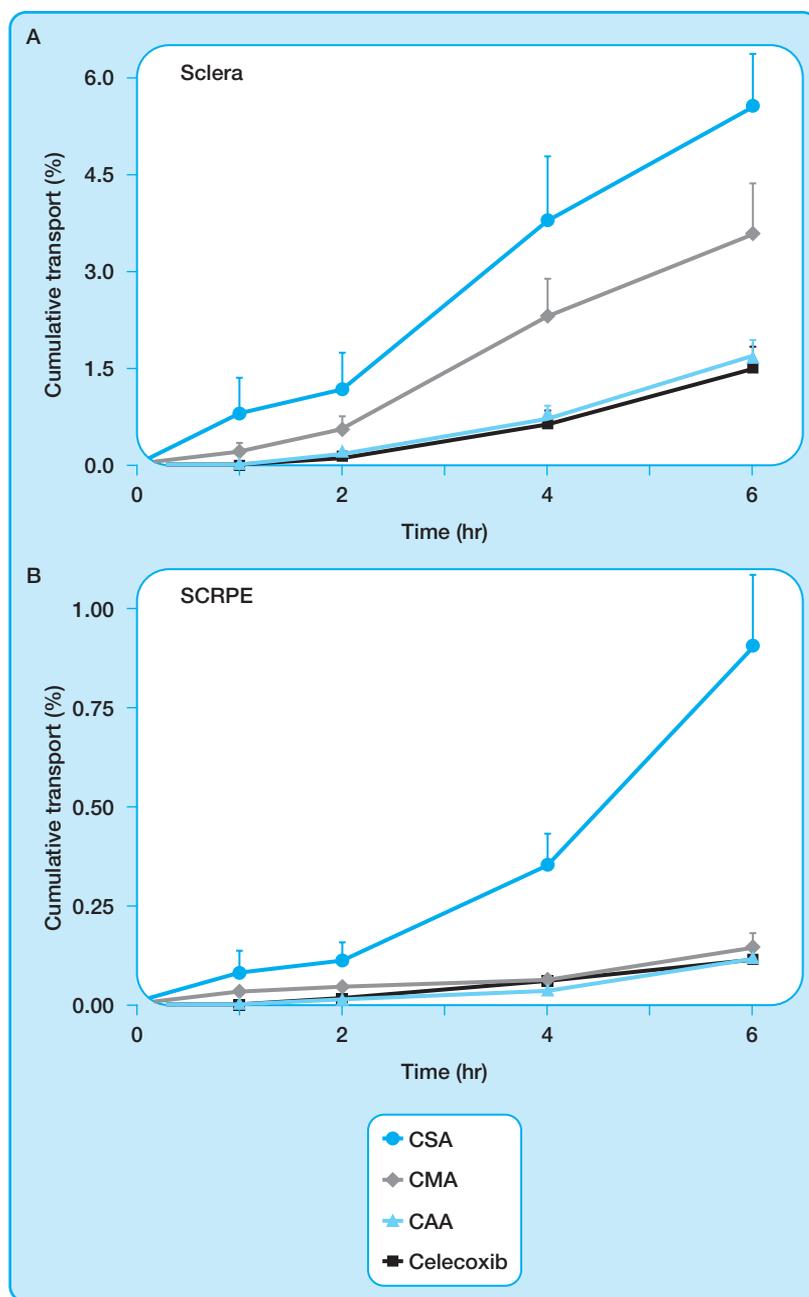


FIGURE 10-4 Cumulative percentage transport showing significantly higher transport for celecoxib succinamidic acid (CSA) than celecoxib across bovine sclera and sclera-choroid-RPE. Cumulative percentage transport of celecoxib and its amide prodrugs across bovine (a) sclera and (b) sclera-choroid-RPE. (Reproduced from Malik P, Kadam RS, Cheruvu NP, Kompella UB. Hydrophilic prodrug approach for reduced pigment binding and enhanced transscleral retinal delivery of celecoxib. *Mol Pharm.* Mar 5, 2012;9[3]:605–614. © American Chemical Society.)

Membrane transporter targeted prodrug design has been the most exciting of all current drug delivery strategies. Prodrugs targeted toward nutrient transporters and receptors expressed on various epithelial cells are designed to enhance the absorption of poorly permeating drug molecules. Influx transporters recognize such prodrugs as substrates and transported the entire cargo across the epithelial membrane. Most studies have been directed toward peptide transporters to improve ocular bioavailability of ACV or GCV after oral, systemic, or topical administrations [73–76]. Mitra and coworkers developed a series of stereoisomeric valine-valine based dipeptide ester prodrugs of ACV. Prodrugs including LLACV, LDACV, DLACV, and DDACV were designed to facilitate enhanced residence time of intact prodrug in the systemic circulation, thus enabling targeting transporters on blood–ocular barriers after oral or systemic administration. Hydrolytic enzymes such as peptidases and esterases responsible for bioconversion of dipeptide prodrugs are stereospecific and show high affinity for L-isomers. Therefore, D-isomers were incorporated into the dipeptide moieties at a particular position to modulate the rate of conversion of the prodrugs. Such incorporation enabled prodrugs to be more stable in the systemic circulation and to facilitate recognition and translocation by the nutrient transporters at blood–ocular barriers. Among these prodrugs, LLACV and LDACV hydrolyzed in Caco-2 cell homogenate and LDACV was relatively more stable of the two compounds (Table 10-1). Incorporation of two D-Valine moieties into a dipeptide moiety, however, enhanced the enzymatic stability but abolished the affinity of these prodrugs (DDACV and DLACV) toward the peptide transporter [77].

Several dipeptide ester prodrugs of GCV (L-valine-L-valine, L-tyrosine-L-valine, and L-glycine-L-valine) were synthesized and evaluated for their vitreal pharmacokinetics in anesthetized rabbit model by an ocular microdialysis technique. These prodrugs appeared to permeate deeper into the retina after IVT administration relative to GCV [78]. Subsequently, vitreal pharmacokinetics of various GCV prodrugs was also studied in a conscious animal model. A comparison of vitreous pharmacokinetic parameters of valine-valine-GCV and regenerated valine-GCV and GCV after IVT administration of valine-valine-GCV in conscious and anesthetized rabbit models is summarized in Table 10-2 [79]. The data suggest lowering the exposure of drug and prodrug levels in conscious animals, although vitreal half-lives did not change.

A novel prodrug strategy that imparts lipophilicity and site specificity has been designed. This study used a lipid raft with one end conjugated to the parent drug (ACV) molecule to impart lipophilicity and the other end to a targeting moiety (biotin)

TABLE 10-1 Stability in Caco-2 homogenate—first-order degradation rate constants and half lives of all prodrugs

Drug	$K \times 10^3 \text{ (h}^{-1}\text{)}$	$t_{1/2} \text{ (h)}$
LLACV	92.23 ± 4.79	7.52 ± 0.40
LDACV	13.33 ± 1.96	52.80 ± 8.42
DLACV	*	*
DDACV	*	*
D-VAI-ACV	29.86 ± 4.71	23.56 ± 3.42

*No degradation during the time of study. Each value is represented as mean \pm S.D. (n = 3).

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TABLE 10-2 Conscious animal vitreous pharmacokinetic parameters of Valine-Valine-Ganciclovir and regenerated Valine-Ganciclovir and Ganciclovir after intravitreal administration of Valine-Valine-Ganciclovir

Parameters	Val-Val-GCV		
	Conscious animal	Anesthetized animal	P Value (<0.05>)
AUC (mg · min · mL ⁻¹)	6.3 ± 0.3	29.4 ± 3.2	*
λ_z ($\times 10^{-3}$ /min)	17.4 ± 6.9	10.1 ± 2.2	NS
$t_{1/2}$ (min)	44.8 ± 19.2	68.6 ± 12.3	NS
V_{ss} (mL)	1.9 ± 0.2	1.3 ± 0.3	*
Cl (μ L/min)	35.3 ± 1.8	8.9 ± 1.8	*
MRT _{last} (min)	50.5 ± 6.4	138.4 ± 25.6	*
C_{last} (μ g/mL)	0.8 ± 0.2	7.2 ± 1.1	*
T_{last} (min)	293.3 ± 11.5	600	*
Regenerated Val-GCV from Val-Val-GCV			
AUC (mg · min · mL ⁻¹)	1.5 ± 0.6	2.2 ± 0.5	NS
C_{max} (μ g/mL)	3.6 ± 1.9	3.9 ± 1.6	NS
T_{max} (min)	120 ± 105.8	460 ± 105	*
MRT _{last} (min)	304.5 ± 15.3	333 ± 28	NS
Regenerated GCV from Val-Val-GCV			
AUC (mg · min · mL ⁻¹)	2.7 ± 1.0	3.5 ± 1.2	NS
C_{max} (μ g/mL)	12.5 ± 7.2	6.3 ± 1.6	NS
T_{max} (min)	140 ± 69.3	420 ± 96	*
MRT _{last} (min)	231.8 ± 64.6	349 ± 48	*

*Represents significant difference at $P < 0.05$.

Abbreviations: AUC, area under curve; C_{last} , last measured plasma concentration; GCV, ganciclovir; MRT, mean residence time; NS, not significant; Val, valine; V_{ss} , volume of distribution at steady state.

Reproduced from Janoria KG, Boddu SH, Natesan S, Mitra AK. Vitreal pharmacokinetics of peptide-transporter-targeted prodrugs of ganciclovir in conscious animals. *J Ocul Pharmacol Ther*: the official journal of the Association for Ocular Pharmacology and Therapeutics. Jun 2010;26(3):265-271.

that can be recognized by a specific transporter/receptor (SMVT). Lipophilic prodrugs readily diffused across the cell membrane by facilitated diffusion, whereas transporter/receptor targeted prodrugs translocated compounds across the cell membrane via active transport by transporter recognition. Marginal improvement in cellular uptake was evident from both approaches. However, this novel approach combines both lipid and transporter/receptor targeted delivery to generate a synergistic effect. Compared with ACV, the uptake of targeted lipid prodrugs (biotin-ricinoleic acid-acyclovir [B-R-ACV] and biotin-12hydroxystearic acid-acyclovir [B-12HS-ACV]) increased by 13.6 and 13.1 times, respectively, whereas the uptake of B-ACV, R-ACV, and 12HS-ACV was higher by only 4.6, 1.8, and 2.0 times, respectively, in HCEC cells (Figure 10-5) [80]. The targeted lipid prodrugs B-R-ACV and B-12HS-ACV exhibited much higher cellular accumulation than B-ACV, R-ACV, and 12HS-ACV. Both the targeted lipid prodrugs

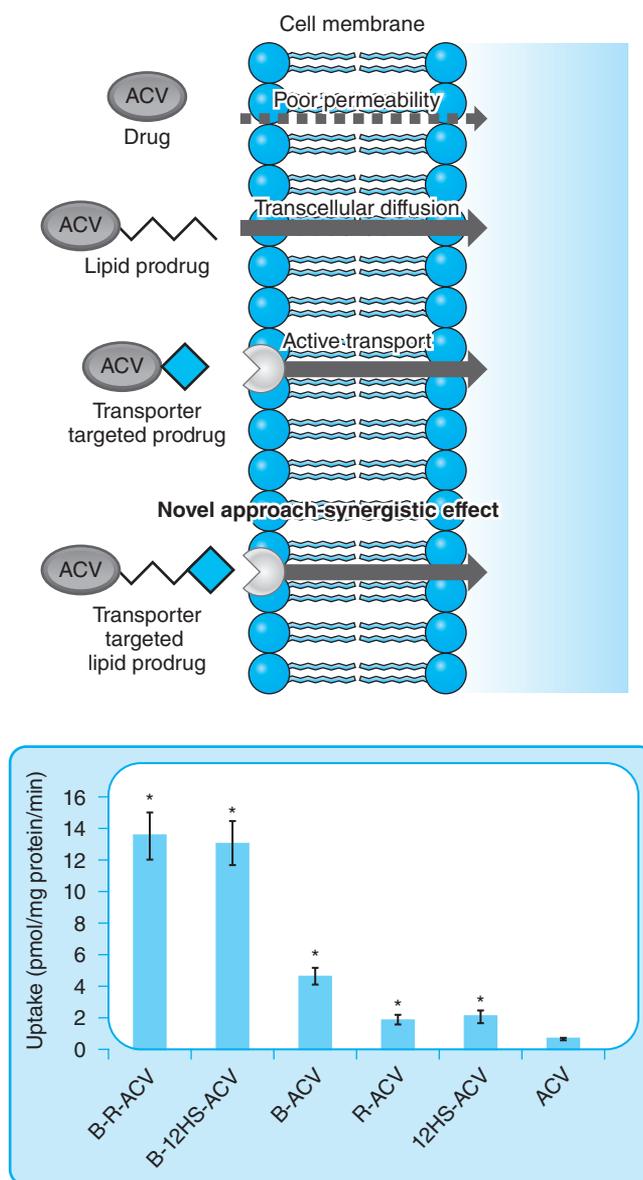


FIGURE 10-5 Novel targeted lipid prodrug strategy showing advantage of this strategy over lipophilic prodrug derivatization and transporter targeted prodrug approaches. Cellular accumulation of B-R-ACV, B-12HS-ACV, B-ACV, R-ACV, 12HS-ACV, and ACV on HCEC cells. (With kind permission from Springer Science+Business Media: Vadlapudi AD, Vadlapatla RK, Earla R, et al. Novel biotinylated lipid prodrugs of acyclovir for the treatment of herpetic keratitis [HK]: Transporter recognition, tissue stability and antiviral activity. *Pharmaceutical research*, May 9 2013.)

B-R-ACV and B-12HS-ACV demonstrated higher affinity toward SMVT than B-ACV. These promising results suggest that the lipid raft may facilitate enhanced interaction of prodrugs with membrane transporters/receptors probably assisting docking of the targeted ligand into the binding domain of transporter/receptor protein. The net effect observed is rapid translocation of the cargo across the cell membrane. This novel prodrug design may also allow for enhanced plasma membrane uptake of hydrophilic

therapeutic agents such as genes, silent interfering RNA, nucleosides, nucleotides, oligonucleotides or antisense oligonucleotides, peptides, and proteins [48].

COLLOIDAL DOSAGE FORMS FOR ENHANCING OCULAR DRUG ABSORPTION

DENDRIMERS

Dendrimers may be defined as artificial macromolecular core shell-like structures consisting of three architectural components: a central core; an interior shell made of repeating branch of monomeric units; and peripheral functional groups. In general, dendrimer/dendrons are synthesized from branched monomer units in a stepwise manner following two approaches: convergent and divergent methods (**Figure 10-6, A and B**). Examples of dendrimers that find application in drug delivery include poly(amidoamine) (PAMAM) and polypropylenimine. Because these macromolecules are artificially synthesized, it is possible to precisely control their molecular size, shape, dimension, density, polarity, flexibility, and solubility with appropriate selection of building units and surface functional groups. Dendrimers with terminal primary amine functional groups are named as “full generation” (G2, G3, G4, etc.), whereas those with carboxylate or ester terminal functional groups are named as “half generation” (G2.5, G3.5, G4.5, etc.) dendrimers, where “G” stands for generation. As the number of branches in dendrimers increase, it leads to the development of higher generation dendrimers. Low generation dendrimers possess empty core and open conformations, which helps to encapsulate hydrophobic drug molecules [81]. In addition, the presence of high surface density functional groups ($-\text{NH}_2$, $-\text{COOH}$, $-\text{OH}$) such as in PAMAM, allows improved solubility for many drugs and also permits surface conjugation of targeting ligands and/or drugs (**Figure 10-7**)

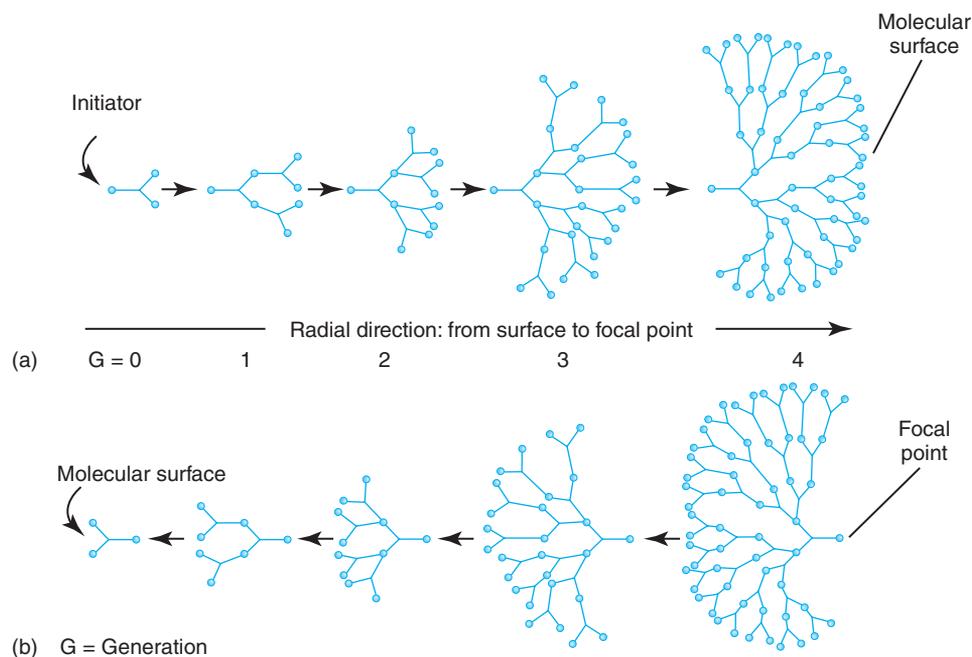


FIGURE 10-6 Synthesis of dendrimers: (a) divergent method and (b) convergent method.

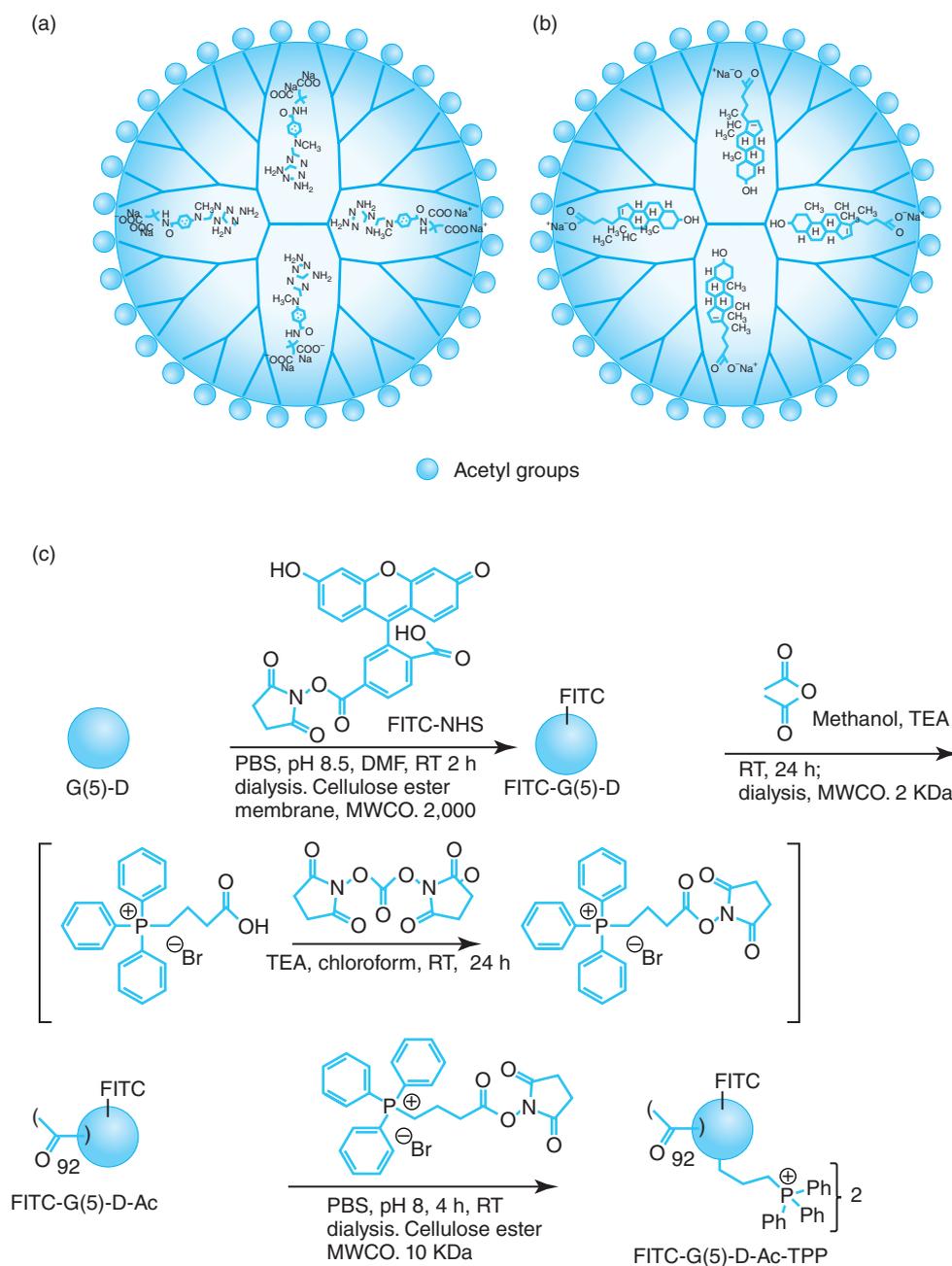


FIGURE 10-7 (i) Proposed inclusion structures of acetylated G4 polypropylenimine dendrimer/ drug complexes: (a) methotrexate sodium; (b) sodium deoxycholate. (ii) Synthesis of triphenylphosphonium (ligand) conjugated, fluorescently-labeled, acetylated G(5)-PAMAM dendrimer. (Part a reproduced from Wang F, Cai X, Su Y, et al. Reducing cytotoxicity while improving anti-cancer drug loading capacity of polypropylenimine dendrimers by surface acetylation. *Acta Biomater.* Dec. 2012;8[12]:4304–4313, with permission from Elsevier. Part b reproduced from Biswas S, Dodwadkar NS, Piroyan A, Torchilin VP. Surface conjugation of triphenylphosphonium to target poly[amidoamine] dendrimers to mitochondria. *Biomaterials.* Jun 2012;33[18]:4773–4782, with permission from Elsevier.)

[82–85]. Different shapes may be observed depending on the dendrimer generation. The PAMAM dendrimers of low generation (G0 to G3) with an ethylenediamine core have ellipsoidal shape, whereas high generations (G4 to G10) with well-defined core display roughly spherical shapes [86]. Encapsulation of active agents into dendrimers is drawing a lot of attention in drug delivery. Mode of interactions with dendrimers may be subdivided into three types: simple encapsulation, electrostatic interactions, and covalent conjugation.

Dendrimers may be suitable as vehicles for ocular drug delivery. These dendritic polymers can accommodate hydrophobic agents in their cavity and provide sustained release. Vandamme and Brobeck [87] studied a series of PAMAM dendrimers for controlled ocular delivery of pilocarpine nitrate and tropicamide. PAMAM dendrimers with primary amino (G2, G4), hydroxyl (G2, G4), and carboxylate (G1.5, G3.5) terminal group were studied. Miotic and mydriatic effects have been evaluated in New Zealand albino rabbits. The duration of miotic/mydriatic response was defined as the time interval between topical administration of drug treatment and the time at which the pupil

TABLE 10-3 Notation of the ocular lesions (conjunctiva, iris and cornea), determination of the maximum ocular irritating index, 1-hour post-instillation and evaluation of the mean residence time post-instillation of different solutions on the cornea

Notation of Ocular Lesion					
Formulation	Conjunctiva	Iris	Cornea	Ocular Irritating Index	Mean Residence Time (min) ± SE
DG2 (2.0%)	2.7	0	0	2.7	100 ± 60.5
DG2.5 (0.25%)	—	—	—	—	300 ± 34.5
DG2.5 (0.5%)	—	—	—	—	260 ± 17.5
DG2.5 (2.0%)	3.3	0	0	3.3	260 ± 0.0
DG4 (2.0%)	4.7	0	0	4.7	203 ± 83.5
DG4.5 (2.0%)	1.3	0	0	1.3	220 ± 69.0
DG2(OH) (2.0%)	0.7	0	0	0.7	300 ± 0.0
DG4(OH) (2.0%)	0	0	0	0	300 ± 0.0
Carbopol® 980 NF (0.2%)	7.3	1.7	0	9	270 ± 30.0
HPMC (0.2%)	4.7	1.7	0	6.3	26 ± 11.0
Phosphate buffer, pH 7.4	4.7	0	0	4.7	21 ± 5.0

Dendrimers and linear polymers are in different pH solutions as described in the text. DG, dendrimer generation; HPMC, hydroxypropylmethylcellulose; carbopol 980 NF, linear poly(acrylic) acid.

Reproduced from Vandamme TF, Brobeck L. Poly(amidoamine) dendrimers as ophthalmic vehicles for ocular delivery of pilocarpine nitrate and tropicamide. *J Control Release*. Jan 20 2005;102(1):23–38, with permission from Elsevier.

diameter returned to its normal pretreatment value. *In vivo* studies have been conducted with topical instillation of various generation dendrimer solutions. All formulations tested were weakly irritant with minor lesions to anterior ocular tissues (up to a concentration of 2.0% w/v). The dendrimer formulations improved fluorescein ocular mean residence time relative to phosphate buffer (**Table 10-3**) [87].

Low polymer concentrations (dendrimer G2.5) demonstrated better ocular compatibility than high concentrations (2.0% w/v). Miotic and mydriatic activities are improved and prolonged with dendrimers G4 amino and hydroxyl terminal groups (2.0% w/v). Pharmacokinetic parameters for miotic/mydriatic activities with topical dosing demonstrated statistically significant improvement in bioavailability for both drugs. Dendrimer G4 with amino and hydroxyl group terminals encapsulating pilocarpine nitrate and tropicamide exhibited higher area under the curve (AUC) of 213.5 ± 7.8 and 559.5 ± 4.3 min mm \pm SE, respectively, compared with phosphate buffer (134.5 ± 3.8 min mm \pm SE). The nature of surface functional groups on the dendrimers helps to improve bioadhesion properties. Therefore, higher drug bioavailability is due to bioadhesive property of dendrimers and subsequent slow release of encapsulated drug from the core.

Dendrimers not only sustain drug release but also translocate drug molecules across ocular tissues. To demonstrate the application of dendrimers in enhancing drug permeation, Yao et al. [88] conducted studies across excised cornea with dendrimer: drug physical mixture and complex. Cationic dendrimers of generations (G3, G4, and G5) with amino terminal and half generation (G3.5 and G4.5) with carboxylate terminal functional group have been studied. A mixture was prepared with peurarin and dendrimer in phosphate-buffered saline, whereas a complex was prepared by solvent evaporation with methanol. Peurarin has low aqueous solubility of 4 mg/mL. The solubility of peurarin is dependent on dendrimer concentration. The dendrimer complex sustained peurarin release (*in vitro*) and reduced burst release by ~25%. Higher generation dendrimer caused ~85% of peurarin released in less than 2 hours [89]. Corneal permeability of dendrimer is dependent on molecular weight, size, and method of formulation preparation. Molecular weight and size of dendrimer have shown inverse relationships with peurarin permeability across the cornea (i.e., G3 > G4 > G5) with cationic dendrimers. Apparent permeability of peurarin is reduced approximately 50% from 3.07 ± 0.07 to 1.68 ± 0.02 cm.h⁻¹ \times 10⁻², respectively, for G3 and G5 PAMAM dendrimers. The physical mixture generated two times higher peurarin permeability (Papp) relative to complex. The reason for the higher permeability of peurarin with a dendrimer physical mixture is that PAMAM dendrimer incorporates into the corneal lipid epithelial bilayer, thereby loosening epithelial tight junctions. Peurarin released from PAMAM dendrimer core then permeates across the cornea through narrow epithelial intercellular spaces through the paracellular pathway. Aqueous humor pharmacokinetic studies with cationic dendrimer physical mixtures (G3, G4, and G5) have been conducted in rabbits following a microdialysis technique [90]. The dendrimer physical mixture G4 demonstrated higher AUC, C_{max}, and maximum time of 107.32 ± 23.83 μ g mL⁻¹min⁻¹, 1.11 ± 0.31 μ g mL⁻¹, and 90 minutes, respectively, relative to G3 and G5. Increasing dendrimer size and molecular weight improved corneal bioavailability but lowered bioavailability with the G5 physical mixture. It can be attributed to the G5 physical mixture causing ocular irritation, which can lead to higher blinking and drug drainage from the ocular surface.

Cationic dendrimers composed of poly-L-lysine (PLL), polyamidoamine, and polyethyleneimine (PEI) can encapsulate plasmid DNA, gene, or short hairpin RNA and deliver the cargo to RPE and retinal ganglionic cells [91]. Marano et al. [91] designed and synthesized a lipid-lysine dendrimer to encapsulate an anti-VEGF agent, ODN-1, and deliver the complex into retinal cell nuclei. *In vivo* studies were conducted in a

neovascularized rat model with IVT injection of the complex. The composition was well tolerated, and a significant suppression of VEGF expression was achieved. The complex was retained in retinal tissues for more than 2 months.

Drug delivery and *in vivo* performance of dendrimers can be further improved with surface modification. Several dendritic conjugates such as PEGylated, guanidinylated, and glucosamine conjugated dendrimers have been studied for ocular drug delivery [92, 93]. Cationic dendrimers such as PAMAM carry a high density of positive surface charge, due to the amino functional group. The surface density is dependent on the generation (i.e., G5 has greater density than G3). The positive surface of PAMAM expresses higher affinity or electrostatic interaction with negatively charged components of bacteria cell membrane, such as lipopolysaccharide or lipoteichoic acid. Binding of dendrimer to the bacterial surface does not induce antimicrobial effect. Dendrimers induce their antibacterial effect probably by disrupting the bacterial cell membrane. Dendrimer penetration and diffusion across the bacterial cell membrane into the cytoplasm may also be required to induce antibacterial effect. To facilitate this process, a net balance between the positive charge, size, and hydrophobicity is required. It is known that G5 has higher amino group density than G3 but suffers from larger size, which prevents its permeation across the bacterial cell wall. Therefore, G3 appears to induce more significant antimicrobial effect than G5. This dendrimer is toxic to normal corneal cells. To reduce toxicity, Lopez et al. [93] synthesized polyethylene glycol (PEG) conjugated dendrimers (G3 and G5). Different PEG chain lengths with varying degrees of PEG surface density have been synthesized and evaluated for antibacterial activity and cytotoxicity. Surface PEGylation demonstrated reduction in antibacterial activity due to smaller number of free $-NH_2$ groups and partial shielding by PEG. An enhancement in antibacterial effect with longer PEG chain lengths has been revealed. Reasons can be attributed to cationic character of dendrimer and dual lipophilic/hydrophilic character of PEG chains. This study confirmed that surface PEGylated dendrimers serve three purposes: reduced toxicity induced to corneal cells, development of a new antimicrobial polymer, and optimization of PEG chain length and density leading to maximal antimicrobial effect.

CYCLODEXTRINS

Cyclodextrins (CDs) are biodegradable, biocompatible cyclic oligosaccharides available as α , β , and γ depending on the number of sugar units 6, 7, and 8, respectively (Table 10-4). Derivatives of CDs with cyclic ring structures appear to improve delivery properties (Figure 10-8). A comparison between the naturally available CDs and their derivatives show that β -CD is most commonly and widely used. The ring size of CD enlarges with the number of sugar units (Table 10-4). These cyclic sugars consist of an outer hydrophilic corona of hydroxyl groups and an inner hydrophobic core (Figure 10-8) [94]. CDs have a wide range of applications in pharmaceutical

TABLE 10-4 Cyclodextrins and their properties

Cyclodextrin	Glucopyranose Units	Mol. Wt. (Da)	Central Cavity Diameter (ext./int., Å)	Aqueous Solubility (at 25°C, g/100 mL)
A	6	972	5.3/4.7	14.5
B	7	1135	6.5/6.0	1.85
Γ	8	1297	8.3/7.5	23.2

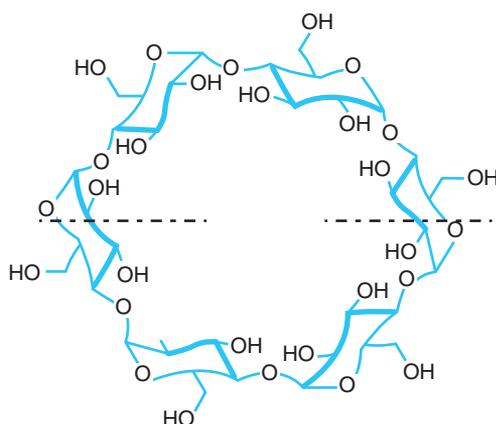


FIGURE 10-8 Structure for cyclodextrins (interior hydrophobic and corona hydrophilic).

compositions and are commonly used as solubilizers and stabilizers. CDs can also lower local drug-induced irritation, sustain drug release, and improve *in vivo* performance [95, 96]. CDs improve aqueous solubility of hydrophobic drugs by complexation. These substances are not capable of modifying the permeability of the biological barrier relative to surfactants. One example of a CD that was modified to improve aqueous solubility is hydroxypropyl (HP)- β -CD. Modification of β -CD with the HP group not only improved its aqueous solubility but also demonstrated high ocular tolerability in rabbits up to 12.5% with ophthalmic preparations [97]. This new CD derivative demonstrated improved solubility for various other drug candidates, such as but not limited to carbonic anhydrase inhibitors (acetazolamide, ethoxzolamide), steroids (hydrocortisone, dexamethasone acetate), and nonsteroidal anti-inflammatory drugs (diclofenac sodium). Also, HP- β -CD has been found to improve stability of drugs such as tropicamide, GCV, and mycophenolate mofetil [98–105].

Fungal infections affecting the eye to date have no specific ocular formulation available. Drugs are administered systemically to treat ocular fungal infections, but local administration with topical drops is preferred. Ketoconazole, an imidazole derivative, possesses fungistatic activity against various pathogens. Its delivery, however, is limited due to poor aqueous solubility. Zhang et al. studied ocular (aqueous humor) pharmacokinetics of topically administered ketoconazole solution containing HP- β -CD in New Zealand albino rabbits [106a]. Ocular ketoconazole aqueous drops were prepared by dissolving with HP- β -CD and compared with 1.5% ketoconazole suspension. Both ketoconazole aqueous solution and suspension were instilled topically to the eye. Both formulations were well tolerated with no irritation. Ketoconazole containing HP- β -CD showed significantly higher corneal permeability relative to suspension. Aqueous humor pharmacokinetics showed a significantly higher ketoconazole level at 10 to 120 minutes relative to suspension. The ketoconazole AUC versus time profile for aqueous humor and cornea was found to be ~ 8.42 and ~ 12.85 times higher than suspension. The highest concentration (C_{\max}) detected in aqueous humor and cornea was 0.44 ± 0.46 and 2.67 ± 2.9 $\mu\text{g/mL}$ at 30 and 20 minutes, respectively, after a topical single dose. The addition of CD to ketoconazole significantly increased drug levels (above minimum inhibitory concentrations) in aqueous humor and cornea than suspension. Local drug administration may avoid systemic ketoconazole complications and result in therapeutic drug concentrations at the target site (ocular tissues).

NANOPARTICLES

Nanoparticles are colloidal dispersions with a size range between 10 and 1000 nm. Depending on the method of preparation, nanospheres or nanocapsules can be obtained. Nanospheres differ from nanocapsules in that they are matrix form, in which particle entire mass is solid. Nanocapsules are reservoir form, in which a solid material shell encircles a liquid or semisolid core at room temperature (15–25°C). The central core is composed of either oil or aqueous solvent. These colloidal systems have been used as drug carriers in ocular drug delivery. In general, nanoparticles offer several advantages, including increased longevity and stability of the carrier system and encapsulated drug, improved drug bioavailability, possibility to obtain a cell targeted release, and ability to overcome ocular physiological barriers. Also, a sustained or controlled release, restricted drug biodistribution, reduced drug clearance, low ocular irritation, and retarded drug metabolism can be achieved. Nanoparticle surface coated with a protective polymer can further alter all the earlier mentioned parameters [106]. Another strategy to sustain the drug release is embedding the nanoparticles in biodegradable and biocompatible thermosensitive gels [107]. Thermosensitive gel-based strategy is used for local drug delivery. More details are provided below in *In Situ* Gelling Systems.

Nanoparticles composed of lipids, proteins, and natural or synthetic polymeric systems have been studied for ocular drug delivery. Most widely used polymeric systems include but are not limited to albumin, hyaluronic acid (HA), sodium alginate, chitosan, poly(lactide-co-glycolic acid) (PLGA), poly(lactic acid) (PLA), polycaprolactone (PCL), PEG, and poly(glycolic acid) (PGA). Ocular administration of drug-loaded nanoparticles into the cul-de-sac/precorneal pocket or vitreous humor causes rapid elimination, mostly similar to aqueous solutions. To overcome such rapid elimination and improve residence time, nanoparticles are surface coated with PEG, chitosan, and/or HA or embedded in thermosensitive gels. Surface modification enhances mucoadhesive property for nanoparticles, sustains drug release, and imparts higher residence time in the eye. Suspending nanoparticles in thermosensitive or hydrogel systems reduces the rate of nanoparticle elimination, retains the particles for longer time in the precorneal pocket, improves bioavailability, and produces better therapeutic effect. To improve ocular bioavailability and retention time of dexamethasone, Gan et al. [108] prepared self-assembled liquid crystalline nanoparticles (cubosomes) following the emulsification method with monoolein and poloxamer 407 polymers. Precorneal clearance parameters for nanoparticles have been evaluated with noninvasive fluorescence imaging in rabbits. Nanoparticles labeled with ethyl rhodamine B (Rh B) have been used for studies using carbopol and Rh B solutions served as controls. A 3.5- and 2.5-fold increase in

TABLE 10-5 Precorneal ethyl Rh B clearance parameters in rabbits, after topical drop administration

Sample	AUC 0→180 minutes (% min)	k (min ⁻¹)	A90 (%)
Rh B solution	2196.7 ± 920.1	0.031 ± 0.008	10.4 ± 5.7
Rh B carbopol gel	3104 ± 1267.9	0.026 ± 0.015	14.2 ± 5.7
Rh B cubosome F1	7715.8 ± 1050.9* [†]	0.013 ± 0.002* [†]	37.8 ± 2.8* [†]

k, clearance rate constant; A90, activity remaining at region of interest at 90 minutes postdosing.

*P < 0.05, statistically significantly difference from Rh B solution.

[†]P < 0.05, statistically significantly difference from Rh B carbopol gel.

Reproduced from Gan L, Han S, Shen J, et al. Self-assembled liquid crystalline nanoparticles as a novel ophthalmic delivery system for dexamethasone: Improving preocular retention and ocular bioavailability. *International Journal of Pharmaceutics*. Aug 30 2010;396(1-2):179–187, with permission from Elsevier.

AUC was observed with nanoparticles relative to Rh B solution and Rh B carbopol gel (Table 10-5) [108]. No significant difference for clearance rate between Rh B carbopol gel and aqueous solution was observed. This indicates that nanoparticles improve ocular residence time and bioavailability of drug into ocular tissues.

To further improve precorneal residence time, nanoparticles are surface coated with chitosan. Chitosan coating imparts a positive charge to nanoparticles and hence they bind to the negatively charged corneal surface. This improves precorneal residence and decreases clearance. For instance, natamycin-lecithin nanoparticles with chitosan coating exhibited higher ocular bioavailability in rabbit relative to marketed suspension. After topical dosing, AUC (0-∞) increased by 1.47-fold and clearance was reduced by 7.4-fold, where marketed suspension served as control [109]. Musumeci et al. [110] studied melatonin-loaded PLGA nanoparticles for an IOP-lowering effect in rabbits. Melatonin nanoparticles surface coated with PEG demonstrated a significant IOP-lowering effect relative to blank PLGA nanoparticles and aqueous solution (Figure 10-9). Reduced zeta potential of nanoparticles fabricated from PLGA-PEG relative to PLGA allows better and longer interaction between nanoparticles and ocular surface. This results in effective and higher hypotensive effect for prolonged period. Other parameters for nanoparticle ocular drug delivery include size and surface property. Particle size-based studies were conducted for posterior ocular tissue drug delivery following the transcleral route. *In vivo* studies were conducted after periocular administration of fluorescent polystyrene nanoparticles with 20 nm, 200 nm, and 2 μm in Sprague-Dawley rats. Twenty-nanometer particles were rapidly cleared from periocular tissues and circulatory systems (conjunctival, episcleral, or other periocular circulatory systems), suggesting that small sized particles could not be retained at the depot site for at least 2 months [111, 112]. Hence, particle size plays an important role in drug delivery from periocular tissues to retina. An optimal particle size is necessary for their ability to overcome the ocular barriers and deliver the cargo to retinal tissues.

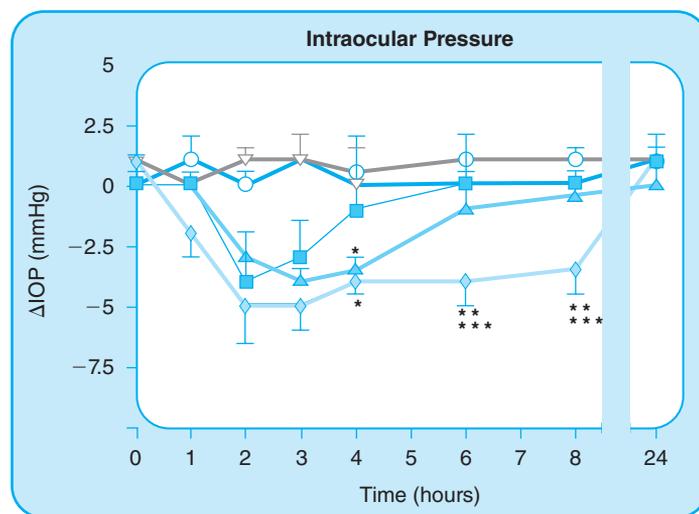


FIGURE 10-9 IOP in normotensive rabbit after topical instillation of melatonin (MEL) aqueous solution (■) or nanoparticles (NPs): RG (PLGA NPs) (○), RGP (PLGA-PEG NPs) (▽), RG-MEL1 (MEL loaded PLGA NPs) (▲), or RGP-MEL1 (MEL loaded PLGA-PEG NPs) (◆). * $P < 0.01$, ** $P < 0.001$ vs. melatonin; *** $P < 0.001$ vs. RGP-MEL1. (Reproduced from Musumeci T, Bucolo C, Carbone C, Pignatello R, Drago F, Puglisi G. Polymeric nanoparticles augment the ocular hypotensive effect of melatonin in rabbits. *International Journal of Pharmaceutics*. Jan 20,2013;440(2):135-140, with permission from Elsevier.)

Another method to improve ocular bioavailability is overcoming ocular barriers and delivering high drug levels to retinal tissues is with an IVT injection. After IVT dosing, nanoparticles migrate through the retinal layers and tend to accumulate in RPE cells. The PLA nanoparticles, <350 nm, accumulated in rat RPE and were detected up to 4 months after a single IVT injection. IVT administration of nanoparticles shows great potential for achieving steady and continuous delivery to the back of the eye tissues. Zhang et al. [113] demonstrated better pharmacokinetics and tolerance for dexamethasone-loaded PLGA nanoparticles in rabbits after IVT injection. Dexamethasone levels were maintained at a steady state in vitreous humor for more than 30 days, with a mean concentration of 3.85 mg/L. On the contrary, only trace amounts of dexamethasone were detected at day 7 with IVT dexamethasone solution. Dexamethasone nanoparticles sustained drug release, thereby reducing the dosing frequency. Surface charge of nanoparticles also plays an important role in ocular drug delivery. Kim et al. [114] demonstrated that anionic surface charged human serum albumin (HSA) nanoparticles penetrated the whole rat retina and formed a depot inside RPE relative to cationic counterparts, after IVT administration. Also, movement of IVT nanoparticles depends on retinal injury. In the injured retina, HSA nanoparticles reached the choroid through the disruption site of RPE and Bruch's membrane. Therefore, the anionic surface charge on nanoparticles imparts greater permeability across ocular tissues such as the choroid region to provide effective treatment for choroidal neovascularization. Similarly, Koo et al. [115] demonstrated a correlation between nanoparticle surface property and their distribution in vitreous body and retina after IVT injection. Heterogeneous PEI/glycol chitosan (GC), HSA/GC, and HSA/HA nanoparticles have been studied. The zeta potential for nanoparticles were 20.7 ± 3.2 , -1.9 ± 4.1 , and -23.3 ± 4.4 for PEI/GC, HSA/GC, and HSA/HA nanoparticles, respectively. Nanoparticles were IVT injected in Long Evans rats and vitreous/retinal distribution was evaluated. **Figure 10-10** shows that PEI/GC nanoparticles easily permeated the vitreal barrier and reached the inner limiting membrane [115]. PEI/GC and HSA/GC nanoparticles, however, could not readily be

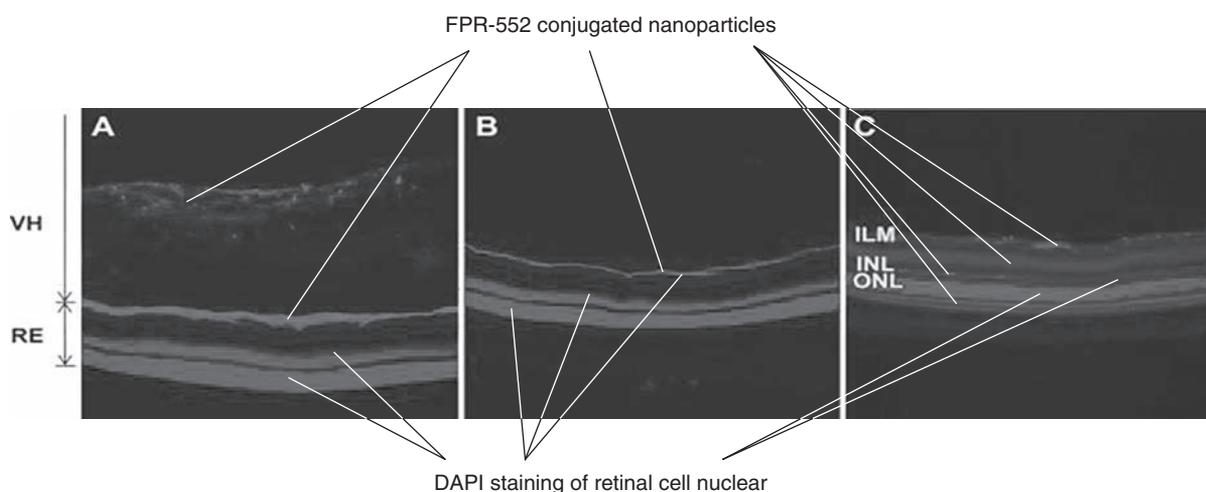


FIGURE 10-10 Vitreal and retinal distribution of intravitreally administered (A) PEI/GC heterogeneous nanoparticles, (B) HSA/GC heterogeneous nanoparticles, and (C) HSA/HA heterogeneous nanoparticles 6 hours postinjection. VH, RE, ILM, INL, and ONL represent the vitreous, retina, inner limiting membrane, inner nuclear layer, and outer nuclear layer, respectively. All images were captured at $\times 10$ magnification. (Reproduced from Koo H, Moon H, Han H, et al. The movement of self-assembled amphiphilic polymeric nanoparticles in the vitreous and retina after intravitreal injection. *Biomaterials*. Apr 2012;33[12]:3485-3493, with permission from Elsevier.)

transported to deeper retinal layers and form aggregates in vitreous. It is hypothesized that inhibition of the interaction between HSA and the Müller cells in retina by GC limits nanoparticle translocation. Negatively charged HSA/HA nanoparticles penetrated the entire retinal structures and reached the outer retinal layers (i.e., photoreceptors and RPE). Enhanced permeation may be attributed to interactions between anionic membrane groups and Müller cells [115].

LIPOSOMES

Liposomes are vesicles/carrier systems composed of one or more phospholipid bilayers enclosing a central aqueous compartment. Liposomal carrier systems offer unique advantages in ocular drug delivery by encapsulating both hydrophobic and hydrophilic drugs. The vesicles also exhibit excellent biocompatibility due to their phospholipid bilayer, which is similar to the cell membrane. In general, size ranges between 0.08 and 10 μm . Liposomes are classified as small unilamellar vesicles (10–100 nm), large unilamellar vesicles (100–300 nm), and multilamellar vesicles (contains more than one bilayer) [116]. Attempts have been made to deliver drugs to anterior and posterior ocular tissues with liposomes as drug delivery vehicles. Despite their advantages, liposomes suffer from certain drawbacks such as low stability (both physical and chemical), sterilization, encapsulation efficiency, and intracellular lysosomal degradation.

Anterior ocular drug delivery suffers from a limitation, that is, loss of a topically applied dose in tears. Natarajan et al. [117] developed a latanoprost-loaded liposomal formulation and demonstrated long-term IOP lowering in rabbits. A single subconjunctival administration of latanoprost liposomal formulation produced somewhat similar and comparative IOP lowering with daily topical dosing up to 50 days. Further precorneal residence time of liposomes is improved by positively charged lipids forming cationic liposomes. The cationic liposomes exhibit better performance as ocular delivery systems than negatively charged and/or neutral liposomes. Positively charged cationic liposomes show higher binding with the negatively charged corneal surface. Examples of cationic lipids include didodecyldimethylammonium bromide, stearylamine, and *N*-(1-(2,3-dioleoyloxy)propyl)-*N,N,N*-trimethylammonium chloride.

Diebold et al. [118] prepared a liposome–chitosan nanoparticle complex for ocular delivery. *In vivo* studies in rabbits demonstrated higher uptake in conjunctiva relative to cornea. Law et al. prepared ACV-loaded cationic and anionic liposomes with stearylamine and dicetylphosphate as cationic and anionic charge-inducing agents, respectively [119]. *In vivo* studies in rabbits demonstrated higher corneal ACV concentrations with cationic liposome relative to anionic and free ACV solution. Concentrations of ACV in cornea were 253.3 ± 72.0 ng/g, 1093.3 ± 279.7 ng/g, and 571.7 ± 105.3 ng/g from ACV solution, ACV loaded cationic, and anionic liposomes, respectively. Also, the extent of ACV transport through the cornea was higher with cationic liposomes. The higher binding of cationic liposomes with negatively charged corneal surface through electrostatic interactions demonstrated increased residence time and improved ACV absorption [119]. Cheng et al. [120] prepared self-assembling liposomes of lipid prodrug of GCV, 1-O-hexadecylpropanediol-3-phospho-GCV. *In vivo* studies were conducted in rabbits with IVT administration of GCV, blank liposomes, and prodrugs in liposome. IVT injection of 1-O-hexadecylpropanediol-3-phospho-GCV showed 0.2 mM vitreal concentration in the rabbit retinitis model. In another study, Shen and Tu [121] prepared GCV liposomes. *In vivo* studies were conducted in rabbits with topical dosing. No significant difference in precorneal clearance was observed with liposomal formulation and GCV solution. AUC with liposomal formulation was enhanced 1.7-fold over GCV solution. Ocular tissue concentrations were 2 to 10 times higher in the sclera, cornea, iris,

lens, and vitreous humor relative to GCV solution. This study indicates that liposomal formulations improve delivery across ocular tissues. Coenzyme Q10-loaded liposomes surface coated with mucoadhesive trimethyl chitosan were studied [122]. *In vivo* topical dosing studies in rabbits demonstrated a 4.8-fold increase in precorneal residence time.

Liposomes as carrier systems have been investigated for posterior ocular tissue delivery. These carrier systems have been developed to improve drug half-life, lower vitreous humor clearance, and provide protection from degradation of enzymatically labile drugs like peptides and oligonucleotides. Also, encapsulation of these molecules in liposomes sustains drug release. Gupta et al. [123] demonstrated improved fluconazole vitreal half-life from 3.08 to 23.40 hours with liposomal encapsulation. Also, the terminal vitreal elimination constant for fluconazole-loaded liposome was seven times less than drug solution. Another study by Zhang et al. [124] demonstrated better efficacy of tacrolimus (FK 506) liposomal formulation in the treatment of autoimmune uveoretinitis with IVT administration to Lewis rats. Tacrolimus concentrations were maintained over ~14 days, and the regimen suppressed experimental autoimmune uveitis. Sasaki et al. [125] developed liposomal topical drops surface modified with PLL for back of the eye delivery. Varying molecular weights and concentrations of PLL were added to surface coat liposomes. The authors identified PLL molecular weights of 15 to 30 kDa, and concentrations of 0.005% were optimal for liposomal coating. Liposomal formulation encapsulating coumarin-6 demonstrated better fluorescence intensity in the inner plexiform retinal layer (**Figure 10-11**). Enhanced intensity lasted for 30 to 90 minutes relative to uncoated liposomes. PLL-coated liposomes delivered payload to the retina during

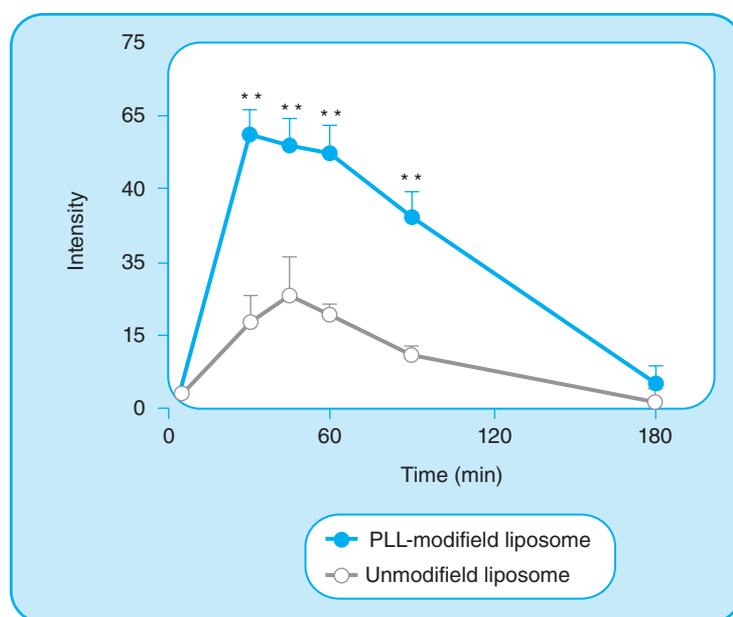


FIGURE 10-11 Time course of accumulated fluorescence intensity in the inner plexiform layer after administration of eye-drop preparations of 0.005% poly-L-lysine (PLL) 15000-modified liposomes (egg phosphatidylcholine [EPC]:dicetyl phosphate [DCP]:cholesterol = 8:0.5:1). Data are shown as mean \pm SEM. Comparisons were made with the unmodified liposome preparation; ** $P < 0.01$. (Reproduced from Sasaki H, Karasawa K, Hironaka K, Tahara K, Tozuka Y, Takeuchi H. Retinal drug delivery using eyedrop preparations of poly-L-lysine-modified liposomes. *Eur J Pharm Biopharm*. Vol 83. Issue 3. April 2013 pp. 364–369, with permission from Elsevier.)

the first 60 minutes and were subsequently cleared within 3 hours after topical dosing. Fluorescence quenching was attributed to diffusion of liposomes from topical drops into the circulatory system. Further, PLL-coated liposomes delivered higher coumarin-6 (higher fluorescence intensity) relative to uncoated liposomes, indicating better *in vivo* performance with surface-modified PLL (Figure 10-11) [125]. Surface modification did not influence liposomal clearance. These results suggest that liposomal formulations may be suitable as potential ocular drug delivery systems.

NANOMICELLES

Nanomicelles are colloidal structures formed from self-assembly of amphiphilic monomers/molecules with sizes from 5 to 200 nm. Monomers tend to initiate self-aggregation (i.e., micelle formation at certain concentration in solvent system). The concentration at which self-assembly of monomers is initiated is defined as the critical micellar concentration. Nanomicelles offer unique advantages such as simplicity, ease of manufacturing, and reproducibility at small and bulk scale. Monomers allow surface conjugation of targeting moiety or specific ligand allowing for targeted drug delivery. But nanomicelles suffer from a few limitations. Drug-loaded nanomicellar formulations encounter rapid tear dilution upon their topical administration. Premature drug release and lack of sustained/controlled release limit their applications. Another major disadvantage of micelles is their inability to entrap hydrophilic small molecule drugs and macromolecules. The advantages include ease of sterilization by simple filtration process, an aqueous formulation of fairly insoluble compounds. Nanomicelles can be either prepared from surfactants (ionic, nonionic, zwitterionic) or block copolymers.

Amphiphilic monomers carrying a charge (anion or cation) on their head groups are referred to as ionic surfactants. Examples include sodium dodecyl sulfate and dodecyltrimethylammonium bromide as anionic and cationic surfactants, respectively [126]. On the contrary, monomers that carry no charge on the head group are referred to as non-ionic surfactant such as n-dodecyl tetra (ethylene oxide) ($C_{12}E_4$). Amphiphilic monomers that carry both positive and negative charges on the head group are referred to as zwitterionic surfactants (e.g., dioctanoyl phosphatidyl choline). Block copolymers have been synthesized to form new amphiphilic polymer monomeric units with U.S. Food and Drug Administration-approved polymers. These polymer blocks are arranged in different ways, such as diblock (A-B type), triblock (A-B-A type), and grafted/branched type copolymers, in which A and B represents any of the polymers like, but not limited to, PEG, PLL, polyethylene oxide, poly(D,L-lactic acid), polypropylene oxide, PCL, PGA, and poly(amino acids), such as poly(aspartic acid), poly(glutamic acid), poly(L-lysine), and poly(histidine).

Nanomicelles as carrier systems have drawn attention from ocular drug delivery scientists because of their ability to translocate drugs across ocular tissues and significantly improve bioavailability. Nanomicelles have been evaluated for elevating anterior ocular drug bioavailability. A twofold increase in ocular bioavailability was achieved for ketorolac when loaded in block copolymeric nanomicelles [127]. These nanomicelles are composed of copolymers of nisopropylacrylamide, vinyl pyrrolidone, and acrylic acid cross-linked with *N,N'*-methylene-bis-acrylamide. Topical administration showed no corneal damage, and the suspension was well tolerated. PEG and/or carbon chain (C16) conjugated polyhydroxyethyl-aspartamide system has been used to encapsulate and prepare dexamethasone nanomicelle for ocular delivery. Higher dexamethasone permeability was achieved with polyhydroxyethyl-aspartamide-C16 and polyhydroxyethyl-aspartamide-PEG-C16 micelles across primary cultured rabbit conjunctival and

corneal epithelial cells relative to solutions and suspensions. *In vivo* ocular bioavailability in rabbits demonstrated higher dexamethasone bioavailability from micelles, relative to its aqueous suspension [128]. Similarly, methoxy poly(ethylene glycol)-hexylsubstituted poly(lactides) micellar formulations of cyclosporine A demonstrated excellent *in vitro* and *in vivo* ocular biocompatibility, transparency, and stability. Pilocarpine-loaded triblock copolymer pluronic F127 (poly[oxyethylene]/poly[oxypropylene]/poly[oxyethylene]) demonstrated improved pharmacokinetic parameters (duration of miotic response and area under the miosis time curve) relative to standard pilocarpine solutions [129]. Further, to improve precorneal residence time, mucoadhesive polymers like chitosan have been used in micellar formulation. Dexamethasone-loaded polyoxyethylated non-ionic surfactant pluronic F127 (F127) and chitosan demonstrated significant enhancement in ocular bioavailability (i.e., 2.4-fold relative to dexamethasone suspension) [130]. A novel photodynamic therapy with dendrimer porphyrin-loaded polyion complex micelles has been evaluated for selective accumulation in pathological corneal neovascularization. Nanomicelles demonstrated an enhanced permeability and retention effect with selective accumulation in corneal neovascularization with no detectable level in normal limbal vessels [131].

Nanomicellar constructs have been used for posterior segment drug delivery. Fluorescein isothiocyanate-poly-lysine (FITC-P[Lys]) loaded PEG-block-poly- α,β -aspartic acid micelles were used to deliver the cargo to posterior ocular tissues (choroid). *In vivo* studies were conducted in rats postinduction of choroidal neovascularization (CNV) with a diode laser photocoagulator. Rats intravenously administered with free FITC-P(Lys) died within 1 hour, suggesting high toxicity, but no death occurred in animals administered FITC-P(Lys)-polyion complex micelles. Polyion complex micelles were retained up to 7 days and showed a C_{\max} at 4 hours. Micellar constructs demonstrated selective accumulation at the pathological neovascular site, due to an enhanced permeability and retention effect [132]. Mitra and colleagues [133, 134] made significant strides to deliver drugs to posterior ocular tissues with mixed nanomicellar formulations. Voclosporin-loaded mixed nanomicelles demonstrated better ocular tolerability than Restasis[®] in New Zealand White rabbits. Single- and multidrop instillation of voclosporin micelles in rabbits demonstrated C_{\max} at 48 and 76 hours, respectively [135]. Further, dexamethasone (0.1%) and rapamycin (0.2%) mixed nanomicellar formulations demonstrated therapeutic drug concentrations in retina-choroid with single-drop instillation [136]. Therefore, small size and hydrophilic corona probably assist nanomicelles to cross ocular barriers and deliver drugs to posterior ocular tissues. A topical aqueous optically clear mixed nanomicellar formulation composed of vitamin E, D- α -tocopheryl polyethylene glycol succinate (TPGS), and octoxynol-40 loaded with 0.1% B-12HS-ACV was successfully developed for the treatment of herpetic keratitis. B-12HS-ACV-loaded nanomicelles are relatively small in size, spherical, homogenous, and devoid of aggregates. The formulations were perfectly transparent and comparable with water. Moreover, ocular biocompatibility studies indicated that mixed nanomicelles were non-toxic and noninflammatory to corneal epithelial cells [137]. Similarly, diclofenac-loaded methoxypoly(ethylene glycol)-PCL micelles were investigated for ocular delivery [138] and demonstrated no irritancy in rabbit eyes. *In vitro* corneal penetration studies across rabbit cornea demonstrated a 17-fold increase in permeability with nanomicelles relative to diclofenac phosphate-buffered saline solution. *In vivo* pharmacokinetic profile of the micelles demonstrated that diclofenac-loaded methoxypoly(ethylene glycol)-PCL micelles generated a twofold increase in AUC (0–24 hours) than diclofenac phosphate-buffered saline solution eye drops. Therefore, nanomicelles have significant potential in improving various ocular pathologies.

MICRONEEDLES

Microneedles have been used to administer drugs into ocular tissues. This administration technique has been developed to deliver drugs to the back of the eye tissues in a minimally invasive manner. Microneedle-based drug administration may reduce the risk and complications associated with IVT injections, such as retinal detachment, hemorrhage, cataract, endophthalmitis, and pseudo-endophthalmitis. It can circumvent BRB and provides an efficient treatment strategy for age-related macular degeneration (AMD), diabetic retinopathy (DR), and posterior uveitis. Microneedles are custom designed to penetrate into the sclera or suprachoroidal space (SCS), the region between sclera and choroid, so damage to deeper ocular tissues may be avoided, and deposit drug solution there. Drug solution or carrier system depot in this region may facilitate drug diffusion into deeper ocular tissues (i.e., choroid and neural retina). Various compounds have been delivered to back of the eye tissues with surface-coated microneedles in cadaver eyes and rabbits [139]. Studies have been initiated to examine sclera as a static barrier to microneedles and intrascleral dissolution of microneedle from surface-coated sulforhodamine. After microneedle scleral penetration, surface-coated drug rapidly accumulated in the microneedle penetration region, forming sulforhodamine depot. Similarly, microneedles have been used for anterior segment ocular delivery. *In vivo* studies with pilocarpine surface-coated microneedles in rabbits demonstrated rapid drug dissolution and depot formation. Such depot can sustain pilocarpine release from the cornea, thereby improving bioavailability and resulting in rapid and extensive pupil constriction [139].

Moreover, to sustain drug release, microparticles and nanoparticles are used. Microneedles have also been used as a mode of drug administration to infuse and deposit drug solutions, microparticles, and nanoparticles into sclera [140]. Carrier systems if placed near the back of the eye tissues (i.e., in SCS) can deliver high drug concentrations to the retina–choroid. Patel et al. [141] attempted to deliver drug solution, nanoparticles, and microparticles in the SCS of rabbit, pig, and cadaver eyes with microneedles and suggested that the procedure is safe, minimally invasive, and forms drug/drug carrier depot at the administration site. Evidence of drug reaching the inner retinal tissues from SCS, however, has not been firmly established [142]. *In vivo* pharmacokinetics of SCS deposited solution/suspension demonstrated longer half-lives for soluble molecules, macromolecules, nanoparticles, and microparticulates. The exact mechanism or pathway, however, for drug solution or micro/nanoparticles clearance from SCS has not been delineated, and further studies are needed. Microneedle-based administration may allow minimally invasive drug delivery to back of the eye tissues (i.e., neural retina, choroid, Bruch's membrane).

IMPLANTS

Implants are drug-eluting devices specially designed for local sustained drug release over long periods. These devices circumvent multiple ocular punctures with needles (IVT injections) and associated complications [143, 144]. Devices are surgically implanted into the vitreous humor by incision at the pars plana and drug placement near the retina. Implants are preferred over IVT injections because of sustained and local drug release for prolonged periods, reduced side effects, and ability to circumvent the BRB [143]. Two types of ocular implants are biodegradable and nonbiodegradable.

NONBIODEGRADABLE IMPLANTS

Nonbiodegradable drug-eluting implants are prepared by using polymers like polyvinyl alcohol (PVA), ethylene vinyl acetate, and polysulfone capillary fiber [144]. Implants from such polymers offer prolonged, controlled drug release with near zero-order kinetics [145]. Examples of nonbiodegradable, U.S. Food and Drug Administration–approved, and commercially available implants include Vitrasert® and Retisert®. Other implants under clinical trials include I-vation, Renexus, NT-503, pSivida, brimonidine, and ODTx intraocular implants.

Vitrasert® is a controlled GCV-releasing intraocular implant for the treatment of cytomegalovirus retinitis. The implant/device is composed of PVA or ethylene vinyl acetate polymer containing GCV (4.5 mg) that provides drug release over 5 to 8 months [145,146]. Moreover, intraocular implants like Vitrasert® may circumvent GCV-induced systemic toxicity. Retisert® is a controlled fluocinolone acetonide–releasing implant for the treatment of chronic uveitis. The device is composed of fluocinolone acetonide encapsulated in a silicone–laminated PVA platform sustaining fluocinolone acetonide release up to 3 years. This implant was shown to effectively control inflammation, reduce uveitis recurrences, and improve visual acuity. The major side effects associated with intraocular implants include cataract formation [145–147].

I-vation (Surmodics Inc.) is a titanium helical coil surface coated with triamcinolone acetonide, poly(methyl methacrylate), and ethylene vinyl acetate for sustained triamcinolone acetonide release over a period of 2 years. The implant has completed phase I clinical trials in patients with diabetic macular edema (DME). Patients were divided into two groups, a slow-releasing group and a fast-releasing group. The slow-releasing group patients demonstrated improvement in visual acuity (greater than 0 Early Treatment Diabetic Retinopathy Study [ETDRS] letter gain from baseline) of 68% relative to the fast-releasing group of 72%. About 28.6% of patients in the fast-releasing group gained more than 15 letters. The mean macular thickness decreased in the slow-releasing group and fast-releasing group by 201 μm and 108 μm , respectively.

Renexus (formerly NT-501) is an IVT implant composed of a sealed semipermeable hollow-fiber membrane capsule covered by six strands of polyethylene terephthalate yarn loaded with genetically modified RPE cells. This implant was developed for the treatment of retinitis pigmentosa and geographical atrophy associated with AMD. RPE cells secrete recombinant human ciliary neurotrophic factor. The implant protects the contents of the device from immune response, human ciliary neurotrophic factor, and other cell metabolites in vitreous humor and also regulates nutrient exchange. Phase I results showed that Renexus was well tolerated for 6 months in patients with late-stage retinitis pigmentosa [148]. Visual acuity was stabilized and was accompanied by corresponding structural changes that were dose dependent ($p < 0.001$). The geographic atrophy (GA) area growth rate was minimized in treated eyes compared with contralateral eyes at 12 and 18 months. Also, Renexus was found to prevent secondary cone degeneration in patients with retinitis pigmentosa [149]. Phase II studies are underway for Usher syndrome (type 2 or 3) and a phase I study for macular telangiectasia.

NT-503 is currently in the development stage. It encapsulates VEGF receptor Fc–fusion protein (VEGFR–Fc)–releasing cells. VEGFR–Fc has been found to be ~20-fold more efficient in neutralizing VEGF relative to ranibizumab. *In vivo* vitreous

NT-503 implantation in rabbits demonstrated VEGFR-FC continuous release up to 12 months. Currently, this implant is under phase I clinical trial for neovascular AMD outside the United States [150]. Drug-loaded multiple reservoir systems/implants are biocompatible and nonresorbable and release optimized injectable doses triggered by laser activation. The composition of the drug reservoir implants is not known. Such reservoirs are capable of loading both small and large molecules. These agents are released via a standard, noninvasive laser activation procedure. The multiple-reservoir system allows ophthalmologists to control drug delivery by activating specific reservoirs. The inactivated reservoirs, however, remain intact for future activation and drug release. Reports indicate that the device demonstrated promising *in vivo* results for release of both small molecules and proteins.

Long-term drug release has been achieved with single nonbiodegradable implant systems. The use of implants is limited, however, because these devices have to be surgically implanted and periodically removed after drug depletion. This procedure makes the treatment expensive and noncompliant. In some cases, the drug-depleted devices are left in the vitreous and a new drug-loaded implant is introduced. Adverse events such as endophthalmitis, pseudo-endophthalmitis, vitreous haze and hemorrhage, cataract development, and retinal detachment limit their applications.

BIODEGRADABLE IMPLANTS

Biocompatible and biodegradable implants are gaining more attention currently. These biodegradable implants are not required to be surgically removed, which signifies a distinctive advantage over nonbiodegradable implants. PLA, PGA, PLGA, and PCL are the most commonly used polymers selected for fabrication of implants [144]. Examples include Surodex™ and Ozurdex®, which are now commercially available. These implants offer sustained dexamethasone release for the treatment of intraocular inflammation and macular edema [145].

Surodex™ is composed of dexamethasone enclosed in PLGA and hydroxypropyl methylcellulose implant. The implant is inserted into the anterior chamber to control postoperative inflammation in cataract patients. This implant demonstrated sustained dexamethasone release over 7 to 10 days with improved anti-inflammatory effect comparable with that of topical steroid administration [145]. Ozurdex® is another dexamethasone ocular implant indicated for the treatment of macular edema. This implant is composed of dexamethasone-enclosed PLGA polymer matrix. The implant prolonged the release of dexamethasone for up to 6 months. Randomized clinical trials have demonstrated its efficacy to stop vision loss and improve visual acuity in eyes with macular edema associated with branch retinal vein occlusion or central retinal vein occlusion. Moreover, clinical trials for treatment of diabetic retinopathy and Irvine-Gass syndrome suggested that the implant is a promising treatment option [145].

Brimonidine IVT implant is a biodegradable, rod-shaped, drug-eluting device for the treatment of retinitis pigmentosa and atrophic AMD. Currently, the implant is in phase II/III clinical trials. Brimonidine is a β_2 -adrenergic agonist that can release various neurotrophins including brain-derived neurotrophic factor (BDNF), human ciliary neurotrophic factor [151], and fibroblast growth factor-basic (b-FGF) [152]. These neurotrophins provide protection to photoreceptor cells and/or RPE from apoptosis. The implant is composed of brimonidine tartrate in PLGA for sustained release. Recently, ocular inserts composed of brimonidine tartrate, 7% PVP K-90, 1.5% low-molecular-weight sodium alginate with or without ethyl cellulose

coat were shown to sustain *in vitro* release of brimonidine. *In vivo* therapeutic efficacy, regarding IOP-lowering effect, showed superior and sustained effect compared with brimonidine solution in rabbits. Moreover, due to mucoadhesive property and sustained effect, one-side-coated ocular inserts have shown higher IOP-lowering effect relative to noncoated or dual-side-coated counterparts [153]. Thethadur is a biocompatible, biodegradable, honeycomb-like nanostructured porous silicon (bio-silicon) device under development. The implant also offers sustained drug release and can carry a variety of drugs (i.e., small molecules, peptides, proteins, and other biologics).

CONTACT LENS

Contact lenses are thin, curve-shaped plastic discs designed to be placed over the cornea [154]. After placing over the cornea, contact lenses adhere to the surface of the eye because of interfacial tension. Both the cornea and contact lens are separated from each other by a thin tear fluid layer called postlens tear film. In general, contact lenses are prepared with polymers such as silicone hydrogel (composed of *N,N,N,N*-dimethylacrylamide, 3-methacryloxypropyltris[trimethylsiloxy]silane, bis- α , ω -[methacryloxypropyl] polydimethylsiloxane, 1-vinyl-2-pyrrolidone, and ethylene glycol dimethacrylate) and poly(hydroxyethyl methacrylate) (p-HEMA) [155, 156].

Contact lenses loaded with drug and/or drug-loaded nanocarriers have been developed for ocular delivery [157, 158]. Several active drugs such as timolol, dexamethasone, dexamethasone 21 acetate, beta-blockers, antihistamines, and antimicrobials have been loaded for anterior delivery [156]. Drug-laden contact lenses exhibit longer precorneal residence time. In the postlens tear film it causes high corneal drug flux with minimal drug flow into nasolacrimal duct. Generally, contact lenses are loaded by soaking the lenses in drug solutions. Such drug-loaded contact lenses demonstrate slow drug release, minimal drug loss due to precorneal clearance, improved corneal bioavailability, and higher efficiency in delivery relative to conventional topical drops. As an example, dexamethasone loaded in p-HEMA contact lenses demonstrated higher ocular bioavailability relative to conventional topical eye drops [159].

Contact lenses surface coated with surfactants have been developed to improve drug loading and achieve sustained release. Optimizing the interactions between contact lens polymer matrices with hydrophobic tails of ionic surfactants helps to adsorb surfactant molecules on the polymer. A tight packing creates high surface charge on the contact lens. Ionic compounds adsorb on the charged surfactant-coated contact lens surfaces with high affinity. Such interactions cause reduced transport rates, leading to extended release. Anionic drug dexamethasone 21-disodium phosphate binds to p-HEMA contact lenses through cationic surfactant (cetalkonium chloride). Drug molecules adsorbed on the surfactant-covered polymer demonstrated slow diffusion along the surface with diffusivity at a lower rate than free drug. The surfactant loading accelerated duration of release from about 2 to 50 hours within 1 day using Acuvue® contact lenses [155]. These drug-loaded contact lenses have advantages over conventional topical drops but suffer from limitations such as inadequate drug loading and short-term drug release (few hours). To further improve drug loading and extend drug release, colloidal drug delivery systems such as liposomes and nanoparticles are laden into contact lenses and molecularly imprinted contact lenses have been developed.

For the preparation of particle-laden contact lenses, initially the therapeutic agent is entrapped in colloids such as liposomes, nanoparticles, or microemulsions. Furthermore,

these loaded carrier systems are dispersed in the contact lens material. For example, particle-laden contact lenses have been prepared by dispersing lidocaine entrapped in microemulsions and/or liposomes followed by incorporation in p-HEMA hydrogels. Particle-loaded contact lenses demonstrated extended lidocaine release over 8 days [160, 161]. After use, contact lenses are required to be stored in drug-saturated solutions to avoid drug loss during storage. Stimuli responsive or “smart” particles that release drug only upon contact with the ocular surface may overcome such a problem.

Molecular imprinted contact lenses have demonstrated improved drug loading and drug release. Soft contact lenses fabricated by the molecular imprinting method demonstrated ~1.6 times higher timolol loading than contact lenses prepared by a conventional method. Also, molecular imprinted contact lenses demonstrated sustained timolol release that produced higher ocular tear fluid bioavailability for ketotifen fumarate relative to drug-soaked contact lens and commercially available topical drops [162]. The ketotifen fumarate molecular imprinted contact lenses demonstrated improved ocular relative bioavailability (about threefold higher) than nonimprinted lenses. The AUC for ketotifen fumarate in imprinted lens, nonimprinted lens, and eye drops were $4365 \pm 1070 \mu\text{g h/mL}$, $493 \pm 180 \mu\text{g h/mL}$, and $46.6 \pm 24.5 \mu\text{g h/mL}$, respectively [163]. Thus, molecular imprinted contact lenses have been demonstrated to possess more effectiveness over conventionally prepared drug-loaded lens and topical eye drops.

IN SITU GELLING SYSTEMS

In situ gelling systems or hydrogels refer to polymeric aqueous solutions that undergo a sol to gel transition in response to environmental or physiological stimuli. In general, thermosensitive gels are prepared with biocompatible and biodegradable polymers. Gel formation can be initiated by temperature variation, pH change, and ionic balance and/or with ultraviolet irradiation. For ocular drug delivery, thermosensitive gels (i.e., solutions that respond to temperature changes and transform into gels) have been developed [164]. Polymers used for the development of thermosensitive gels include poloxamers and multiblock copolymers made from PCL, PEG, PLA, PGA, poly(*N*-isopropylacrylamide), and chitosan. These polymeric systems form temperature-dependent micellar aggregation followed by gelation at higher temperatures due to aggregation or packing [165]. Drug-loaded thermosensitive polymeric solution once in contact with the ocular surface transforms to an *in situ* gel depot. Because of the unique transition from sol-to-gel phase at physiological temperature, these gelling systems cause improvement in ocular drug bioavailability.

Effects of hydrophilic and hydrophobic polymeric additives on sol–gel transition and release profile of timolol maleate from PEG-PCL-PEG-based thermosensitive hydrogels have been studied [166]. PCL (hydrophobic additive) and PVA (hydrophilic additive) lower critical gel concentrations of the PEG-PCL-PEG triblock polymer. The effect of PCL on sol–gel transition is more pronounced than PVA (Figure 10-12) [167]. The effect of PVA on the release profile was more pronounced and the cumulative percentage timolol maleate release was improved from $86.4 \pm 0.8\%$ to $73.7 \pm 1.8\%$ over 316 hours. Thermosensitive gels appear to be noncytotoxic in rabbit primary corneal epithelial culture cells with/without additives, indicating PEG-PCL-PEG hydrogel matrix to be a viable drug delivery system.

Dexamethasone acetate-loaded thermosensitive gel comprising the triblock polymer PLGA-PEG-PLGA has been developed as an ocular delivery system [165]. *In vivo*

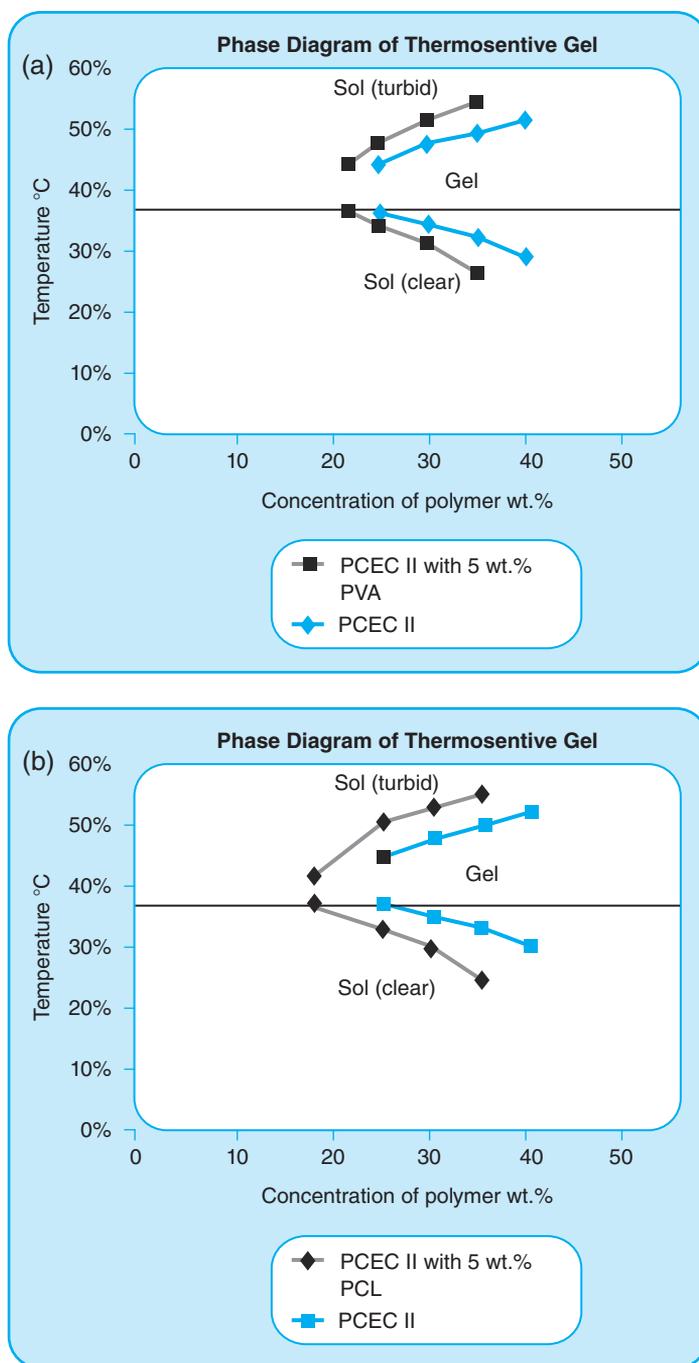


FIGURE 10-12 Sol-gel transition phase diagram. (a) $\text{PEG}_{750}\text{-PCL}_{3750}\text{-PEG}_{750}$ (PCEC II) triblock copolymer aqueous solutions alone and with 5 wt.% PVA. (b). PCEC II triblock copolymer aqueous solutions alone and with 5 wt.% PCL. (Reproduced with kind permission from Springer Science+Business Media: Mishra GP, Tamboli V, Mitra AK. Effect of hydrophobic and hydrophilic additives on sol-gel transition and release behavior of timolol maleate from polycaprolactone-based hydrogel. *Colloid and polymer science*. Sep 2011;289[14]:1553–1562.)

studies with topical drop administration to rabbit eye demonstrated significantly higher C_{\max} (125.2 $\mu\text{g}/\text{mL}$) for dexamethasone acetate in the anterior chamber with PLGA-PEG-PLGA solution relative to dexamethasone acetate solution (17.6 ± 2.18 ng/mL). Moreover, AUC was higher with PLGA-PEG-PLGA over topical solution. C_{\max} and AUC were higher by ~ 7 - and 7.98-fold from PLGA-PEG-PLGA *in situ* gel than solution. The triblock polymer comprising PLGA and PEG (ReGel™) has been studied for delivery of macromolecules such as ovalbumin [168]. *In vivo* studies with subconjunctival administration in rat eye demonstrated sustained drug release. Ovalbumin concentrations were maintained at measurable levels in the back of the eye tissues (sclera, choroid, and retina) over 14 days.

Other polymeric systems comprising cross-linked poly(*N*-isopropylacrylamide)-PEG diacrylate hydrogels have been synthesized for sustaining bovine serum albumin and immunoglobulin G release [169]. *In vitro* studies demonstrated 3-week sustained bovine serum albumin release from gel. Therefore, thermosensitive gels offer significant advantages in providing sustained drug release, improving residence time on the cornea, minimizing dosing frequency, lowering side effects, and increasing ocular bioavailability. Furthermore, drug-loaded carrier systems such as nanoparticles suspended in thermosensitive gels can be used [170]. Steroids such as dexamethasone-, hydrocortisone acetate-, and prednisolone acetate-loaded PLGA nanoparticles suspended in thermosensitive gel demonstrated zero-order drug release kinetics. The burst release from nanoparticles was minimized. *Ex vivo* studies across rabbit sclera demonstrated longer sustained drug release relative to drug-loaded nanoparticles. For targeted drug delivery, thermosensitive gels composed of PLGA-PEG-PLGA were studied. Doxorubicin-loaded micelles (PLGA-PEG-folate [FOL]) were suspended in thermosensitive gel that sustained doxorubicin release for a period of 2 weeks. *In vitro* uptake studies across Y-79 cells demonstrated about four times higher uptake than PLGA-PEG micelles [171]. These studies indicate that thermosensitive polymers form gels at physiological temperatures sustain drug release and aid in targeted delivery to both anterior and posterior ocular tissues.

GENE DELIVERY

Gene delivery to targeted cells involves safe and effective delivery of a specific, efficacious, and safe genome. The delivered genome is expected to elicit robust expression, coupled with high efficiency, high persistent levels, and long duration of expression. Two types of gene transfer vectors, viral and nonviral, have been evaluated in preclinical and clinical settings. Viral gene transfer vectors include adenovirus, adeno-associated virus (AAV), retrovirus, lentivirus (LV), alphavirus, herpes virus, and baculovirus vectors [172–174]. Of these viral vectors, adenovirus, AAV, and LV are commonly used for ocular gene transfer. These viral vectors have specific molecular sites on the capsid coat that causes it to track specific cell types of a target organ known as tropism [175]. Nonviral gene transfer vectors include plasmids, cationic lipids, and polymers. These nonviral vectors form a complex with DNA and then condense to form particles in nanometer range (<25 nm) so the complex can pass to the cell nucleus. Gene transfer vectors have specific advantages and limitations. Viral vectors are more efficient in translocating into target ocular cells. These viral vectors, however, are limited in application due to heightened concern for long-term safety and immunogenicity problems, which have not yet been overcome. Nonviral vectors do not usually elicit such safety and immunogenicity concerns. Clinical trials have revealed nonviral vectors to

be considered safer than viral vectors but much less efficient in transduction relative to viral vectors [176].

ADENOVIRUS VECTORS

Adenovirus-based gene therapy can be directed to the back of the eye tissue (retina) and anterior segment tissues. Adenoviruses are nonenveloped double-stranded DNA viruses with several serotypes [177]. These viruses are obtained by partial deletion of the viral genome. Adenoviruses can accommodate 36 kb of exogenous sequences and do not integrate in the target cell. Because of the transient nature of the transgene expression in adenovirus vector, immune-mediated elimination is identified. Therefore, the desired long-lasting therapeutic gene expression required for ocular diseases is absent. Adenovirus vectors expressing the herpes virus thymidine kinase have been demonstrated to phosphorylate the GCV into the triphosphate form. This cytotoxic product of the herpes simplex thymidine kinase reaction is transported to adjacent cells through gap junctions, inhibits DNA replicates, and destroys transduced cells [178].

AAV VECTORS

Recombinant AAV (rAAV) is a small, nonpathogenic, single-stranded DNA virus that exists in over 100 distinct variants. Ocular gene transfer with AAV has received much attention recently. The rAAV vectors demonstrated several advantages, such as long-term transgene expression in the eye and lack of pathogenicity [179]. More than eight different AAV serotypes are known. These vectors have specialized tracking mechanisms to target cells that have demonstrated robust, persistent gene expression and profiles of differing kinetic expressions. The versatility, efficacy, low immunogenicity, and nonpathogenicity render rAAV vectors highly efficient for ocular gene transfer. These vectors have only one limitation: their gene-carrying capacity is restricted to 4.7 kb [180]. rAAV vectors have been used to mediate efficient transduction by deletion of coding sequences and insertion of expression cassettes between the inserted terminal repeats. Tissue specificity and expression kinetics of rAAV vectors highly depend on the vector serotype and anatomical compartment of delivery within the globe. rAAV vectors have been used for efficient transduction across RPE, photoreceptor cells, and retinal ganglionic cells [181]. In *in vivo* studies in large animal models, rAAV vectors demonstrated stable expression in the retina that was maintained over several years [180, 182, 183].

LV VECTORS

The LVs are integrating, complex, single-strand, positive sense, enveloped RNA retroviruses. These vectors possess the necessary cellular and molecular components required for stable transduction of dividing and postmitotic cells. The vectors are widely used in ocular gene delivery because of their ability to transduce nondividing cells and generate and maintain long-term transgene expression [184]. Advantages of LVs include absence of immunological complications after intraocular delivery and relatively large transgene carrying capacity (8–10 kb) compared with rAAVs, which permits multicistronic codelivery of several therapeutic genes. Such LVs have certain limitations, such as, the potential generation of replication competent lentiviruses during LV production, *in vivo* recombination with lentiviral polynucleotide sequences, and insertional addition of proviral DNA in or close to active genes, which may elicit tumor initiation or promotion [185].

In vivo studies in rodents with intracameral administration of vesicular stomatitis virus glycoprotein (VSV-G) pseudo-typed HIV-1 and equine infectious anemia virus demonstrated efficient transduction in corneal endothelium and trabecular meshwork [186–189]. Other studies with different species established that VSV-G- pseudo-typed feline immunodeficiency virus vector specifically target the trabecular meshwork [190–192]. Rabies-G pseudo-typed equine infectious anemia virus vector can transduce neuronal cells within the anterior ocular tissues with low efficiency [187]. IVT delivery of VSV-G pseudo-typed HIV-1, equine infectious anemia virus, VSV-G pseudo-typed feline immunodeficiency virus, and rabies-G pseudo-typed equine infectious anemia virus vector failed to produce efficient intraocular expression in the corneal endothelium. On the contrary, few studies suggest widespread intraocular transduction after IVT delivery of these vectors [193–195]. Subretinal delivery of LVs have been found to demonstrate efficient and stable transduction of rodents and nonhuman primate RPE cells [172].

NONVIRAL VECTORS

Nonviral vectors have been and continue to be of interest due to their safe, effective, and promising strategy for ocular gene transfer. Vectors such as compacted DNA nanoparticles may mediate gene therapy safely with sustained expression over several months [196]. These systems appear to be promising in the treatment of genetic ocular diseases. Han et al. demonstrated that DNA (CK30PEG) nanoparticles can be retained in the eye after subretinal injection. On the other hand, AAV injection exhibited vector DNA and green fluorescent protein (GFP) expression in the visual pathway of the brain. It appears that nonviral nanocarriers are a potential successful alternative to viral gene therapy [197].

Similarly, cationic core-shell liponanoparticles demonstrated higher uptake in human conjunctival cells than plasmid-laden chitosan nanoparticles and lipid-coated chitosan nanoparticles [198]. *In vivo* studies conducted in rabbits revealed that cationic core-shell liponanoparticles demonstrated ~2.5-fold rise in enhanced green fluorescent protein (EGFP) expression, indicating an alternative to viral vectors. Nanomicellar constructs prepared from polyethylene oxide-polypropylene oxide-polyethylene oxide were evaluated as a carrier for plasmid DNA with the *lacZ* gene [199]. These nanomicellar constructs demonstrated efficient and stable transfer of functional gene via topical drops in mice and rabbits, indicating the potential of block copolymers for DNA transfer. Kurosaki et al. [200] evaluated ocular gene expression using anionic ternary/plasmid DNA-PEI complex [200]. A coating of plasmid DNA-PEI complex with ternary complexes such as γ -polyglutamic acid and chondroitin sulfate demonstrated no cytotoxicity and aggregation. *In vivo* intravenous administration of ternary complex to rabbits showed high gene expression in the retina, indicating the complex to be a suitable carrier for effective and safe ocular gene therapy.

SUMMARY

Effective management of ophthalmic diseases is a challenging task for pharmaceutical scientists because of the complex nature of various diseases and the presence of ocular barriers. Challenges in ocular drug delivery have been partially met by the identification of transporters on ocular tissues and chemical modification of drug substances to target such transporters. Transporter specificity helps in targeting specific tissues, thereby

lowering side effects and improving bioavailability. Ideally, therapeutically effective drug levels should be maintained for prolonged periods of time after a single application.

Invasive modes of drug delivery cannot be considered safe, effective, and patient compliant. Drug delivery via the periocular route could overcome many of these limitations and also offer sustained drug levels in various ocular pathologies affecting both the anterior and posterior segments. Targeted lipid prodrug strategy can be a promising approach for many drug molecules with poor absorption across ocular barriers. Colloidal drug delivery systems could significantly improve the state of current therapy and may emerge as an alternative after periocular administration.

Advances in nanotechnology and noninvasive drug and gene delivery techniques will remain in the forefront of designing and developing novel ocular drug delivery systems. More emphasis needs to be given to noninvasive sustained drug delivery for both anterior and posterior segment eye disorders. Further, continuous innovation in gene therapy appears to be a very exciting area for a gamut of ocular diseases. Nevertheless, a clear understanding of the complexities associated with normal and diseased conditions, physiological barriers, and pharmacokinetics would significantly hasten further drug development in this field.

ACKNOWLEDGMENT

This work was supported by National Institutes of Health grants R01 EY09171 and R01 EY10659.

REVIEW QUESTIONS

1. List the various layers of the cornea.
2. Describe the advantages and disadvantages of topical ocular drug administration.
3. What are cyclodextrins?
4. Define nanomicelles and their advantages and disadvantages.
5. What are ocular implants? Describe briefly implants used in ocular drug delivery.
6. What is gene delivery? List the vectors commonly used in gene delivery.

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