Jayvadan K. Patel · Vijaykumar Sutariya
Jagat Rakesh Kanwar · Yashwant V. Pathak
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Drug Delivery for the Retina and Posterior Segment Disease

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Foreword

Drug delivery systems are in the forefront of research in disease manipulation. With the advent of nanotechnology applications in drug delivery systems, targeted drug delivery to the disease site is becoming a reality. Application of this exciting new science to eye diseases in general and poster segment diseases in particular is long overdue. Drug delivery to the posterior segment of the eye has long been a challenge. Injections have become the mainstay of treatment for a variety of posterior segment diseases. Topical application of medications is unlikely to reach the posterior segment because of multiple barriers, i.e., both static and dynamic biological ocular barriers. These barriers include anterior segment static barriers, such as cornea, conjunctiva, blood aqueous barrier, and efflux pumps, while anterior segment dynamic barriers include tear drainage, conjunctival lymph and blood flow, and aqueous humor. The posterior segment eye barriers include sclera, Bruch's membrane, blood retinal barrier, and choroidal blood and lymph circulation.

The book addresses various issues related to the posterior eye segment drug delivery covering the challenges and potential solutions to the disease treatment. The editors of the book, known experts, namely, Drs. Jayvadan K. Patel, Vijaykumar Sutariya, Jagat Rakesh Kanwar, and Yashwant V. Pathak, have assembled an outstanding team of contributors to make this book a great resource for the research community.

The book has 25 chapters dealing with different aspects of posterior eye segment drug delivery including anatomy and physiology of posterior eye, pharmacotherapy of diabetic macular edema and retinopathy, oxidative stress in ocular disorders (exploring the link to pesticide exposure and potential for using nanotechnology for antioxidant delivery), nanomedicine-based gene delivery for the retina and posterior segment diseases, receptor-targeted prodrug approach for retina and posterior segment disease, stereoisomeric dipeptide prodrug approach for retina and posterior

segment disease, and biodegradable polymeric implants for retina and posterior segment disease.

It is my belief this book will be very useful for the ophthalmologists and researchers working in this research. I congratulate the editors and the many contributors on their efforts to bring this very useful resource to fruition.

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Ramesh Ayyala

Preface

The posterior segment or posterior cavity is the back two-thirds of the eye that includes the anterior hyaloid membrane and all of the optical structures behind it: the vitreous humor, retina, choroid, and optic nerve. Patients with retina diseases require frequent eye exams and a variety of imaging studies to assess for any signs of early damage to the posterior eye segment. Some of these diseases can often be treated with medication, laser treatment, or surgery. Treatment sometimes may prevent further damage to the retina, and in some cases damage can be reversed. Some of the common diseases of posterior eye segment include diabetic retinopathy, macular degeneration, retinal detachment, retinal hemorrhages, retinal blood vessel occlusions, retinal cancer, retinal and intraocular infections, and so on.

Since last decade, significant advances are made in formulating and optimizing the delivery of drugs to target tissues within the eye and in maintaining effective drug doses within those tissues. Most pharmacologic management of ocular disease, however, continues to use the topical application of solutions to the surface of the eye as drops. Factors that can limit the usefulness of topical drug application include the significant barrier to solute flux provided by the corneal epithelium and the rapid and extensive pre-corneal loss that occurs as the result of drainage and tear fluid turnover. Currently, the treatment of posterior segment disease is to a significant extent limited by the difficulty in delivering effective doses of drugs to target tissues in the posterior eye.

This book is edited with a focus on drug delivery for the retinal and posterior segment diseases. We had published earlier a book entitled *Nano-Biomaterials for Ophthalmic Drug Delivery* (ISBN 978–3–319-29,344-8) edited by Yashwant V. Pathak, Vijaykumar Sutariya, and Anjali Hirani. This is our second effort in the area of ophthalmic drug delivery systems to deal with recent advances in treatment of posterior eye segment diseases. We are pleased to submit this second book on ophthalmic drug delivery edited by Drs. Jayvadan K. Patel, Vijaykumar Sutariya, Jagat Rakesh Kanwar, and Yashwant V. Pathak. The objective of the book is to address issues related to novel advances in drug delivery for posterior segment diseases of the eye. It covers the different novel drug delivery systems to the eye such as liposomes, nanoparticles, transscleral iontophoresis, implant, and prodrug approach.

This book is divided in to six major parts; the first part with five chapters covers the introduction and basic concepts of drug delivery to retina and posterior eye segment. The chapters address the anatomy and physiology of retina and posterior eye segment, pharmacotherapy of diabetic macular edema and retinopathy, drug delivery to posterior segment of eye (conventional delivery strategies and their barriers and restrictions), penetration routes to retina and posterior segment, and drug delivery diabetic retinopathy.

The second part deals with nanotechnology-based formulations for retina and posterior eye segment. It includes six chapters. The chapters cover liposomes for retina and posterior segment disease, nano−/microparticles for retina and posterior segment disease, nonviral delivery systems for gene therapy for retina and posterior segment disease, oxidative stress in ocular disorders (exploring the link to pesticide exposure and potential for using nanotechnology for antioxidant delivery), advances in the microbial infection in cornea and role of nanotechnology to cure ocular surface, and nanomedicine-based delivery to the posterior segment of eye (brighter tomorrow).

The third part covers transscleral iontophoresis for retina and posterior segment disease and includes three chapters. These chapters address transscleral drug delivery to retina and posterior segment diseases, colloidal carrier systems transscleral drug delivery, and transscleral iontophoretic drug delivery for treating retinal diseases.

The fourth part deals with implant formulation for posterior eye segment and includes two chapters, biodegradable polymeric implants for retina and posterior segment disease and nanomedicine-based gene delivery for the retina and posterior segment diseases.

The fifth part talks about prodrug strategies for retina and posterior segment disease and includes four chapters covering transporter targeted prodrug approach for retina and posterior segment disease, lipid prodrug-based delivery for retina and posterior segment disease, injectable prodrug approach for retina and posterior segment disease, and stereoisomeric dipeptide prodrug approach for retina and posterior segment disease.

The last part discusses other advances for retina and posterior segment diseases and includes five chapters. These cover receptor-targeted prodrug approach for retina and posterior segment disease, intravitreal injection drug delivery for retina and posterior segment disease (challenges and future ahead), thermoresponsive gel drug delivery for retina and posterior segment disease, peptide synthesis and delivery for retina and posterior segment disease, and corneal haze, refractive surgery, and its implications on choroidal neovascularization.

This book is targeted toward academic researchers as well as industry experts involved in the development of drug delivery for retinal disease as well as posterior segment diseases of the eye. This book will be a great resource for students as well as professors. Additionally, this will be a useful tool for industrial scientists investigating novel drug delivery and device for the retinal and posterior segment diseases of the eye.

We sincerely hope this book gets similar response as the first book on ophthalmic drug delivery.

We also would like to thank and acknowledge our respective families for their support, the publishers, and our wonderful team of contributing authors. A special thanks to Dr. Priyanka Bhatt and Ms. Priya Narvekar and Ms. Gulimire Rouzi for their help in the final compilation.

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Gujarat, India — Jayvadan K. Patel
Tampa, FL, USA — Vijaykumar Sutariya
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Part I
Introduction and Basic Concept of Drug Delivery for Retina and Posterior Segment Disease

Chapter 1
Anatomy and Physiology of Retina and Posterior Segment of the Eye

Orhan E. Arslan

Abstract The fact that the retina is readily accessible has a key role in constructing visual images and interacting with the environment and that most of the sensory input is of visual nature affirms the significance of investigative exploration of this part of the visual system. The goal of this work is to recognize the unique cellular characteristics and the neural circuitry of the retina in the posterior segment in primates. To that end an attempt has been made we have attempted to examine the retinal pigment epithelium and its role in the barrier system and the metabolic activity of the retina. We also discussed the characteristics of the photoreceptors and the foveal structure with associated reflexes. The blood flow and associated regulatory mechanisms as well as laminar organization of the optic disc have been explored.

Keywords RPE · Optic disc · Fovea · Hyaloid · Photopic · Scotopic

Introduction

Developmentally, the retina and the optic nerve are considered an extension of the central nervous system. The eyeball consists in succession from the exterior inward of the tunica fibrosa, tunica vasculosa, and tunica nervosa. The tunica fibrosa consists of the cornea and sclera, while the tunica vasculosa (uvea) is comprised of the choroid, ciliary body, and iris. Examining the tunica nervosa reveals a retinal pigment epithelial (RPE) layer in apposition to the retinal sensory layer [1–6].

The RPE is loosely bound to the retina but provides major source of nutrients, ions, and water in addition to physical support by secreting factors that maintain the integrity of the retina. It plays a vital role in the absorption of excess light, protection against photooxidation, and in the visual cycle that produces 50% of the rhodopsin byre-isomerization of trans-retinal into 11-cis-retinal. Embryologically, the

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RPE develops from the neuroectoderm, which is also the source of the sensory layers of the retina [7, 8].

There are nearly 3.5 million RPE cells with a perceptible variation between the diameter of the central and the peripheral RPE cells, which is estimated to range between 14 and 60 mm, respectively. The variable diameter of the RPE cells correlates inversely with their density, as the density of RPE cells in the fovea centralis, estimated around 5000 cells per mm, outnumbers by two and half-folds that of the cells in the peripheral retina [9–11].

The cytoarchitectural organization enables the RPE cells to become an important element of the outer blood-retinal barrier (BRB) that selectively allows the movement of the molecules between the choroid layer and the photoreceptor cells. To adequately accomplish this function, the RPE is arranged into single tightly joined cuboidal epithelial cells in the central part of tunica nervosa [12–17].

The RPE cells mediate immune responses and thus complement the BRB by the expression of cytokines, FasL, major histocompatibility complex (MHC), and adhesion molecules. They possess high metabolic activity as evident by the large number of endoplasmic reticulum, free ribosomes and mitochondria, and polarity [15, 16]. The efficacy of the tightly joined RPE cells as a barrier and ability to maintain selective transport of molecules is facilitated by the enfolding of the basal and apical surfaces of these cells [18, 19]. In order to maintain the excitability of the photoreceptors, the RPE buffers the ionic composition in the subretinal area. Thus, conditions, such as proliferative diabetic retinopathy (DR), induces RPE cell dysfunction, degenerative changes in the retina and subsequent visual disorders including total blindness [20, 21]. While the proliferative diabetic retinopathy (PDR) is considered a common etiology of visual deficit, diabetic macular edema (DME) remains the principal etiology of poor vision. The pathogenesis of these two conditions also markedly differs as the PDR is associated with hypoxia-induced neovascularization, whereas disruption of the blood-retinal barrier (BRB) and subsequent vascular leakage are regarded as the important components in the pathogenesis of DME [22–24]. Despite this difference, both conditions may invariably coexist in diabetics. The RPE cells lack the ability to undergo mitosis but show proliferation under pathological processes [25, 26].

The dark brown to black discoloration exhibited by the RPE cells is attributed to the oxidation of amino acid tyrosine and polymerization through the tyrosinase pathway, and tyrosinase catalytic properties remain dependent on its binding to copper [24, 27, 28]. This process starts early around the fifth week and concludes by the end of the sixth week of gestation. The process of pigmentation of RPE cells differs from that of the choroidal melanocytes in that it starts late around the 20th week of gestation and continues into the postnatal life. Further, unlike the melanocytes of the choroidal layer, which are neural crest derivatives, the RPE cells pigmentation does not manifest racial-bias variations. The RPE and the Bruch's membrane are the sites of lipofuscin deposition, which is produced by the degradation of the outer segment of the photoreceptors. Although a predominantly age-related pigment, lipofuscin can readily be in trace amounts in the RPE of children.

Sensory Retina

The retina, a derivative of the optic vesicle, is comprised of optic and nonoptic components. The optic part is where the photoreceptors reside, whereas the nonoptic component consists of the a ciliary part that shields the ciliary body, and an irideal part that stretches under the iris. The optic part contains the photoreceptors that obtain nutrients and mechanical support from the loosely linked pigment epithelium. Congenital hypertrophy of the retinal pigment epithelium (CHRPE), seen in pediatric patients with familial adenomatous polyposis (FAP), is an autosomal dominant condition that also exhibits multiple adenomatous colic benign polyps that may transform to malignant cancer at later stages.

The delineation between the optic (sensory) retina and the plane non-optic (pars plana) part on the ciliary body is delineated by the ora serrata, a site where the neural retina is reduced to a single row of cells that continue with the nonpigmented ciliary epithelium, while the pigmented ciliary epithelium replaces the RPE. The gap between the RPE and the neuroretina, the subretina, is occupied and sealed by the occluding junctions that connect the pigmented and nonpigmented ciliary epithelia and renders the pars plana a secure zone for surgical intervention. Also, expansion of an exudate in this space in a diseased retina is resisted by a barrier formed by union of the photoreceptors and the pigment epithelium at the ora serrata.

When the optic part of the retina separates partially or completely from the pigment epithelial layer as consequence of trauma or disease processes, the condition is known as detachment of the retina. Trauma-induced condition is termed rhegmatogenous retinal detachment. In patients with chronic retinal detachment, a tear and rarely a peripheral retinal sea-fan neovascularization may occur that can be countered by cryopexy and a segmental scleral buckle directed at the sites of retinal tear [29]. An inflammatory fluid produced by pathologic processes may also produce exudative retinal detachment by sweeping from the choroid layer into the subretinal space. Non-traumatic processes can also cause retinal detachment by the excessive pull exerted through the fibrovascular bundles that stretches between the retina and vitreous body leaving the retina unaffected. This is seen in diabetic vitreoretinopathy.

Microscopic view of the neuronal part of the retina demonstrates a total of ten layers and nine types of cellular elements with three primary neural layers and three synaptic (plexiform) layers. The photoreceptors are considered the visual neuronal antennae that induce phototransduction of the photons into nerve impulses. Although the number is variable according to various sources, it has been estimated to be around five million cones and 100 million rods in human retina [30–32].

Each photoreceptor is comprised of two cilium interconnected segments: an outer segment with photopigment and an inner segment that encloses mitochondria with the endoplasmic reticulum, inner fiber, a nucleus, and a synaptic terminal [33].

Opsin, a transmembranous protein that connects the photopigment to the plasma membrane, resides within the outer segment as flat discs in the form of cavernous

cavitation or as independent entities from. Discs increase the concentration of opsin photopigment in each photoreceptor and continuously shed to be phagocytosed by the RPE. These segments also differ structurally and relatively to metabolic activity. The outer ellipsoid portion is full of mitochondria that produce ATP through the process of oxidative phosphorylation, in contrast to the inner myoid part that contains microtubules proven to be essential for intracellular transport as well as endoplasmic reticulum utilized for synthetic activity. The non-mitochondrial DNA is confined in the photoreceptor nucleus. The synaptic endings of the inner fiber of the photoreceptor enable signal transmission from the photoreceptors to the outer plexiform layer (OPL). The highest concentration of rods occurs in an area defined as the "rod ring" and estimated to be about 4.5 mm from the foveola. The cones are positioned primarily in the center, with some located in the periphery among the rods. Cones are categorized into red cones (63%) of the total cones, outnumbering the green cones (32%) and blue cones (5%) as an overall percent. Cones enhance daytime (photopic) vision and color discernment, exhibit higher threshold of excitability, and maintain a one-to-one synapse ratio with the bipolar cells in the outer plexiform lamina (OPL). Additionally, cones also exhibit specific characteristics that maximize light detection including narrow and long pattern [34–37].

Foveal Cytoarchitecture

The fovea centralis, a depression with 0.35 mm width, is devoid of the plexiform and inner retinal layer and lies within the macula lutea on the temporal side of the optic disc. The macula is a horizontally positioned yellow oval disc in the center of the retina that measures 2 mm in diameter and contains xanthophyll. Central position of the macula immediately inferior to the horizontal meridian is utilized in the study of ocular torsional rotation.

The foveola is approximately 0.33-mm-wide depression in the center of fovea and lies approximately 1 mm below the horizontal meridian and 3 mm from the temporal edge of the optic disc. It is delineated from the fovea by the clivus, an oblique sloping area that produces the foveal annular light reflex when the healthy retinal fovea is illuminated by an ophthalmoscope. This reflex is characterized by a bright circular pinpoint spot of light approximately 1.5 mm in diameter that appears at the center of fovea and develops at 34–36 weeks of development and matures by the 42nd week of gestation. Reduced or absence of this reflex may prove to be significant in the early detection of macular disease [38].

As the long processes of the cones radiate outward of the foveola, they establish the Henle's layer, and subsequent opposing outward shift of the inner nuclear and ganglion cell layers occurs. The fovea is the "rod-free zone" of the retina that harbors only cones and few Müller cells. Despite the proximity, the temporal branches of the central retinal artery encircle the foveola but do not run through it, and thus the central part of the fovea remains avascular and dependent solely on the blood flow from the electrocapillary of the choroid layer. Fluorescein angiography has

been proven to be an optimal imaging method that demarcates the foveal avascular zone [38–41]. Since the foveolar inner fibers lack the inner nuclear layer, they pursue a peripheral course in the macula and project to the outer plexiform layer to establish synaptic contact and initiate depolarization. The latter is associated with the release of glutamate and occurs for the most part in darkness, whereas hyperpolarization is induced by phototransduction. Conveyance and early modulation of the visual retinal input is mediated by the intrasynaptic link of the photoreceptors through connections with the horizontal and bipolar neurons. In contrast, the rods which predominate throughout the remaining retina are stimulated by low and dim light and are the most numerous photoreceptors. The nuclei of the photoreceptors contained in the outer nuclear layer appear to achieve maximum thickness in the foveola [42, 43].

A low-threshold excitability of the rods is conducive for scotopic (achromatic) vision, enabling visualization of black, white, and gray colors. Lack of foveolar rods may explain the physiological central scotoma seen under unusually low light circumstances. The capacity to expand the surface area exposed to light by contracting in low light is an exceptional feature of the rods. Additionally, proximity of the rods to the RPE allows the removal of the sloughed discs from the outer segments of these photoreceptors.

Synaptic Connectivity

Photoreceptors secure synaptic connections in the inner nuclear layer through the dendrites of the horizontal cells. Retinal layers adhere to each other in the outer limiting membrane formed by processes of the large glial (Müller) cells. Despite its name, the external limiting membrane lacks the characteristics of a true membrane as it is formed by the unique synaptic junctional complexes among Müller cells as well as between Müller cells and the photoreceptors. It is separated from the outer blood-retinal barrier by a subretinal space, a potential gap identified earlier between the RPE and sensory retina [44, 45].

Synaptic contacts between the bipolar and horizontal cells in the inner nuclear layer and with the photoreceptors in the outer nuclear layer represent a significant step in enabling signals with circular receptive areas and antagonistic center-surround pattern to be transported from the cones to the bipolar cells within the inner plexiform layer. As discussed earlier the OPL is the site of synaptic connections between the photoreceptors, bipolar, and horizontal cells. As indicated earlier, the processes of the photoreceptor in the central retina convey signals to the OPL from the Henle's fiber layer and then through the rod spherules and cone pedicles of the terminal endings of the photoreceptors to the bipolar and horizontal cells [46, 47].

The inner nuclear layer harbors several types of cells that are unique structurally and functionally: the horizontal, the bipolar, the amacrine, the interplexiform, and the Müller cells. These cells are arranged in a manner that the nuclei of the horizontal

cells lie alongside the exterior of the inner nuclear layer and opposite the OPL; the amacrine cells are positioned opposite the IPL, whereas the bipolar nuclei, interplexiform, and Müller cells assume transitional middle positions [48]. The bipolar, amacrine, and ganglion multipolar cells establish connections in the multilaminar inner plexiform layer that facilitates signal processing from photoreceptor, allowing specific synaptic connections to be established between the bipolar, amacrine, and ganglion multipolar neurons.

Within the multipolar ganglionic layer, nearly one million multipolar neurons as well as "displaced" endothelial and amacrine cells exist as well as astrocytes and pericytes [49]. The presence of nearly ten rows of nuclei, a reduction to a single row outside the macula, and eventual absence from the foveola lead to a dramatic increase in the ganglion cell layer thickness within the perifoveal part of the macula. Most of the ganglion cell layer is formed by the midget and the parasol cells with ON-OFF center-surround, a pattern of receptive field that is also seen in the outer plexiform layer. This arrangement is dependent on the position of their dendritic branches in the ON or OFF zones of the inner plexiform layer.

The axons of the multipolar ganglionic neurons converge toward the optic disc, accompanied by astrocytes and divided into minute fascicles by Müller cell processes. Although the precise pattern of arrangement of the axonal fibers emanating from the temporal vs. the central ganglion multipolar neurons of the retina remains controversial, the axons on the temporal side of the optic disc exhibit the highest density. Those fibers that emanate from the macula lutea proceed directly to the optic disc forming papillomacular bundle, while those that arise from the rest of the temporal retina are around the papillomacular bundle as they proceed toward the optic disc [29, 50–54]. Due to this arrangement, the axons of the temporal ganglion cell outside the macula are most likely to be positioned into dens easily visible superotemporal and inferotemporal bundles within the optic nerve, superior and inferior to the papillomacular bundle fibers. The thickness of the supero- and inferotemporal fibers makes the fibers more visible in ophthalmological examination, using red-free filter light. Like the papillomacular bundle, the axons of the ganglionic neurons of the medial half of the retina assume relatively a straight course toward the medial half of the optic disc. One additional characteristic of the axons of the ganglion multipolar neurons is the fact that they don't run across the horizontal meridian.

The inner limiting membrane of the retina, which is formed by the expansion and flattening of the innermost processes of the Müller cells on the vitreal side, allows the vitreous collagen fibrils to attach to it and eventually places the retina under the impact of vitreoretinal traction forces [55].

The macula lutea, which occupies the posterior pole of the retina, constitutes less than 5% of the entire retina but accounts for virtually the entire central and greatest portion of daylight vision. Histologically this area is divisible into the perifovea, the parafovea, foveal slope, and the foveola. These regions are arc-shaped with a common central point. The perifovea, which is the most exterior region of the macula, lies near the peripheral part of the retina and contains a large network of capillaries. It shows increased cone density with a cone-rod ratio of 1:33–1:30, as well as more

than a single-layer increase in the ganglion cell layer thickness. The parafovea is part of the macula inserted between the fovea and the perifovea. Interestingly, a reduction in the rod/cone ratio and retinal capillaries paralleled with an increase in the concentration of ganglion neurons are observed in the parafovea. The foveola is a plane central depression in the fovea that retains highest visual acuity and is encircled in the clivus. The latter foveal slope marks the shift between vascular and avascular parts of the retina (FAZ), separating a highly concentrated rod zone from that of cone zone [56]. The foveola is formed by contrasting migratory processes: a gradual outward movement of the inner nuclear and ganglion multipolar neurons and a central shift toward the foveola by the red and green cones from the external retina. Subsequent to these migratory processes, the fovea becomes thinner and develops a central foveolar depression which is populated by the cones and Müller cells. However, blue cones, rods, multipolar ganglion neurons, inner retinal cells, capillaries, and some neuroglial cells are not included in the foveolar depression [57]. This process produces a substantial number of cones with a density considered the highest in the adult human retina.

The cone density shows a consistent reduction starting from the foveola and extending to the margins of the fovea and reaching the lowest level on the exterior of the fovea. The rod-free zone in primates begins to undergo reduction in density at the 22nd week of prenatal development and continues until 4 years of age. The cones within the highly dense foveola are elongated to nearly 70 mm and pecked to about 1–1.5 mm. During the peripheral displacement of the foveolar cones, the inner fibers show considerable elongation and maintain contact with the bipolar and horizontal cells. Further, the centrifugal pattern of the processes within the clivus leads to the formation of Henle's layer.

The lack of branches of the central retinal artery in the foveolar avascular zone enhances vision by eliminating any optical barrier from this zone and enables the thermocapillary to assume substantial role in the blood supply of this area. These unique features are augmented by the fact that axons of the multipolar neurons of the temporal retina form follow a circuitous round around the fovea bypassing the center of the foveola. Despite this exquisite foveal organization which fosters highest visual acuity under photopic environment but remains functionally blind in extreme scotopic conditions, visual acuity is dramatically reduced relative to changes in the degree of eccentricity.

With the exception of the foveolar avascular zone (FAZ) and the peripheral retina that receives nourishment by diffusion, the rest of the retina is supplied by both the central retinal artery and the choroidal capillaries. This difference in the blood supply is most likely attributed to the fact that the time required for diffusion to occur multiplies by the square of the length traversed by diffusion. It has been estimated that a molecule of glucose diffuses 10 mm within 50 milliseconds [58]. In view of the above, the capillaries of the choroidal layer provide blood supply to the peripheral retina, whereas the central retina receives blood from the central retinal artery [59, 60]. Due to competition between these diverse blood supply, the oxygen level in the watershed area appears to be the lowest.

Uveal Arterial Circulation

It has been estimated that 80% of the blood supply is provided to the choroid layer as compared to the iris/ciliary body and retina which receives 15% and 5%, respectively [61]. Diffusion from the choroid layer is considered the source of nutrients to the outer avascular part of the retina, including the photoreceptors and the RPE, while the inner part of the retina receives particular vascular support from the capillaries the cilioretinal vessels.

The medial and lateral posterior ciliary branches of the ophthalmic artery, further divide into a single long and several short ciliary arteries, and remain the primary source of blood supply to the choriocapillaries. Despite the fact that the contribution of the long posterior ciliary arteries is minimal when compared to the short ciliary branches that gain access to the eye through the lamina cribrosa sclera. In addition to the choroid layer, the short posterior ciliary arteries also provide blood supply to the optic disc via the vascular circle of Haller and Zinn. The thickness of the choroid layer exhibits variation and consists in succession from the exterior inward of the layer of Haller, the intermediate layer of Sattler, and the choriocapillaries [62–64].

The Bruch's membrane separates the single-layered densely packed capillaries from the retinal pigment epithelium. Rapid diffusion is facilitated by the proximity of the choriocapillaries to the photoreceptors. Studies conducted on monkeys have demonstrated that the blood flow to the choroid layer is considered voluminous when compared to other tissue, with the highest share received by the fovea and the optic disc. There is a mutual interdependence between the choriocapillaries, which supplies both the RPE and the photoreceptor layers, as the formation, integrity, and functional role of the choriocapillaries intimately correlate to an intact RPE.

Development and Anatomy of the Central Retinal Artery

A derivative of the primitive dorsal ophthalmic artery, the hyaloid artery, gains access to the center of the optic disc by traversing the optic fissure. The growth and expansion of the hyaloid artery toward the lens guided by the vascular endothelial growth factor (VEGF) expressed by the lens leads to the formation of the pupillary membrane and the tunica vasculosa lentis. As a result, an anteroposterior multilayered series of structures are formed consisting of the hyaloid artery, the vasa hyaloidea propria, tunica vasculosa lentis, and pupillary membrane. This multilayered involution of the hyaloid circulation is a programmed event that occurs between 3rd and 9th months of gestation. It has been demonstrated that the progressive degeneration of the hyaloid vessels includes both programmed cell death (apoptosis) and necrosis mediated by the Juxtavascular hyalocytes in the vitreous body. When the involution is complete, the canal of Cloquet becomes the sole remnant of the hyaloid vessels. Failure of involution seen nearly in 3% of full-term babies leads to the formation of persistent pupillary membrane, persistent fetal vasculature (PFV), and

persistent hyaloid artery. It also results in the formation of the Mittendorf dot (small opacity on the medial side of the posterior pole of the lens that constitutes the remnants of the lenticular attachment of the hyaloid artery), vitreous cysts, Bergmeister's papilla (small tufts of fibrous tissue in the center of the optic disc), and persistent primary hyperplastic vitreous [65–69].

Despite the transitory nature of the hyaloid artery as it undergoes gradual involution during the second and third trimesters, it remains the early source of nutrients to the developing lens. Additionally, prior to involution, the non-vitreal part of this vessel converts to the central retinal artery that reaches the optic disc via the optic fissure and eventually occupies the center of the optic nerve. A gradual replacement of the primary vitreous (formed by the hyaloid vasculature) by the secondary or mature vitreous will be followed by the tertiary vitreous that consists of the lens zonules and vitreous mass.

On and around 4–5 months of development, the vascularization of the retina begins centrally near the optic disc and expands toward the peripheral parts to be concluded before delivery. Although retinal vascularization is believed to be an angiogenic phenomenon by some, the commonly recognized model of vascularization relies on the development of new endothelial cells by vasculogenesis from the vascular precursor cells, followed by the propagation of the endothelial cells through angiogenesis [70–75]. This process is mediated by the vascular endothelial growth factor (VEGF), a signaling protein which originates from the platelet-derived growth factor family that governs both embryonic vasculogenesis and angiogenesis. The expression of VEGF by the retinal astrocytes and Müller cells appears to be precisely programmed and remains a crucial factor in the development, remodeling, and maintenance of the blood flow in the healthy or diseased retina.

A branch of the internal carotid artery after its exits from the cavernous sinus, the ophthalmic artery, gives rise to the central retinal, anterior and posterior ciliary, ethmoidal, supraorbital, and lacrimal branches. The central retinal artery pierces the dura and arachnoid mater and then the orbital part of the optic nerve just posterior to the posterior pole of the eyeball. As the central retinal artery emerges through the optic disc, it divides into superior and inferior temporal and superior and inferior nasal retinal branches and proceeds toward the peripheral retina through the ganglionic layer. These end arteries divide into arterioles that further divide into horizontal and deep capillaries. The horizontal group provides blood supply to the superficial nerve fiber layer, while the deep group converts to a single perifoveal or four peripapillary horizontal capillary layers based on the thickness of the retina [76–80]. With the exception of the avascular photoreceptors supplied by diffusion, the primary source of blood supply to the retina remains the central retinal artery. The venous blood of the retina returns through the retinal venules to the central retinal vein, which drains to the cavernous sinus via the ophthalmic veins. The connection of the inferior ophthalmic vein to the pterygoid venous plexus allows retinal venous blood to drain into the external jugular vein.

There is evidence that the pattern of arrangement of the retinal vasculature bears important clinical implications. Both retinal vasculature and uveal circulation compete to provide blood supply to the retina. Despite this duality of arterial supply, the

territory of each circulation remains confined, allowing watershed zone to form, rendering the retina more prone to infraction. Occlusion of a branch of the central retinal artery interrupts the blood flow to the retina at the arteriolar and capillary bed levels. Due to the proximity of the branches of the central retinal artery and vein, hypertension may result in compression of the adjacent central retinal vein by the corresponding artery. The thin wall and low hydrostatic pressure of the central retinal vein make it particularly vulnerable to compressive forces generated by the increase in the intraocular, intracranial, or intraorbital pressure. Another characteristic is that the temporal retinal vessels encircle the fovea centralis and the foveolar avascular zone during their course to the periphery of the retina. The notion that the fovea is initially vascularized by the retinal circulation and then regress and lose this circulation has not received much support by the recent studies indicating that avascularity remains valid throughout development [81–84].

Barrier System of the Retina

The retina is protected by a blood-retinal barrier, which is analogous to the blood-brain barrier. This barrier consists of inner and outer parts and acts as a filter allowing only lipophilic and small molecules, such as O2 and CO2, to cross. The inner part controls the intercellular spaces and is established by the occluding junctions between the non-fenestrated retinal endothelium, while the outer part is formed by the occluding junctions between the retinal epithelial cells. Tight control of the rate of retinal blood flow has a vital role in the maintenance of the retinal milieu [85, 86].

The rate of blood flow in the retinal capillaries is regulated at the precapillary arteriolar level by central and local mechanisms. The central factors include the autonomic nerve fibers, adrenal gland secretions and humeral elements, such as angiotensin and vasopressin. While the local mechanisms encompass myogenic reflex, defined either as vasoconstriction in response to heightened transmural hydrostatic pressure and stretch or as a vasodilation in reaction to a decrease in pressure orreaction to metabolic variables of oxygen, carbon dioxide, pH, temperature, and adenosine triphosphate. Although the uveal blood flow lacks autoregulation, the regulatory effect of the sympathetic nervous system maintains robust on this circulation. Unlike the choroidal circulation, the retinal circulation is autoregulated and lacks sympathetic control [24, 87, 88].

The total blood flow to the retina follows Murray principle in that when a vascular trunk divides into branches, the cube of the radius of the vascular trunk is equal to the sum of the cubes of the radii of its branches. According to this principle, a cost function is a total energy cost of the blood in a vessel and the energy cost of propelling it through the vessel. Pathological processes can disrupt this organizational scheme as well as the fractal geometric dimensions that maintain normal retinal blood flow [89–91].

It has also been proposed that the bifurcation angles of the retinal vessel show variations in proportion with the density of the retinal microvascular. Therefore, a

low density of the microvasculature produces a narrower angle as is seen in individual with low birth weights and reduced microvasculature density. In the same manner, the low microvascular density in the peripheral retina produces acute angles of the temporal retinal vessel and consequently a considerable degree of interocular symmetry between the pattern of distribution of the peripheral and that of the central retinal vasculature [92].

Optic Disc and Cup

Due to the retinotopic organization of the axons of the multipolar ganglionic neurons of the retina that sweep within the retina toward the optic disc, a typical elliptical optic disc emerges with a mean vertical diameter of less than 2 mm, which is slightly greater than the mean horizontal diameter. Despite this pattern, a considerable variation in the optic disc size and shape remains [93–95]. There have been various definitions of the optic dis, optic nerve head, and optic papilla. Some authors consider the "optic disc" as the entire optic nerve head others list the anterior-most part of the optic nerve head. The latter appears to combine the ganglionic axon layer and the prelaminar area. Whereas the term "optic papilla" is used as a substitute for both terms with some studies pointing to the papilla as the elevated anterior part [96–98]. The optic nerve head is estimated to be around 1 mm in length and 1.5 mm in width, with the average horizontal diameter as 1.6 mm and the vertical diameter as 1.7 mm. The diameter of the chorioscleral canal at the Bruch's membrane expands posteriorly or centrally but remains proportional with the diameter of the optic disc [99–101]. This becomes apparent in funduscopic examination in which the shape, direction, and size of the chorioscleral canal may determine the size and shape of the optic disc and physiologic cup. Plausible explanation is that when the canal is narrow, a limited space will be present to accommodate the optic nerve fibers, glial tissue, and vasculature without a visible optic cup, whereas in individuals with a wide canal, an additional space will be available for a larger physiologic cup. The way that the retinal ganglionic axons leaves the eyeball may also correlate with the shape of the cup in that oblique course of the axons cause changes in the shape of the optic disc and size of the physiologic cup. The optic disc reaches three quarters of that of the adult size at the end of gestation and attains most of that size by the end of the first year of postnatal life [102].

The axons of the multipolar ganglionic neurons leave the retina and make a 90° angle in the superficial most layer of the optic disc and then join the optic nerve. The glial tissue of the prelaminar part of the optic disc provides the principal support to the optic nerve fibers during their abrupt turn. A microscopic examination of the optic nerve reveals distinct bundles consisting of axons separated either from each other or from an astrocytic process by a narrow intercellular space.

In addition to its role in segregating axonal bundles, the astrocytes provide covering to the vessels that enter the optic disc in this region and form a glial column around the chorioretinal canal [103]. During their course, the axons exhibit

retinotopic organization in which the central axons are positioned in the inner part, while the axons from the ganglionic neurons of the peripheral retina remain exterior to the optic disc rim. Absence of the retinal axons from the center of the optic disc results in the formation of the optic cup.

The floor of the central depression formed by the physiologic cup is separated from the vitreous body by the inner limiting membrane of Elschnig. The central depression may or may not extend to the level of the lamina cribrosa. Failure to establish contact with the lamina cribrosa enables the central connective tissue to send anchoring fibers to the floor of the depression and finally reach the internal limiting membrane of Elschnig. Although the normal cup-disc ratio is 3–4 to 10, considerable variations exist in normal healthy eyes, and the variation in this ratio becomes considerable in pathological conditions. The structural organization of the optic disc reveals, in an anteroposterior direction the nerve fiber, the prelaminar, laminar, and postlaminar regions.

The nerve fiber layer is formed by the most anteriorly positioned ganglionic retinal axons that converge and make a right angle turn before joining the rest of the optic nerve. This layer contains a dense capillary network, and large retinal and venous channels, and is delineated from the vitreous body by the astrocytic inner limiting membrane of Elschnig. Rarely, human optic disc contains the Bergmeister's papilla and embryologic remnant of the hyaloid artery. In the eyes with a narrow physiological cup, the thickened membrane of Elschnig will be known as the central meniscus of Kuhnt [45, 104, 105].

Laminar Organization of the Optic Disc

Examination of the region posterior to the nerve fiber layer reveals, in anteroposterior direction, a glial prelaminar, mixed transitional zone, and laminar connective tissue regions. The transitional zone is located between the prelaminar and the lamina cribrosa regions. A postlaminar region has also been proposed.

Prelaminar Part

The prelaminar part of the retinal axons lies between the lamina cribrosa sclera and the vitreous body and is surrounded by the astrocytes. Electron microscopic studies demonstrate that the prelaminar part is demarcated from the vitreous body by the internal limiting membrane of Elschnig and the central meniscus of Kuhnt (neuroglia in the lining of the optic cup), from the retina by a ring of astrocytes known as the intermediary tissue of Kuhnt, and from the adjacent choroid by the marginal tissue of Jacoby, a continuation of the intermediate tissue [106–113]. The loosely arranged glial framework and the connective tissue fibers in this region may render

the optic disc vulnerable to edema in arteritic and/or nonarteritic anterior ischemic optic neuropathy, optic disc edema, and glaucoma.

The thin glial fibers are arranged in a perpendicular fashion to the optic disc fibers and attach to the choroid and to the elastic membrane as well as to the tissue around the central retinal vessels. Due to large number of glial cells in this region, they are densely packed, flattened, and organized in transverse fashion. The optic nerve fiber bundles are separated by glial tissue partitions that contain capillaries with the perivascular spaces. Lack of uniformity in glial separation of axons in this region is responsible for placing the axons in proximity to each other. The nerve fibers in this part are unmyelinated, show differences in diameter, and are arranged in bundles that are densely packed.

It has also been demonstrated that the prelaminar part consists of the optic nerve fibers arranged in bundles enveloped by a cap-like basket of spider cells, which are specialized astrocytes that maintain close connection at their bases and apices with the lamina cribrosa and with the vitreal surface of the optic disc, respectively. They serve as a source of nutrition and protection to the optic nerve fibers, although a clearly defined anterior boundary of this basket has been debated. An exception is the connective tissue that continues with the capillaries of the optic disc. Studies also show that glial fibers not only envelop the optic nerve bundles but also the individual nerve fibers within. Other studies conducted through electron microscopy have demonstrated that the astrocytes send their long processes into the optic disc fascicles at right angles to the nerve fibers, while others concluded that astrocytic extensions into the optic nerve are not constant and that the presence of such processes is rather rare [114].

Laminar Region

The laminar (scleral) region of the optic disc is associated with lamina cribrosa sclera, consisting of concentrations of collagen fibers of sclera that follow the contour of the eyeball and thus shows convexity posteriorly and concavity anteriorly. A transitional zone separates the anterior prelaminar glial from the posterior connective tissue regions. The connective tissue of the laminar part is fairly extensive, occupies the entire thickness of the optic disc, and stretches across the scleral canal. Peripherally, it is compactly organized and as a result appears as a dense band in longitudinal sections of the optic disc. The foramina that transmit the optic nerve fibers become evident in cross sections of the lamina cribrosa.

The lamellar structure of this layer is attributed to the collagen bundles with alternating glial fibers. The collagen bundles become distinctly obvious on the posterior aspect. Presence of variable amount of elastic fibers within this layer has been demonstrated. Studies using immunofluorescent techniques have shown that the laminar region consists of elastin, laminin, and collagen III and IV and that the elastic fibers are arranged in circuitous manner bundles containing astrocytic processes. This study also showed that aging produces increase in the density of

collagen types I, III, and IV as well as elastin. The connective tissues, containing fine autonomic nerve fibers, accompany and form a cylindrical covering around the central retinal vessels as they pierce the optic nerve and course centrally within this layer toward the optic disc [115]. The course and distribution of the nerve of Tiedemann formed by the autonomic nerve fibers can be seen on the surfaces of the vessels and resemble that of the prelaminar region. These nerve fibers are separated from the sclera by the border tissue of Elschnig, a layer of dense collagenous tissue intermixed with glial and elastic fibers as well as pigment cells, which is considerable on the temporal than on the nasal side. The Elschnig membrane also separates the choroid from the prelaminar region. The presence of astrocytic glial structure in the optic disc accounts for most of its mass. The nerve fibers are separated from the connective tissue in this region by a glial membrane that resembles that of the postlaminar region. The laminar region shows structural variations with regard to the size of the foramina and thickness of the connective tissue extensions. In contrast to the superior and inferior parts, the nasal and temporal parts of the laminar region contain smaller foramina and thicker connective tissue partitions. The difference in the density of the connective tissue and the structural composition of the glial cells is also evident when viewed superoinferiorly versus mediolaterally. It has been demonstrated that the connective tissue and structural elements of glial cells are robust in the nasal-temporal quadrants when compared to the feeble presence of these elements in the inferior and superior quadrants [116–118].

The foramina that transmit the optic nerve fibers show variability in diameter and are lined by astrocytes and crossed by glial fibers that divide them into smaller compartments. Although their shape also varies between oval and round, larger openings are usually partitioned by connective tissue septa. Like the prelaminar region, the glial membrane envelops the nerve fiber bundles and separates them from adjacent connective tissue. Presence of fibrous astrocytes in the center of the foramina and absence of myelin sheath render these openings smaller, oval, or rounded than that seen in the interseptal space of the postlaminar region which appears polygonal [119].

Postlaminar Region

This portion is predominantly a glial structure consisting of the oligodendrocytes and connective tissue. In this portion, the axons of the ganglionic neurons fibers acquire myelin, and the optic nerve receives additional covering from the dura, arachnoid, and pia mater with intervening cerebrospinal fluid.

Following its course through the optic disc, the axons of ganglionic neurons pursue a path in the orbit as the intraorbital, within the optic canal as the intracanalicular, and then in the cranial cavity as the intracranial optic nerve. Following the formation of the optic chiasma by the crossed nasal retinal fibers, the ipsilateral temporal fibers join the crossed contralateral nasal fibers to form the optic tract, which establishes synaptic connection primarily with the neurons of the lateral

geniculate nucleus (LGN), although some fibers that mediate light and accommodation reflexes also terminate in the pretecum, the tectum, and the hypothalamus.

The arterial supply of the optic disc is dependent on dual sources: the arterial circles of Zinn and Haller and the peripapillary arterial supply within the choroid layer derived from the branches of the posterior ciliary artery. The arterial blood contribution of the central retinal artery to the superficial part of the ganglionic axons of the optic nerve and to the posterior optic disc remains substantial. In the same context, the posterior ciliary arterial circulation maintains blood flow to the peripheral retina and to the greatest part of the optic disc. However, several differences exist between the capillaries of the choriocapillaries and that of the optic disc, as the capillaries of the optic disc are non-fenestrated with occluding junctions in contrast to the choriocapillaries which are fenestrated. As a result, the capillaries of the optic disc retain the ability to autoregulate the blood flow, a functional feature which is lacking from the choriocapillaries. Although the blood supply to the optic disc derives from the posterior ciliary circulation, its predominant reliance on retinal circulation is apparent from close functional resemblance of the blood-retinal barrier and autoregulation to that of the retinal circulation and not the choroidal circulation.

The fact that the axons of the retinal ganglion neurons and the central retinal artery converge to course in the optic disc, though in reverse directions, accounts for the devastating clinical consequences of demyelinating patches in the optic nerve or small vascular lesions at the optic disc. Also, the localization of a lesion can be determined by the specific visual field defects that emerge when a lesion disrupts the multipolar neuronal axons along the path between the retina and lateral geniculate nucleus and by the manner of neuronal loss and morphologic changes of the optic disc [120–126].

Myelination of the Ganglionic Axons

The retinal ganglionic axons acquire myelin after they leave the eyeball enabling faster and energy effective conduction. Through this process, multipolar ganglionic neurons limit the ion channels to the nodes of Ranvier, initiating saltatory conduction in the internodal segment and production of all-or-none action potentials without depolarizing the axon along its course. Myelination is a gradual process in the optic tract and the intracranial part of the optic nerve that completes by birth or extends beyond to postnatal life as is the case of the orbital part of the optic nerve [127]. The mechanism that underlies myelination is poorly understood, and more research is needed to be fully identified unlike that of the physiologic and pathologic axonal growth, which appears to be well settled [128–131]. It has been proposed that migration of the CNS myelin-forming oligodendrocytes and the oligodendrocyte progenitor O-2A cells into the retina is hindered by the optic disc, accounting for the absence of myelin sheath around the axons of the multipolar ganglionic neurons prior to their exit from the sclera.

Due to the initial development of large number of ganglionic neurons, death of excess of these neurons is predictable and appears to be necessary. Ganglionic neuronal death usually occurs between 4 and 7 months of fetal life, and cell death ranges between 4.5 and 4.8 million of the human fetal ganglionic cells [132]. The death of excess of ganglionic cells in the prenatal stage of development follows an initial rapid process followed by gradual and steady reduction in the amount of 5000 axons per year. This balance between prenatal production of large number of retinal ganglion neurons and eventual death of the great majority of them possibly form the basis of the selection of axons that can successfully establish synaptic connections with the specific neurons in the central nervous system.

Metabolic Activity of the RPE and the Photoreceptors

In the human retina, a single RPE cell establishes contact with an average of 45 photoreceptors. Although RPE does not directly participate in the processing or transmission of visual impulses, its nutritive and mechanical supportive roles are crucial for the survival and optimum functions of the photoreceptors. Accordingly, degeneration of RPE, which possesses a high metabolic activity, can cause the death of the photoreceptors and irreversibly impair the blood flow through the choriocapillaries. As part of this role, the RPE provides the optimum functional environment in the subretinal space, clears the debris, and eliminates the shed outer segments of the photoreceptors involved in retinoid metabolism and the visual processing.

Outer segment shedding of the photoreceptors is a regularly occurring phenomenon in which the lipid membranes of the damaged disc of the photoreceptors shed under the effect of the free radicals produced by light irradiation in an oxygen-saturated milieu. This phenomenon becomes a necessity considering that the photoreceptors don't divide and thus become vulnerable to damage by peroxidation. The outer segment shedding is not consistent but a process that exhibits fluctuations and speed variation. This is attributed to the fact that rods undergo more rapid conversion than that of the cones. Experimental studies in primates also revealed variation in the time required to replace a shed segment in the peripheral as compared to the central retina. According to this study, this replacement may take longer in the parafoveal retina, when compared to the peripheral retina.

This massive connection enables RPE microvilli to eliminate the distal part of the outer segments by phagocytosis. The remaining parts of the outer segment are eliminated from Bruch's membrane by the choriocapillaries. The connection of the RPE and the vital role of the choriocapillaries enable the rapid removal of large amounts of outer segment lipid membranes in a short period of time. Additionally, the RPE maintains the regeneration of the chromophores (oxy-hemoglobin, deoxy-hemoglobin, and melanin) in the photoreceptors, which are crucial elements in the production and recycling of vitamin A and other retinoids involved in visual processing [133].

Retinol (vitamin A_1), a hydrocarbon molecule, belongs to isoprenoid family that also includes vitamins E and K and can only be obtained by dietary supplement as its in vivo synthesis is not feasible. Retinol also exists as isomers in the form of 11-*cis*-retinal and 13- *cis*-retinoic. Retinal is transported hematogenously from the liver, where it is stored, to the RPE via retinal-binding protein. In turn, RPE delivers the absorbed retinal to the photoreceptors and covalently links to a transmembranous protein, opsin, in the discs of the outer segments of the photoreceptors. It can be converted to retinal, an aldehyde group, or to a retinoic acid, a carboxylic acid group through oxidation. Retinal is transformed to all-trans-retinol and then transported to the RPE to be isomerized and eventually reoxidized to retinal to be finally returned to the photoreceptors to recombine with opsin. The transport of retinol from the RPE and retinal from the RPE to the photoreceptors is maintained by the interphotoreceptor retinoid-binding protein (IRBP). Further, the intracellular retinol and retinal are protected by both the cellular retinol-binding protein (CRBP) and cellular retinaldehyde-binding protein [15].

Modulatory and Protective Role of the RPE

In the presence of the occluding junctional complexes between the RPE cells and their location in proximity to the exterior of the retinal sensory neurons, the RPE forms the outer blood-retinal barrier that selectively allows movement of ions and other substances between the choriocapillary layer and the RPE [16]. In addition to the barrier function, the RPE is also actively involved in the transport of ions and molecules which enables balancing of the extracellular milieu in outer retina [19]. The RPE also exhibits secretory, immunomodulatory, and protective functions. The RPE secrets growth factors, such as platelet-derived growth and ciliary neurotrophic factors, as well as immunomodulatory chemicals and cytokines. The latter plays an important role in maintaining an immune-privileged ocular site by preventing inflammatory reactions when exposed to antigens. Thus, the antiinflammatory function of the RPE is derived from the antioxidants they containin, such as glutathione, catalase, ascorbate, superoxide dismutase, and melanin, among many others [10]. Melanin contained in the RPE acts as a barrier by absorbing excess of light and blocking the light that pierces sclera from reaching the retina. Further, maintaining a dehydrated subretinal environment, an essential factor in keeping the RPE in apposition to the photoreceptors, is dependent upon the sliding or pump action of the RPE.

The presence of a weak bonding force of the interphotoreceptor matrix (IPM) between the RPE and the outer segments of the photoreceptors renders the pumping feature of RPE crucial in this process. It should be noted that ischemic conditions can rapidly disrupt the retinal adhesive force, an energy-dependent mechanism, across the subretinal space.

Optic and Non-optic Retina

The cellular organization and the retinal neuronal connections of the retina is delicately designed to facilitate the initiation, propagation, organization, and processing of visual input within various ranges of illumination. A complex array of synaptic connectivity occurs at the retina and the visual cortex in order to construct binocular vision. Through these series of intricate neural processes and sophisticated integrative mechanism of various inputs from the brainstem, cerebellum, diencephalon, and cerebral cortex, highly refined visual images can be produced [134–136]. In order to recognize images in the visual field with different physical characteristics including color, depth, and temporal resolution as well as motion, the retina is equipped with functionally and structurally unique light-detection components. These components reside in neural layers of the retina and include the photoreceptors, the interneurons, and plexiform networks [137–144].

The nonvisual component utilizes the retinal neurons, optic nerve, and optic tract as well as the reticular formation to mediate pupillary light and accommodation reflexes, pineal gland secretion, and circadian rhythm [145–150]. The latter uses the retinal input to the suprachiasmatic nucleus of the hypothalamus and also the reticular formation. A subset of photosensitive melanopsin-expressing retinal ganglion cells (pRGCs) has been identified that are capable at modulating behavioral and physiologic responses to light and enabling these cells to transmit image-based input from the retina directly to the cerebral cortex without following the usual pathway of the photoreceptors [146, 151–156].

Physiologic Properties of the Photoreceptors

The photoreceptors initiate the process of phototransduction by facilitating the detection of photons by the neuroretina and production of a nerve signal [157–160]. The structural organization and the chemical composition of the photoreceptors enhance this process as such they contain visual pigments with two distinctive compositions: opsin and a chromophore, also known as 11-cis-retinal.

The retinal-opsin complex is formed by covalent bonding of retinal to opsin. All photopigments have the same basic retinal molecule; however, opsin exhibits diverse spectra and absorption peaks due to the presence of retinal-opsin complexes in relationship to rods and cones. There is only one rod photopigment (rhodopsin) ranging between blue and green and three cone photopigments. The latter is categorized into “blue” or S cones sensitive to short wavelength, “green” or cones sensitive to medium wavelength, and “red” or L cones sensitive to long wavelength. Based on this, the cones can be green, red, or blue, while the rods are one type and remain unaffected by the wave lengths. A developmental correlation to this is the fact that S opsins are expressed earlier than L/M opsins [161, 162]. The tendency of some cones to change their expression from S opsin to L/M opsin is possibly related to the

spread of L/M opsin expression in an increasing wave front to the temporal retina, which expresses S opsin. Further, during the fetal and neonatal period, small percentage of cones that advance in L/M opsin wave front expresses both S and L/M opsins before dramatic reduction diminishing in the adult retina [163].

Rods are adapted for scotopic vision, remain light sensitive, and able to discern a single light photon. They are achromatic and associated with gray color vision that has low resolution. In contrast to the cones which are relatively light insensitive, it requires more photons to produce an electrical signal and can only be activated by increasing illumination. Due to these characteristics, activation of the cones leads to incorporation of temporal and spatial resolution as well as color to the images. Presence of functionally and structurally different photoreceptors in the retina (duplex retina) bestows variable sensitivity and enhanced scope that enable night and daylight vision as well as color discrimination.

Light absorption and G protein activation are two principal functions of opsin, which mediates conversion of a photon into an electrical signal, an initial step in transduction cascade. Opsin precursor in the endoplasmic reticulum of the inner segment is transformed to opsin in the Golgi apparatus and then through vesicular transport that reaches and combines with the photoreceptor membrane. Following this merger, a covering is formed for the outer segment disc membrane, followed by movement of the opsin from the inner to the outer segment of photoreceptors, enabling these sensors to detect incoming photons. The surface area for photopigments is increased by the presence of compact array of discs in the outer segments of photoreceptors. A research study demonstrated that a disc in a rod photoreceptor with a limited number of discs may be exposed to one thousands of photons in each second in sunlight illumination but contains millions of rhodopsin molecules.

Detection of light signal in the retina through phototransduction involves a chain of intracellular reactions that transduce the absorbed single photon into a mass response from the entire photoreceptor. The molecular basis of this process may pave the way for understanding of the molecular basis of certain retinal disorders [164–166]. A connection between the site of photons on the exterior of the disc membrane and the site of generation of the membrane potential in the cation channels on the outer segment is established via activation of cGMP (3′-5′-cyclic guanosine monophosphate), an intracellular second-messenger system that conveys signal across the cytosol.

Energy transferred from the photon to the photopigment during light absorption causes a conformational change in the retinal molecule leading to the isomerization and formation of all-trans-retinal. A similar alteration in the structural organization of the opsin molecule results in the stimulation of opsin. The latter bonds to transducin and activates cGMP phosphodiesterase (PDE), catalyzing conversion of transducin into 5′-guanosine monophosphate. This cascade of events leads to the reduction in the level of cGMP and blocking of the cGMP-gated cation channels and the resultant hyperpolarization.

cGMP regulates the influx of sodium and calcium through cGMP-gated cation channels by enhancing a movement of sodium toward the inside of the cell and the calcium out of the cell. As the inward current of the sodium occurs, causing cellular

depolarization, a concomitant wave of outward potassium movement by the potassium-selective channels in the inner segment is seen, which causes cellular hyperpolarization. The active sodium-potassium pumps in the inner segment enable potassium to be driven into the photoreceptor and sodium to be transmitted out of the cell. A sodium-potassium and calcium exchanger in the outer segment enhances sodium inward current into the cell in exchange for potassium and calcium outward movement.

Since the second-messenger cGMP regulatory function is restricted to the cGMP-gated cation channels, no noticeable effect of this messenger is detected on any other potassium-selective openings, the active sodium-potassium pump, or the sodium-potassium and calcium exchanger system. Due to high concentration of the cGMP in darkness, the cGMP-gated cation channels remain open, and the influx of sodium (dark current) and the subsequent depolarization of the photoreceptor occur. In light, the activation of cGMP, a subunit of transducin complex, activates phosphodiesterase (PDE), which hydrolyzes the intracellular cGMP leading to the blocking of the cGMP-gated cation channels and the resultant hyperpolarization. Thus, the response of photoreceptors to light stimulus is not through depolarization or an all-or-none action potential as is seen in neurons but rather a gradation response through hyperpolarization, which blocks the release of glutamate in contrast to the depolarization that facilitates the release of this neurotransmitter.

Stimulation of a rhodopsin can cause the elimination of nearly 2% of all non-binding cGMP, resulting in a perceptible 2 mV photoreceptor hyperpolarization. This enormous biochemical intensification is attributed to the phototransduction cascade which allows a rhodopsin molecule to stimulate a large number of protein molecules within 50 milliseconds. Each in turn is thought to cause degradation of a number cGMP molecules by the stimulation one PDE. Not only activation of this amplification mechanism is crucial for hyperpolarization, but the ability to replicate and sustain the process of phototransduction is equally important. To that end a rapid inactivation of the photopigment must occur through enzymatic phosphorylation, coupling to arrestin, inhibition of transducin and PDE, and production of the cGMP by guanylate cyclase [167].

Retinal Pathways and Synaptic Integration

The visual information processed by the photoreceptors become part of the neurocircuitry of the pathways that assume vertical and horizontal direction within the retina to be conveyed to the ganglion multipolar neurons. Retinal horizontal integration pathway principally occurs within the OPL, where the photoreceptor cells establish direct synaptic connections with the bipolar cells or indirectly through horizontal cells, contributing to contrast enhancement. Bipolar cells enable transmission of the processed signals from the photoreceptors and horizontal cells to the internal plexiform layer (IPL) through several vertical pathways. Some of the pathways may involve rod bipolar and blue cone bipolar cells which are synaptically

connected to rods and blue cones, respectively. It has been shown that red and green cones establish connections with midget bipolar and diffuse bipolar cells. Each midget bipolar cell has short axonal and dendritic branches, possesses a single on/off center, connected to a single cone, and scattered in the central retina. Each diffuse bipolar cell synapses with multiple cones has massive dendritic and axonal branching with evenly distributed on/off centers.

The receptive fields of the bipolar cells exhibit antagonistic center-surround pattern. When the cones are stimulated by the bipolar cells, an ON and OFF parallel pathways are activated, conveying cone signals to the corresponding ON/OFF cone bipolar and ON/OFF ganglion cells. ON bipolar cells' depolarization and OFF-cells' hyperpolarization are based on the premise that OFF bipolar cells have sign-conserving synaptic connections in which the OFF bipolar cells' hyperpolarization and depolarization is dependent upon similar corresponding activities in the photoreceptor. In contrast, ON bipolar cells have sign-inverting connection by which they respond to light-induced photoreceptor hyperpolarization with depolarization and to darkness-induced depolarization with hyperpolarization. These responses correlate with the type of glutamate receptors involved as these receptors couple to and open the ionotropic channel in the OFF bipolar cells causing depolarization. Glutamate receptors function via a G protein in the ON bipolar cells, blocking the ion channels and the resultant hyperpolarization [46]. Due to the synaptic connection of the cone bipolar cells to the amacrine and ganglion cells, parallel transmission of signals from the same photoreceptors can produce diverse retinal plans in the sublaminae of the IPL.

Amacrine cells exhibit morphologic characteristics that show variations in the dendritic field ranges and in regard to their distribution within the sublaminae of the IPL into unistriate, bistriate, or multistriate amacrine cells. This striatal (sublaminar) localization enables synaptic interactions within and between the six stratae of the IPL. Amacrine cells also differ relative to the neurotransmitters as dopaminergic, cholinergic, glycinergic, GABAergic, or peptidergic. Functionally, amacrine cells are categorized into AII amacrine and starburst amacrine cells. The AII amacrine cell, the most common type, performs a substantial function in the rod pathway that connects the rod bipolar cell with the antagonistic ON- and OFF-cone bipolar pathways. It has been reported that starburst amacrine cells may be involved in the directional selectivity/optokinetic eye movements [33].

Eventually all visual input converges on last order of neurons in the retina, the multipolar ganglion cells. These cells vary in regard to the sites of synapse in the IPL with the bipolar and amacrine cells as well as relative to the dendritic branching. Midget and parasol cells are the most common type of ganglion cells, with the midget cells being smaller, outnumbering the parasol cells. The latter group of cells have limited dendritic branching, making synaptic contact feasible with a midget bipolar cell. Most ganglion multipolar neurons establish parallel ON and OFF pathways, exhibiting a receptive field which is concentric with ON and OFF center-surround. The latter organization is significant in enhancing contrast. The cone pathway, which consists of the three layers of neurons, the cone, bipolar, and

multipolar ganglion neurons, diverge into distinct ON- and OFF pathways in the OPL [168, 169].

The rod scotopic pathway transmits visual information to the rod bipolar cell that establishes a link with an AII amacrine cell in the inner nuclear layer, which in turn establishes a link with the ON/OFF-cone bipolar cells, becoming part of the rod and cone pathways. This indicates that the rod bipolar cell does not establishes direct contact with the ganglion cell but uses the AII amacrine cell to accomplish this connection and that the classical rod pathway divides following union with the cone ON and OFF pathways in the IPL. The massive convergence of rods on an amacrine AII cell accounts for the role of classical rod pathway in scotopic vision. The presence of rod pathways that mediate rod function under mesopic vision (night-time outdoor lightening) involving direct rod-cone link or rod/cone bipolar connections has been proposed.

Pupillary Light Reflex and Circadian Rhythm

The visual system not only mediates photopic and scotopic vision but also detects and regulates environmental light levels as in the pupillary light reflex and circadian rhythm. Research reports indicate that circadian synchronization relies on the ocular photoreception by the rods and cones as bilateral enucleation abolishes it [170–172]. However, the presence and the role of the nonrod and noncone photoreceptors in the inner retina that mediate extra-image visual system have also been discussed [173–175].

A distinct group of intrinsically photosensitive retinal ganglion cells has been recognized as pRGCs or photosensitive retinal ganglion cells that react to directed light in the absence of any intervention by other retinal cells. The pRGCs depolarize slowly in response to light, and their maximal sensitivity to light stimulation remains limited, while the photoreceptors hyperpolarize rapidly and exhibit gradated response to the same light stimuli. Unlike the photoreceptors that convey processed visual impulses to relay neurons that eventually reach the brain, the pRGCs' dendritic links remain limited to the IPL. Studies conducted on individuals who lacked photoreceptors have revealed the presence of short-wavelength pRGCs [176, 177].

Melanopsin, the presumed photopigment of the pRGCs, is responsible for the intrinsic photosensitivity, and deletion of the melanopsin gene can abolish this characteristic feature. The ability of melanopsin to function as a photopigment and as an isomerase with self-regenerative capacity has been proposed. This feature proves to be particularly helpful for the inner retinal photoreceptor as RPE in the outer retina possesses the regenerative capacity of the photopigment.

Pupillary light reflex and circadian synchronization, as nonimage-forming mechanisms, can be abolished by the loss of function of melanopsin, rods, and cones; in contrast, expression of melanopsin bestows the ability to react toward photons to cells that are insensitive to incoming photons. It has been estimated that the number of melanopsin-expressing RGCs in the retina forms approximately one fifth to four

fifth of the total number of ganglion neurons. Although their number shows reduction starting in the parafoveal area and extending toward the peripheral retina, their dendrites maintain extensive branching than any ganglion neurons. Additionally, their function is modified by both rods and cones in the retina, a synaptic interaction that identifies the melanopsin-mediated photoreception in the retina.

Conclusions

A conceptual view of the organization and physiology of the retina and posterior segment of the eye is paramount for identifying the disease processes that affect the intraocular structures. The cytoarchitecture of the retinal pigment epithelium allows the barrier system to protect the retina but at the same time opens the horizon for further research to determine the utilization of this system in the administration of medications. Similarly, the intricate synaptic connectivity between various layers of the retina may provide valuable information about visual and nonvisual functions of the retina. In this chapter we have attempted to provide a systematic approach to the retina and examine the histologic makeup, circuitry, and metabolic activities of the retinal pigment and the photoreceptors providing foundation for clinical relevance.

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Chapter 2
Pharmacotherapy of Diabetic Macular Edema and Retinopathy

Ashley Oake, Charles Preuss, and Yashwant V. Pathak

Abstract Diabetes mellitus affects over 20 million people in the United States and that number is growing every year. It is caused by a lack of insulin production which leads to an excess amount of glucose to build up in the bloodstream. Type 2 diabetes mellitus is the most common affecting over 90% of the people with the condition. It occurs when there is a resistance to insulin but can be managed with a healthy lifestyle. Type 1 diabetes mellitus usually is diagnosed in younger adults and occurs when insulin is not produced at all in the body. When these conditions are not properly managed, the patient can develop diabetic retinopathy. Glucose builds up in the bloodstream and causes blood vessels in the eye to swell and produce microaneurysms. Growth factors are released to cause proliferation of new blood vessels in the eye. Fluid starts to leak from these blood vessels into the retinal cavity to cause diabetic macular edema. This disease is the number one cause of blindness in patients diagnosed with diabetes. Proper treatment of these diseases includes management of glucose levels, laser photocoagulation, vitrectomy, anti-VEGF injections, and corticosteroids.

Introduction

Diabetes mellitus is a chronic condition resulting from high blood glucose levels due to complications with production of insulin, also known as hyperglycemia [1]. Insulin is a hormone that is necessary for the conversion of glucose into energy by transferring the sugar into cells [1]. Problems can arise not only with the production of insulin but also with the action it takes [1]. Abnormalities occur when there is an insulin resistance in the cell [1]. These problems together result in hyperglycemia

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and if not treated properly may cause damage to multiple organs [1]. This disease restricts the production of energy in the cells which can cause fatigue throughout the body, followed by weight loss and a weak immune system [1]. As this progress, patients may experience frequent urination and extreme thirst [1].

Diabetes mellitus has affected over 23 million people in the United States alone, which is about 7.2% of the population [6]. It is primarily in adults with only 0.18% of those diagnosed being under the age of 18 [6]. The disease is more prevalent in the American Indian population compared to other races with 15.3% of women and 14.9% of men having the disease [6]. Pertaining to location, the southeast corner of the country has the highest percentage of diabetes [6]. Areas around Montana and Idaho have an average obesity rate of 7–8%, while states like Mississippi and Alabama have an obesity rate of over 12% [6].

Diagnosis of the condition can be determined by the fasting plasma glucose, the 2-h plasma glucose, or the A1C tests [2]. They are all blood tests that will help determine the level of glucose in the blood [2]. The standards of diabetes for these tests are ≥48 mmol/mol (6.5%) glucose in the blood with the A1C test, a level above 126 mg/dL after fasting for 8 h with the fasting plasma glucose (FPG) test, or a level above 200 mg/dL for 2 h when mixed with 75 grams of anhydrous glucose in water for the 2-h plasma glucose test [2]. Race can have an influence with the A1C levels of certain individuals and may even cause them to be much higher, especially with the African American population [2]. Patients with high glucose levels that have not developed diabetes just yet are referred to as impaired fasting glucose and are considered high risk [2]. Changes in lifestyle, including weight loss, may help or even prevent the disease [2]. More than one-fourth of the population in the United States is undiagnosed because most type 2 cases are not discovered until there are serious complications [2]. Testing should start occurring with any individual with a BMI that is considered overweight or obese (>25 kg/m^2). Individuals with type 1 diabetes are not connected to obesity but rather may be seen with a slimmer figure [2].

Type 1 diabetes is believed to result from the immune system causing the destruction of β-cells in the pancreas [2]. This can cause decreased secretion of insulin with abnormally high concentrations of glucose in the blood [2]. Because it is thought to be an autoimmune disease, there is no cure or possible way to prevent this, but it can be managed with insulin [5]. Almost 98% of the patients are shown to have pancreatic antibodies [5]. This type of diabetes only is present in 5–10% of individuals with the disease and usually appears at a very young age [1]. The rate of cell destruction in children is much more rapid than in adults, so children tend to develop ketoacidosis [1]. Ketoacidosis occurs when there are low insulin levels and glucose cannot be transferred into the cells [1]. Instead they must burn fat, which produces high levels of ketones in the blood [1]. Type 1 diabetes is an autoimmune disease, so other complications may arise like celiac or thyroid disease [1]. Celiac disease is another immune disease which occurs in 1–16% of the diagnosed individuals, and one-fourth have issues with thyroid disease [1].

For effective treatment of type 1 diabetes, proper monitoring of blood glucose levels and insulin administration is crucial [3]. Self-monitoring of blood glucose (SMBG) helps individuals track their glucose levels and determine if their treatment

is properly working [1]. With this opportunity, patients can help prevent complications like hypoglycemia [1]. Glucose levels should be monitored multiple times every day [3]. This can be paired with continuous glucose monitoring (CGM) which will also give alarms when levels are too high or low [1]. β-cell replacement therapy and pancreas transplants have been done experimentally to possibly improve the condition and prevent further issues with the disease [2].

Type 2 diabetes is the most common form and accounts for 90–95% of the population diagnosed [1]. Patients with this condition do not have a complete insulin deficiency but rather have an insulin resistance [1]. There is no evidence of early destruction of β-cells, so there is no need for insulin treatment upon diagnosis [1]. Unlike type 1, it is usually diagnosed in adulthood and has a strong correlation with obesity and lack of physical activity [7]. 87.5% of individuals diagnosed were overweight, and 43.5% were shown to be obese [7]. As this condition progresses, patients may develop a hyperosmolar hyperglycemic state where people with high blood sugar have issues with high osmotic concentration [4]. Symptoms for this condition are very similar to those of hyperglycemia, which include extreme thirst and weakness [1]. Diabetic ketoacidosis is less likely to occur with this condition, but it has a very similar treatment that includes fluid replacement therapy [1].

Specific treatments and weight loss can help reduce the effects of this disease and lessen risks for further complications [1]. There are possible ways to reverse the disease completely by the reduction of excess fat [1]. Seventy-three percent of patients reduced their blood glucose levels back to normal while undergoing bariatric surgery, which is reducing the size of the stomach with a band to influence weight loss [1]. Reducing the average daily caloric intake may also normalize glucose levels and reverse the metabolic issues [1]. In a study done involving 11 people diagnosed with type 2 diabetes, individuals were put on a 600-calorie diet [7]. Within a week, internal fat in the liver decreased by 30%, and glucose levels returned to normal [7]. During pregnancy, there are occasionally some signs of issues with high glucose plasma concentrations, which is referred to as gestational diabetes mellitus (GDM) [2]. After birth, conditions may return to normal, but it would still be referred to as GDM [2]. This complication occurs in 7% of all pregnancies and may lead to the mother of the child developing type 2 diabetes [2]. Risk is higher in women who are older, are overweight or suffer from obesity, or have a history of diabetes [2].

Diabetic Retinopathy

When diabetes is not properly managed, certain organs and tissues in the body start to fail or deteriorate [10]. The retina is the tissue in the posterior portion of the eye that converts light rays into impulses that travel to the brain through the optic chiasma [8]. The visual cortex of the occipital lobe converts these impulses into images [8]. This tissue is very light sensitive, and without proper treatment, the high blood sugar can damage the blood vessels in the eyes and cause diabetic retinopathy [8].

These blood vessels may leak fluid into the eye and cause serious vision issues [8]. The retina is surrounded by two other layers which are the sclera, the outer layer of the eye, and the choroid, the middle of the eye that contains most of the blood flow [8]. Located in the retina is the optic nerve which is the connection between the visual cortex and the eye and also contains the retinal vein and artery [8]. The macula is the center of the retina that gives people the ability of sight, and in the center of the macula is the fovea, which contains the photoreceptor layer [36]. The photoreceptor layer is made up of two layers of rods and cones referred to as the ellipsoid zone and external limiting membrane layer [36]. Visual acuity is strongly associated with the ellipsoid zone and its individual strength [36]. Above this zone is the external limiting membrane layer, which encompasses the mechanisms of the Müller cells and separates the photoreceptor nucleus from other compartments of the cells [36].

High blood glucose, which is referred to as hyperglycemia, can cause pathological changes in the bloodstream including inflammation and oxidative stress, which can affect the defense system and cause tissue damage [8]. It is hypothesized to be the number one cause of diabetic retinopathy and macular edema in patients [9]. With such high glucose plasma concentrations, the aldose reductase pathway overworks by converting glucose into sorbitol, which leads to an overabundance of it in the body [8]. Sorbitol in high plasma concentrations can lead to many gastrointestinal issues [8]. These complications trigger the body to activate platelets and repair blood vessels by releasing vascular endothelial growth factors (VEGF) and platelet-derived growth factors [9]. Basement membranes, which hold the tissues to the epithelium, tend to thicken and may cause problems with oxygen transport [8]. Blood cells have a more difficult time flowing through the vessels which therefore start to form plaques and decrease the expression of pericytes [8]. Pericytes contribute to the production of cells in the blood vessel walls, which control repairs [8]. A reduction in these cells can cause problems with blood flow and the narrowing of capillaries [8]. High levels of the neurotransmitter glutamate are shown to have a strong correlation with diabetic retinopathy [36]. Müller cells can regulate this process, but with the destruction of these cells, excess glutamate is produced [36]. With too much glutamate in the retina, the sodium-calcium exchanger is activated, and calcium is removed from the cell until it cannot function properly [36]. This results in cell toxicity and advancement of the disease [36].

A protein also involved in breaking down this barrier through oxidative phosphorylation reactions is kinase C [36]. It is involved in the growth and transcription in cells, causing an overabundance of new blood vessels that may form in the retinal cavity [36]. Excess oxygen is converted into diacylglycerol which activates this protein and other influences including VEGF and TGFB1 [36]. This process eventually leads to excess fluid buildup and inflammation. Reactive oxidative species (ROS) are associated with oxidative stress and the progression of diabetic macular edema [36]. They cause the destruction of pericytes and thickening of the basement membrane [36].

This disease is the leading cause of blindness for individuals diagnosed with diabetes mellitus [8]. Out of the 37 million cases of blindness in the world, 4.8% of

them are a result of diabetic retinopathy, which is about 1.8 million cases [8]. It is common in patients with type 1 and 2 diabetes mellitus but may appear at different stages of the disease [8]. With type 1 diabetes, it is very uncommon within the first 5 years of diagnosis, but 95% of patients show signs of this disease after 15 years of being diagnosed with diabetes [8]. Age is an important factor as well, so signs may show in the initial diagnosis of type 2 diabetes due to the fact that most diagnoses happen at a later age [8]. A study was done in North Hampshire with 464 patients diagnosed with type 1 diabetes that tested the correlation with diabetic retinopathy [35]. 71.5% of the patients tested showed signs of diabetic retinopathy and 6.5% of proliferative diabetic retinopathy (PDR) [35]. These diseases were prevalent in patients after 10–20 years of diagnosis [35].

This disease advances through four main stages: mild nonproliferate retinopathy, moderate nonproliferate retinopathy, severe nonproliferate retinopathy, and proliferate diabetic retinopathy [9]. Mild nonproliferate retinopathy is the earliest stage of this disease that includes minor hemorrhages and microaneurysms, which is minor swelling in the blood vessels [9]. These hemorrhages include intraretinal (small dots that are found in the interior retina), blot (larger hemorrhages), and flame-shaped bleeding (externally found on the nerve fiber) [8]. A hard-yellow plaque-like substance can be found called lipoprotein exudation [8]. In a study involving 300 and 74 patients with mild nonproliferate retinopathy, 68.1% of patients had formation of microaneurysms after 12 months [10]. 28.9% of the patients had signs of subclinical macular edema following this observation [10].

Moderate nonproliferate retinopathy occurs when there is a higher degree of hemorrhaging than in the preceding stage [8]. Transport of blood through the vessels becomes difficult due to swelling [8]. Capillary occlusive disease, also known as pulmonary veno-occlusive disease (PVOD), may start to appear in this stage with flat blood vessels and cotton-wool spots, which also could cause pulmonary hypertension in severe cases [11]. Diabetic macular edema intensifies, and some vision loss is present in certain conditions [8]. When these conditions worsen even further, it leads to severe nonproliferate diabetic retinopathy (NPDR). NPDR can lead to complications with other tissues in the eyes [8]. Fluid may build up in the anterior chamber and damage the optic nerve, referred to as glaucoma [12]. This may lead to cataracts, which is a cloudiness in the lens on the eye, and may be chronic, leading to permanent blindness [12]. Transport of essential cell parts, including mitochondria and lipids, through neurons will be compromised [12]. Severe nonproliferate retinopathy can be diagnosed with the 4-2-1 rule [16]. The 4 stands for determining 20 or more hemorrhages in the 4 quadrants, and the 2 is for severe bleeding in at least 2 of the quadrants [16]. The final determination is the presence of at least one intraretinal microvascular abnormality [16].

The advanced stage of this disease is referred to as proliferative diabetic retinopathy (PDR), as it involves the growth of new blood vessels from the multiple growth factors released in the initial stages [23]. This can disrupt the vitreous, which is the fluid that gives the eye its shape [23]. The anti-angiogenic agents that naturally prevent the growth of blood vessels are inhibited and held in the fluid, which causes neovascularization [23]. These new blood vessels can be found on the sur-

face of the retina or on the optic disc [23]. With the new blood vessels, formation of scar tissue and possible leaking follows, which could cause irreversible blindness [23]. This scar tissue could cause problems with connective tissue, and the retina will peel away from the underlying layers and lead to retinal detachment [23]. If new blood vessels start to form in the iris, it can lead to glaucoma [23].

After 15 years of being diagnosed with type 1 diabetes mellitus, 25% of patients advance to the final stage of this disease PDR, and after 20 years, 56% of patients develop this [23]. It is much less common in patients with type 2 diabetes mellitus [23]. Only 4% of patients show signs of the final stage after 15 years if they are not on insulin treatments [23]. Complications with this disease include narrowing of peripheral vision, macular edema, choroidal effusion, and decreased night vision [23]. Choroidal effusion is a result of excess fluid in the suprachoroidal space, which is the space between the choroid and the sclera [23].

Characteristics of Diagnosis

Diabetic retinopathy may be difficult to detect at first due to the lack of initial symptoms [8]. This disease is detected by multiple exams while the eyes are dilated, which include visual acuity testing (measuring sight at different distances), tonometry (measure of eye pressure), slit-lamp biomicroscopy, and OCT [8]. Optical coherence tomography (OCT) uses light waves to visualize the layers of the retina for any irregularities with the buildup of fluid [26]. This noninvasive technique measures the internal structures through low-coherence reflectometry and can observe structures only micrometers small [26]. It can differentiate between the optic nerve, macula, and fovea to target the complications occurring throughout the disease [26]. The benefits of using this method is that it is highly precise in its action and helps compare imaging throughout time to help determine progression [26].

Ophthalmologists may use more hands on approach referred to as the slit-lamp biomicroscopy method where a high magnification lens is used to observe complications including macular edema [38]. There are two units to this biomicroscope referred to as the illumination unit and the microscope unit [38]. A chin rest is used to properly hold the patients head in place while the examiner operates the optical system [38]. The slit lamp illuminates the eye and adjusts for efficiency of the examination [38]. On the optical system, the axial angle is adjusted to produce an image for the examiner that lets them view the retina and other tissues of the eye [38]. While using the lens at 78-D, 66-D, or 60-D, a clinician can detect the posterior pole and mid-peripheral retina [38]. This can help a clinician determine fluid in the retinal cavity and production of excess blood vessels [38]. Unfortunately, this may place patients and examiners in an uncomfortable position or may not be completely precise compared to other methods [38].

Damaged blood vessels can be observed through fluorescein angiogram by the injection of a fluorescent yellow dye with high absorbance levels into the bloodstream [9]. A blue light is then used to take the picture of the retinal area [36]. It can help determine if there is any leakage in the vessels, fluid buildup, or excess

production of blood vessels in the retina [36]. This method can also help rule out certain complications including loss of capillaries or inflammation in the vessels [9]. Many experiments have concluded that this method may have trouble viewing deep retinal capillaries due to light scattering [36]. When paired with OCT, they can be used to follow the progression of the disease and determine the proper treatment [36]. Complications with these injections may include dizziness, nausea, dry mouth, and increased heart rate [36].

Treatments and Therapy

If detected early enough, the risk of developing diabetic retinopathy can be reversed. The diabetes control and complications trial (DCCT) has determined that patients not only need to continuously control their blood glucose concentrations but also need to get an eye exam every year due to the fact that symptoms of advanced diseases don't always appear [42]. Vascular endothelial growth factors play a role in retinopathy and lead to diabetic macular edema [13]. Most patients suffering from this disease are prescribed anti-VEGF agents to control the symptoms [13]. Nonsurgical treatments are available for patients who need to manage their condition, including glycemic control [42]. A study with the DCCT showed that patients who strictly manage their glycemic control reduce their risk of developing the disease by 76%. Patients with the disease also had a 46% chance of it not advancing [42].

Managing blood pressure in certain patients may also improve the condition of the disease [42]. A study with the United Kingdom prospective diabetes study showed that patients with lower blood pressure, pertaining to systolic and diastolic measurements, reduced their risk of needing further surgical treatment by 35% [42]. Patients with type 2 diabetes mellitus should aim to keep their blood pressure around 140/80 mm Hg compared to individuals without the disease having an average of 120/80 mm Hg [42]. Type 2 diabetes mellitus has shown a link between the development of the disease and systolic hypertension, so it needs to be closely monitored [42]. If control becomes difficult, the use of angiotensin-converting enzyme inhibitors are used to manage the disease [42]. High low-density lipoprotein cholesterol is linked to diabetic retinopathy, so patients with either diabetes or a more advanced state should aim to keep their levels under 2.0 mmol/l [42].

Surgical options for more mild conditions of diabetic retinopathy include the use of laser treatments and intravitreal therapy, which are also referred to as maculopathy [8]. Laser treatment is primarily used to close leaking blood vessels throughout the retina [14]. It is aimed at areas mainly in the macula, but not in the center due to the fact that it could possibly cause further damage to vision [8]. Patients who underwent this treatment reduced their risk of complete vision loss by 60% [14]. Retinal pigment epithelium cells may also be targeted during this treatment to help drain the fluid from the retina and create a barrier [8]. A study done with the early treatment diabetics retinopathy study treated one eye with laser therapy and left one

eye as a control in multiple patients [42]. The vision loss of the course of 3 years reduced 12% in the treated eyes [42].

The two main laser treatments preformed today are scatter photocoagulation and focal laser surgery [18]. Scatter photocoagulation uses many laser spots (1000–2000), and the entire treatment takes multiple sessions to complete [14]. This treatment is usually suggested in patients with more advanced stages of the disease [14]. The macula is avoided during this to prevent any further damage [14]. Focal laser surgery uses less laser spots, but they are more intense, and the treatment only takes one session [14]. During this procedure, the specialist may also use fluorescein angiogram to identify areas with complications [14]. Complications with this treatment may include damage to other areas of the eye tissue including photoreceptors. Micropulse lasers shoot shorter pulses than original lasers to prevent this damage [42]. The short pulses emit less thermal damage which is absorbed by the retinal pigment epithelium cells [42].

Treatment for certain patients follows the guidelines of the early treatment diabetics retinopathy study [18]. A dye is injected into the bloodstream to target problem areas. Grids are placed at the leaking areas to help with fluid buildup and blood flow [18]. The treatment may not help improve certain conditions but will prevent them from progressively getting worse [18].

Diabetic Macular Edema

When diabetic retinopathy goes untreated properly, fluid leaks out of the damaged blood vessels when the inner blood-retinal barrier is disrupted and builds up in the retinal cavity and causes diabetic macular edema [8]. More than 50% of the patients with diabetic retinopathy develop this disease and is the main cause of permanent blindness [8]. At this stage of the disease, vascular endothelial growth factors (VEGFs) induce the formation of excess blood vessels and endothelial cells [8]. With advancement of this disease, ischemia occurs in the fovea and macula which decreases vision in patients [8]. Symptoms of this disease are further advanced to those of retinopathy, which include blurry vision, floating matter, and eventually blindness [8]. The course of this disease initially starts with the Müller cells becoming inflamed followed by death of the cells [36]. Surrounding cells, including ganglion cells, are affected and result in damaged metabolic states [36]. The membrane is unable to keep out surrounding fluid, so the cells fill and form cysts on the inner and outer layers [36]. This can be reversed if the metabolic states return to normal in the affected cells [36]. Blood vessels in the eye start to leak lipid deposits from this intracellular buildup and form masses that can be viewed with optical coherence tomography [36].

There is not one single cause to this disease that has been properly defined, but many factors play a role including damage of the blood-retinal barrier [36]. When the levels of vascular endothelial growth factors are high, new blood vessels attempt to form, and the barrier is disturbed which leads to inflammation [36]. This

inflammation can lead to capillary non-perfusion in the blood vessels, which is when blood is cut off from certain areas of the retina and can lead to apoptosis [36]. There is evidence that microRNAs play a role in influencing the regulation of VEGFs [41]. MicroRNA is single-stranded RNA that acts as a regulator for gene splicing and protein production [41]. They affect the insulin secretion in the blood and VEGFs [41].

There are two layers of the blood-retina barrier which are referred to as the inner and outer barriers [33]. It is responsible for regulating the nutrients that enter the eye and retinal homeostasis [33]. The tight junctions that hold the individual cells together help diffuse molecules from the blood vessels to the tissues of the retina [34]. These tight junctions that are connected to the retinal capillary endothelial cells are referred to as the inner blood-retinal barrier, while tight junctions are connected to retinal pigment epithelial cells [34]. The inner blood-retinal barrier is responsible for removing the endobiotics from the retina [34]. The choroidal vasculature, which is where most of the blood in the eye originates from, is separated from the retina by the outer blood-retinal barrier [34]. The nutrients and molecules enter the eye through the paracellular route or transcellular route [36]. Molecules travel through the endothelial unions that regulate for the needs of the tissue during the paracellular route [36]. Vesicular transporters move molecules across the endothelial cells with membrane cell transporters during the transcellular route [36].

Diabetic macular edema (DME) can be diagnosed into two separate categories: focal and diffuse diabetic macular edema [16]. Focal diabetic macular edema is when microaneurysms in the eye start to leak, which start to harden around the area to form yellow or white masses [16]. Leakage that results over 67% from microaneurysms is classified in this category [16]. Diffuse diabetic macular edema is caused by the swelling of the capillaries in the eye and the breakdown of the barrier, but no hard exudates are found [16]. This classification shows less than 33% of leaking from the abnormal blood vessels [16]. Clinically significant macular edema is when the retinal thickening reaches above 500 μm of the diameter of the fovea, which is about 1/3 the thickness [16]. It can also be classified as the hard exudates leaking from the blood vessels to grow within 500 μm of the foveal center [16].

Cytotoxic edema is when the cells start swelling due to excess intracellular fluid and may lead to the production of vasoactive agents which break down the blood-retinal barrier [36]. This can be caused by excess phosphates, lactate, and sorbitol in the blood stream [36]. It usually leads to vasogenic edema, which is excess fluid in the extracellular space [36]. The rupture of the blood-retinal barrier, free radicals, and release of vascular endothelial growth factors in the bloodstream can lead to this disease [36]. Excess fluid can lead to the detachment of the neurosensory retina and lesions throughout the eye that can cause the decrease in endothelial cells, capillaries, and Müller cells [36]. Müller cells are the glial cells in the retina that help regulate the blood flow and produce glutamate through proteins and enzymes [36]. When these cells are damaged, they are no longer able to transport liquid into the vessels, and the fluid builds up in the extracellular space [36]. Glucocorticoids can be used to restore metabolic function and help alleviate the buildup of fluid [36].

Macular edema can be associated with other retinal diseases including retinal vein occlusion and radiation retinopathy [24]. Retinal vein occlusion occurs when a blood clot blocks the retinal vein in the system [24]. There can be a central retinal vein occlusion or a branch retinal vein occlusion [25]. The central vein runs through the optic nerve, and when this is blocked, blood flow is isolated from the retina, and it is not able to drain [25]. This leads to hemorrhages and leaking from the vessels in the eye, which prevents oxygen and specific nutrients from being delivered [25]. It is diagnosed by optical coherence tomography and can be treated with anti-VEGF injections [26]. Radiation retinopathy is the thickening of the capillary walls due to exposure to radiation [27]. This causes decreased blood flow followed by hemorrhages and exudates [27].

Pseudophakic cystoid macular edema, also referred to as the Irvine-Gass syndrome, is a disease resulting from difficulties during cataract surgery [28]. It can be classified by fluid in the Henle's fiber in a perifoveal petaloid pattern and leaking from the optic nerve [28]. The risk of developing these complications has decreased from 60% to 20% due to phacoemulsification and small incision cataract surgery [28]. Inflammatory mediators that were released from the vitreous along with toxicity are hypothesized to be the main factors of the development of the disease [28]. These mechanisms together break down the blood-aqueous barrier and blood-retinal barrier which result in diabetes or diabetic macular edema [28]. As this disease advances, lamellar macular holes form, when cellular layers detach from each other [28]. This specific condition can be cured within 3–4 months without treatment, and within a year, less than 1% show issues with vision or any symptoms of the disease [28].

Macular telangiectasia is a disease associated with macular edema when the blood vessels around the fovea are damaged [29]. This rare condition can be detected through the use of fluorescein angiography and optical coherence tomography [29]. Symptoms of this condition are cloudiness in the macula, deposits in the retina, swelling in the vessels, and pigment issues [29]. Type 2 macular telangiectasia occurs when the blood vessels leak fluid and the macula thickens while new blood vessels form, which is macular edema [29]. Both eyes are affected, unlike type 1 where only one eye suffers, and vision issues will follow [29]. Type 1 occurs when blood vessels swell, but do not leak. Swelling damages the cells in the macula and leads up to type 2 [29].

Myopic traction maculopathy (MTM) occurs when the retina thickens due to the tissue in the posterior portion bulges out of weak portions of the eye and causes outpouching [30]. This happens mostly in patients with severe myopia, which is nearsightedness with damage to the shape of the eye [30]. Symptoms of this disease include thick macular holes, foveal detachments, stretched retinal vessels, and fluid in the retinal cavity [30]. MTM has strong association with the Bruch's membrane curvature which can be measured with optical coherence tomography [30]. Vitreomacular traction occurs when the vitreous begins to cling to the retina [30]. This causes blood vessels to distort due to pulling and stretching [30]. Swelling occurs in these blood vessels which could lead to microaneurysms [30]. Surgery can be done to remove the tissue that is causing the traction or the internal limiting membrane [30].

Characteristics of Diagnosis

Diagnosis of diabetic macular edema is almost identical to that of diabetic retinopathy and in some situations can be discovered in the same examination. Slit-lamp biomicroscopy can reveal the presence of retinal thickening and excess fluid/exudates [38]. The examiner looks for indications of the progression of the disease including bending vessels, location of the disrupted areas, and fluid that has hardened into yellow or white masses [38]. Color fundus photos can be taken of the areas of complication to track the progression of the disease throughout time [38].

Fluorescein angiography (FA) is the main source of diagnosis for this specific disease if not made during clinical examination by a physician [36]. Advances in technology have allowed ischemia to be diagnosed early on, which is shown to play a role in influencing diabetic macular edema [36]. Unfortunately, side effects may result from the fluorescent dye injected, including gastrointestinal issues, nausea, vomiting, and possible skin reactions at the injection site [36]. While FA is the best test used to diagnose the disease, optical coherence tomography (OCT) is widely used to monitor the progression of the disease [32]. This will help track the thickness of the retina and amount of fluid buildup in the cavity [32]. There are two different instruments used in this detection referred to as the spectral-domain and swept-source OCT [22]. The spectral-domain OCT uses a central wavelength of 840 nm and a spectrometer as a detector, while the swept-source OCT uses a wavelength of 1050 nm and a single photodiode detector [22]. Swept-source OCT has a faster scanning speed to cover larger area and can detect deeper layers of the retina [22]. It is also safer for the eye due to the long wavelength and reduced sensitivity [22].

A study was done involving 45 patients suffering from retinal diseases [32]. The optical coherence test was used to diagnose these patients with the specific diseases suffered from and was 100% efficient in diagnosing 15 patients with DME [32]. Specific OCT scans create noise on the images that make it difficult to read, so B-scan averaging can be used to denoise the image by sparsity-based block matching and 3D filtering [32]. The retinal tissue in the eye naturally curves, so the images taken by the OCT need to be horizontally flattened [32]. The images are compressed to 248 rows and 256 columns during the denoising, and when the problem area is established, the image is further cropped to 150 columns [32]. Histogram of oriented gradient (HOG) descriptors separate images into contrast-normalized regions, and the gradients for each of these regions are calculated. Images are then classified into categories of disease [32].

A newer detection instrument has been introduced called the OCT angiography, which can identify the depth of certain tissues in the eye [36]. Similar to other detection methods, it can identify microaneurysms, proliferation of blood vessels, and non-perfusion zones [36]. The biggest advantage it has is its ability to detect choroidal vasculature without the presence of dye leakage [36]. Previously, an indocyanine green angiography needed to be combined with fluorescein angiography to diagnose this issue [36]. Fluorescein angiography is still used over this method, because the area covered is much greater [36].

Treatments and Therapy

Many therapies used to treat diabetic macular edema are used in combination with a significant lifestyle modification [18]. Patients not only need to treat the initial disease but start to manage their blood pressure and cholesterol by changing eating habits and having a more active lifestyle [18]. Hemoglobin A1c (glycosylated hemoglobin) levels are suggested to stay under 7% [40]. Targeting other aspects of the body instead of trying to treat the disease outright may help reduce the symptoms [18]. The kallikrein-kinin system is a hormone system that controls inflammation and blood pressure which can be a target for pharmacotherapy [18].

To manage the growth factors that are released, patients are prescribed with the anti-VEGF drugs that are injected into the fluid in the eye [13]. This interrupts the proliferation and decelerates the progression of the disease [13]. By blocking the formation of excess blood vessels, fluid in the eye will reduce making vision clearer for the patients [13]. More than 90% of specialists prefer this method over other surgical options offered [17]. For the first 6 months to a year of treatment, monthly injections are needed, but eventually patients are able to conclude the treatment and finish injections [13]. It can also be used before vitrectomies to reduce the risk of vitreous hemorrhaging but may worsen any retinal attachment that may occur post operation [13].

Three of the most common anti-VEGF drugs used today are aflibercept, bevacizumab, and ranibizumab [17]. Ranibizumab and aflibercept are FDA-approved drugs; however, bevacizumab is not yet approved even though it is used in certain cancer treatments globally [17]. The diabetic retinopathy clinical research network has conducted numerous studies on these drugs, and although they all improve vision over time, patients with more severe cases of this disease show more improvements with aflibercept [17]. Not only did it result in recovery of vision, it also decreased the thickness of the retinal area [17]. The use of this drug controls the intraocular pressure in the eye by decreasing the amount of neovascularization, but not in patients that have reached the level of severe glaucoma [17].

Glucocorticoids are another nonsurgical option for the treatment of this disease [17]. Similar to anti-VEGF agents, corticosteroid implants are injected right into the eye which allows for a slow release of the drug [17]. Injected dexamethasone is used for short-term treatment, and fluocinolone acetonide is used for long-term treatment [17]. Dexamethasone treatments may last up to 6 months, while the fluocinolone acetonide treatment can last up to 3 years in the body [17]. Although they are commonly used for many conditions, long-term treatment with these drugs may cause severe side effects including cataract formation or retinal tears [19]. Over long periods of time, exposure to this may cause gastrointestinal conditions, hypertension, and bone fractures, so it is suggested in certain cases that short-term treatment may be the best option [19]. In addition, this treatment even may increase the risk of other non-related eye conditions, including glaucoma and cataracts [19].

A study was done with 15 patients suffering from chronic diabetic macular edema throughout a period of 4 months [15]. Each of these patients were administered a dexamethasone implant, which is a glucocorticoid that treats inflammation, in one of their eyes and returned for monthly examinations [15]. These patients

were previously administered the anti-VEGF injections with no response [15]. After 3 months of the dexamethasone, the thickness of the fovea decreased from 462 to 366 um, and the intraocular pressure increased from 15.38 to 17.5 mmHg [15]. This study may suggest that dexamethasone steroid injections may be a preferred alternative to patients suffering from a more advanced stage of DME compared to anti-VEGF [15].

If nonsurgical treatments do not completely improve the condition, laser surgery or vitrectomy is suggested for stabilization of the disease [20]. Laser surgery helps treat retinopathy and macular edema by targeting leaking blood vessels throughout the retina [20]. When these blood vessels are treated, the fluid will drain from the retinal cavity and help clear vision [20]. The risk of diabetic macular edema is reduced significantly when laser therapies are preformed but only in patients with vision 20/40 and better [8]. Certain patients may experience foveal burns, laser scars, or issues with color vision after the procedure [8].

Vitrectomy is a procedure where the vitreous gel, which is the fluid in the eye between the lens and the retina that gives the eye its shape, is removed from the eye to treat bleeding [39]. It is required when there is severe hemorrhaging or a risk of retinal detachment [39]. By removing this, proinflammatory substances are removed, and the area is able to increase in oxygen levels [39]. This treatment can be completed while the patient is completely aware with local anesthesia or unconscious [39]. A surgeon uses a small light and a vitrector, a small suction tool, to remove the initial vitreous, and it is replaced with a clear saline solution [39]. Eye pressure is maintained while the vitreous is replaced [39]. The recovery for this procedure may only take a few weeks, but the area needs to stay dry and clean with a patch [39]. There may be irritation of the eye and light sensitivity initially, but effects will diminish within 3 months [39]. Complications with this procedure may include vitreous hemorrhaging, retinal tears, and cataracts [39].

A study done by Hayatabad Medical Complex examined the effects of combining laser therapy and intravitreal anti-VEGF injections on 174 patients with diabetic macular edema [21]. 155 eyes out of the 236 that had clinical significant macular edema were treated with the grid laser, and 63 eyes were administered with anti-VEGF injections [21]. After 6 months of the treatments and follow-ups, 65% of eyes with the condition are stabilized, and 30% showed improvement [21]. The results from this study show patients who took advantage of both treatments significantly improved the intraocular pressure and fluid buildup in their eyes [21].

Conclusion

Diabetes mellitus is a chronic condition that affects more than 7.2% of the population in the United States. It is associated with hyperglycemia, which is a condition that occurs when the glucose concentrations in the blood are severely high due to lack of insulin production. Insulin assists in the conversion of glucose into energy in the cells as well as increasing blood glucose uptake into cells. Type 1 diabetes mellitus

occurs when the body does not produce insulin at all (or very little) and is referred to as insulin dependent. It is commonly diagnosed in patients that are younger and only affects 5–10% of the population of diabetes. Type 2 diabetes mellitus is the most common form and occurs when there is insulin resistance in the body. It can be managed with a healthy lifestyle and possibly insulin treatments especially in later years of the disease.

If glucose concentrations in the blood are not managed, patients can develop diabetic retinopathy, which is the swelling of blood vessels in the retina. These blood vessels can cause hemorrhage and inflammation in the eye tissue. This tissue damage results in cloudiness and possible blindness. Proliferative diabetic retinopathy is the formation of new blood vessels because of growth factors released in the prior stages of the disease. These blood vessels are very fragile and may cause microaneurysms, hemorrhages, and scar tissue. Fluorescein angiogram, optical coherence tomography, and slit-lamp biomicroscopy are methods that can be used to diagnose the disease. These techniques can be used in combination to help the diagnoses. Fluorescein angiogram uses dye to detect leaking blood vessels, and optical coherence tomography uses light waves to visualize individual layers. Diabetic retinopathy can be treated by glycemic control and blood pressure management. Surgical treatment includes scatter photocoagulation and focal laser surgery. Scatter photocoagulation is used in patients with a more severe advancement of the disease.

Diabetic macular edema (DME) develops when the fluid leaking from the blood vessels builds up in the retinal cavity and causes vision loss. This condition is the number one cause of blindness in patients diagnosed with diabetes mellitus. It can be classified into two categories referred to as focal and diffuse diabetic macular edema. Focal edema occurs when there is leaking in the main blood vessels and is diffuse when the leaking occurs in the capillaries. It is associated with retinal vein occlusion, pseudophakic cystoid macular edema, macular telangiectasia, and myopic traction maculopathy. OCT angiography can be used to diagnose DME along with fluorescein angiogram. Anti-VEGF drugs and glucocorticoids can be injected into the eye to manage the condition. The three main anti-VEGF injections are aflibercept, bevacizumab, and ranibizumab. Aflibercept shows the most improvement in patients and is recognized by the FDA. Surgical options are available if injections do not adequately help to improve the ocular condition, including laser therapy and vitrectomy.

Future Trends

Many advances in insulin treatments are currently being researched throughout the world. The National Eye Institute is developing new ways to treat and prevent vision loss [31]. Currently, ways to possibly detect diabetic retinopathy at early stages are called adaptive optics [31]. It improves the optical coherence technology to help visualize cells; the changes progress through during the disease [37]. This

technology helps determine the exact sequence that causes the symptoms associated with this disease. Focus is on the outer retina that contains the photoreceptor neurons, retinal pigment epithelial cells, and blood vessels [31].

Researchers are confident that in the future, VEGF suppression through injections will be able to completely replace the need for panretinal photocoagulation for severe cases of diabetic macular edema [37]. This would decrease the risks associated with this procedure including decreased color and peripheral vision [37]. Currently, a third of the patients treated with injections show no improvement, and researchers are looking into this treatment failure [37]. Until resolved, laser therapy will continue to be the follow-up treatment for patients unresponsive to anti-VEGF injections [37].

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Chapter 3
Drug Delivery to Posterior Segment of the Eye: Conventional Delivery Strategies, Their Barriers, and Restrictions

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Abstract Posterior eye segment diseases have been a crucial health ailment for years due to the anatomical structure of the human eye and the challenges to deliver drugs to certain tissues in the eye. This chapter discusses the parts of the posterior segment and its functions along with the possible barriers to conventional treatments. Topical and systematic drug delivery systems are traditional ways of treating most ocular diseases. However, these are not as effective due to low absorption, degradation of the therapeutic agents via enzymes, and toxicity due to higher drug doses to compensate for the low permeability. On the other hand, intravitreal, transscleral diffusion and iontophoresis along with ocular implants are novel techniques that increase the bioavailability as well as precision of the drug delivery.

Introduction

The human eye, the organ of vision, is a complex and delicate organ. Clinically it can be divided into two segments: the anterior and the posterior. Posterior eye segment diseases are a major health concern as these conditions directly impact on the patient's vision and therefore their quality of life. Around 285 million people are estimated to be visually impaired or blind, and this number is increasing by at least seven million per year [79]. The main vision-threatening diseases affecting the posterior segment include age-related macular degeneration (AMD), cytomegalovirus

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retinitis, diabetic retinopathy, posterior uveitis, and retinitis pigmentosa. Drug delivery to the posterior segment of the eye is a highly challenging task. Drug delivery research has significantly increased for other routes such as oral [37, 66, 108, 110], parenteral [19, 43], and transdermal [47, 109, 116] especially by application of nanotechnology [107, 112, 111, 9, 55, 93, 114], whereas progress in the area of ocular drug delivery has been gradually and relatively limited. Lee and Robinson described the majority of ocular drug delivery systems as "primitive and inefficient" [65], referring mainly to solutions, suspensions, and ointments. In 1995 around 90% of the ophthalmic formulations on the market were based on these three systems [62]. Most of the commercial drugs or formulations for ophthalmological disorders are designed for the treatment of the anterior segment illnesses, so there is a permanent need for the medications for the posterior ocular segment.

Available literature documents the difficulties of effective and efficient drug delivery to the posterior eye segment, primarily due to its unique anatomy and the range of ocular barriers that are crucial in maintaining healthy physiological function which pose a variety of challenges for drug delivery. These barriers can be generally categorized into three groups: static, dynamic, and metabolic [59]. The degree to which these barriers affect drug bioavailability, however, is dependent on the route of administration. Static barriers pose a physical barrier to drug diffusion to the target tissue and include the sclera, the choroid–Bruch's membrane, and the retinal pigmented epithelium (RPE). Dynamic barriers to drug delivery to the posterior segment include the lymphatic system, blood vessels, bulk fluid flow, and the active transporters in the RPE. The posterior eye also possesses various metabolic enzymes to defend the eye against xenobiotic entry. As such these enzymes reduce the bioavailability of drugs in the posterior eye. Therefore, the treatment of posterior segment diseases is limited by the difficulty to achieve sufficient effective drug level within the target tissues, most of the methods requiring repeated long-term administration of therapeutic agents. The present chapter discusses the conventional drug delivery strategies for posterior eye segment diseases, their barriers, restrictions, and limitations.

Posterior Segment of the Eye

Posterior eye segment or "back of the eye" comprises sclera, choroid, retinal pigmented epithelium, neural retina, and vitreous humor. Sclera is a tough, avascular, sieve-like elastic tissue present below the conjunctiva and continuous with the cornea. Sclera is commonly called the "white of the eye." Choroid is present between peripheral sclera and inner retinal pigmented epithelium (RPE). It is a highly vascularized and innervated tissue containing melanocytes along with mucus-like extracellular fluid. Each eye contains approximately 3.5 million retinal pigmented epithelium cells [77], which adhere together to form tight junctions

(zonulae occludentes). The inner lining of the eyeball is composed of light-sensitive neural cells, called neural retina, which transmit sensory information to the brain and interact with the external environment. These cells mainly function to capture and convert the photons into a nerve signal [54]. Vitreous humor or vitreous body is avascular and transparent, thick, gel-like fluid that covers the space between lens and retina. It aids in maintaining the structure of the eye globe. Thickness of this fluid decreases with growing age.

Drug Delivery Strategies for Posterior Eye, Their Barriers, and Restrictions

Current drug delivery systems vary in their design and duration of action to suit the route of delivery and properties of the drug, to minimize complications associated with drug delivery, and to improve patient compliance. Topical, systemic, intravitreal, and periocular routes are presently used to deliver drugs to the posterior segment of the eye. Still the challenges of drug delivery to the posterior eye are compounded by growing number of new therapeutic entities and the need for chronic therapy. The topical route has many limitations such as the drug has to follow a longer diffusional distance, also rapid drainage through the nasolacrimal ducts, low permeability of the corneal epithelium, counter-directional intraocular convection, systemic absorption, and the blood–aqueous barrier. Because of these barriers, the development of topical ocular formulations for retinal diseases has become unpredictable [35]. Oral or systemic administration of drugs is not very much effective because of blood–aqueous (BAB) and blood–retinal barriers (BRB) [21]. Intravenous administration is effective to maintain the drug concentrations in the posterior tissues relatively at high doses, but frequent systemic administration of high doses is also likely to exacerbate drug-related toxicities owing to nonspecific distribution [46, 51]. Currently, intravitreal route is widely used to deliver drugs to posterior eye segment for chronic back-of-the-eye conditions. It requires frequent intravitreal injections of drug solutions due to the short half-life and limited tissue permeation of the administered molecules. Periocular route is also a promising alternative owing to the large surface area and the relatively high permeability of the sclera [27, 44]. Yet the blood–retinal barriers and efflux transporters hamper the transport of therapeutic entities to the retina. However, these methods of administration are too invasive and have their own limitations. Sustained-release ocular delivery systems offering reduced administration frequencies have therefore gained popularity over recent years with a few implants already on the market and many more in the pipeline. The challenge in developing a new drug delivery system for the treatment of posterior eye disorders is to deliver the effective dose to the affected tissue and at the same time to minimize the systemic and local side effects.

Topical Drug Application

The easiest route of administration is topical application of ophthalmic preparations in the form of solutions, suspensions, ointments, gels, or emulsions. Topical drug application is the most common method of ocular drug delivery for anterior segment disorders. This route has the highest compliance with patients, but it is estimated that less than 5% of the topically administered drug enters the internal structures of the eye [56]. While being the least invasive method of drug application, topically applied drugs have many barriers to cross to reach the posterior segment. The low fluid-holding capacity of the cul-de-sac, about 30 μL, is one limiting factor. Productive absorption from topical delivery occurs by two routes: the corneal and non-corneal (conjunctival/scleral) pathways. In the precorneal space, lacrimation, tear dilution, nasolacrimal drainage, and tear turnover limit transcorneal penetration [2]. Even though the lacrimal turnover rate is only about 1 μl/min, the excess volume of the instilled fluid is flown to the nasolacrimal duct rapidly in a couple of minutes [103]. This precorneal drainage also reduces the effective contact time between the drug and the corneal epithelium and, thus, decreases the amount absorbed. Viscosity enhancers can be used to increase drug contact time on the cornea and hence drug release to the posterior segment. Systemic absorption also removes a portion of topically applied drug for its effective ocular absorption to posterior segment. Systemic absorption may take place either directly from the conjunctival sac via local blood capillaries or after the solution flow to the nasal cavity [102, 104]. Anyway, most of the small molecular weight drug dose is absorbed into systemic circulation rapidly in few minutes. Drug absorption into the systemic circulation decreases the drug concentration in lacrimal fluid extensively. Then, the drug has to cross the cornea (relatively impermeable to most of the drugs), the anterior chamber, and the crystalline lens area before reaching the posterior segment of the eye. This way is further slowed down by the counter-directional intraocular convection [4, 71]. The non-corneal or conjunctival/scleral route of absorption involves penetration across the conjunctiva and sclera and then into the intraocular tissues. Molecules up to approximately 70 kDa can readily penetrate the sclera, whereas the size limit to pass through the cornea is less than 1 kDa. In addition, the sclera provides a large surface area of 17 cm^2, comprising 95% of the surface area of the human eye. This area provides a large region for transscleral drug absorption [113].

The expression of efflux pumps such as P-glycoprotein (P-gp) on the corneal [17, 23] and conjunctival epithelia [92] also hinders the topical absorption of drugs. All of these barriers and limitations lead to increased dosing and a higher frequency of topical drug application in order to attain therapeutic concentrations in posterior eye segment, making the use of topical drugs relatively inefficient for posterior segment eye diseases [32]. Recent studies have shown that drugs with lower molecular weight have increased permeability into the posterior chamber. On the other hand, drugs with higher molecular weight and superior water solubility (highly charged) may have longer half-lives in posterior segment than those with lower molecular

weights [101]. Thus, lower molecular weight drugs have increased access to the posterior eye and may minimize the risk of toxicity compared with higher molecular weight drugs, which degrade at slower rates.

Penetration of drugs into the cornea and conjunctiva is primarily driven by the molecular weight, lipophilicity of the drug molecule, and concentration gradient. The epithelial layers of the cornea and conjunctiva act as rate-limiting barriers for topical drug absorption. Depending on its lipophilicity, the applied drug will enter the conjunctival or corneal epithelium through the paracellular and transcellular routes. Hydrophilic drugs such as atenolol and inulin enter the epithelial layers via the paracellular route, while lipophilic drugs such as timolol and propranolol enter through the transcellular pathway [16, 45]. The intercellular space of corneal and conjunctival epithelia is sealed by the junctional complexes that hinder the transport of hydrophilic compounds [40]. The rate of paracellular penetration decreases with an increase in molecular size. Conjunctiva is more permeable (15–25 times) to hydrophilic compounds compared to the cornea. Reason for this is the larger paracellular pore diameter of conjunctiva (3.0 nm ± 1.6), through which molecules of size 5–10 kDa can permeate easily. The paracellular pore diameter of corneal epithelium is 2.0 nm ± 0.2, which allows paracellular permeation of molecules with size <500 Da [40]. Though the conjunctiva covers approximately 8% of the ocular surface, its pore density is 16 times higher than the cornea, and the total paracellular space in the conjunctiva is approximately 230 times greater than in the cornea [99]. Surprisingly, memantine HCl and brimonidine tartrate have been shown to reach the vitreous and retina after topical ocular delivery. Memantine appears to exploit a scleral route of delivery to the posterior segment. While their route of penetration is unclear, brimonidine tartrate and dorzolamide both achieve therapeutic posterior concentrations from topical delivery [46].

Systemic Drug Delivery

Systemic administration of drugs is another method of access to the posterior eye segment. The drugs are administered orally or intravenously (tablets, capsules, intravenous solutions, etc.), enabling distribution throughout the body via bloodstream. From blood, the drugs can enter the choroidal extravascular space as the choroid has an extensive vascular network and leaky walls. However, the entry of the drug into the posterior segment is often limited by the outer and inner blood–retinal barrier (BRB) made up of retinal pigmented epithelium (RPE) and endothelial cells of the retinal blood vessels, respectively [95]. The BRB acts as a significant diffusional barrier, a characteristic dependent on the physiochemical properties of the compound and its interaction with BRB efflux proteins. The permeability of most compounds across the blood–retinal barriers is very low. It is impermeable to polar or charged compounds. However, extremely lipophilic compounds such as chloramphenicol, tetracycline, and benzyl penicillin can penetrate the blood–retinal barrier achieving appreciable concentrations in the vitreous humor after systemic administration [46].

The RPE and retinal vessels also contain several efflux pumps including P-glycoprotein and multidrug resistance-associated protein that actively transport compounds from the vitreous toward the plasma. Organic acids such as the penicillins, iodopyracyte, and fluorescein are actively transported out of the vitreous across the RPE [46]. The active transport of these compounds presents a further barrier to the systemic delivery of drugs to the vitreous. Therefore most pharmacologically active compounds used in the treatment of posterior segment disorders are not able to achieve therapeutic concentrations. Moreover, compared to topical instillation, extremely large doses have to be administered systemically in order to generate the targeted therapeutic concentrations in the eye, if at all possible. This can lead to nonspecific systemic exposure and consequential side effects [97]. It can lead to serious adverse effects due to accumulation of drug in other tissues. Systemic administration of drugs has other limitations including potential reduced time of therapeutic effects and potency due to the dilution and degradation of the drug before reaching posterior eye segment [51]. Moreover, drug–drug interactions in patients being treated for coexisting medical conditions also influence the administration of systemic drugs for the treatment of retinal disease.

Despite all these limitations, systemic delivery remains the standard route of administration for many drugs for posterior eye. Ocular therapies with protein kinase C inhibitors, VEGF inhibitors, retinoids, antibiotics, and antivirals have all been successfully delivered systemically [46]. Other drugs with lipophilic properties like chloramphenicol, minocycline, and cephalosporins that can penetrate the barrier are administered systemically in the treatment of serious ocular infections. Likewise, mannitol and carbonic anhydrase inhibitors, such as acetazolamide, are administered intravenously to reduce intraocular pressure [15].

Intravitreal Route

Direct drug administration into the vitreous offers distinct advantage of a straightforward access to the vitreous and retina. The drug is directly injected into the vitreous humor using a 30-G needle [67]. Therefore intravitreal injection is gold standard to treat posterior eye segment conditions. Today, various pharmaceutical forms are administered by intravitreal injection, such as aqueous solutions, viscous solutions prepared with hydrophilic polymers, suspensions, micro- and nano-particulate systems, and biodegradable and non-biodegradable depots or implants [70]. This method of drug application eliminates the barriers common with topical and systemic administration. This method provides increased drug concentrations at the neural retina and minimizes systemic side effects. However, multiple injections may be necessary as a result of the limited half-life of many compounds in the vitreous. This typically requires frequent visits by the patients who may be of advanced age or in poor health, and there is associated discomfort. Nonetheless, frequent administration of drugs via this route can lead to serious side effects like retinal detachment, retinal hemorrhage, cataract formation, endophthalmitis, and increased

intraocular pressure [51, 82, 106]. Accordingly, in formulating a drug delivery system for intravitreal administration, the pharmacokinetics of the drug substance must be considered so as to avoid repeated administrations. To minimize some of these complications, novel drug delivery systems have been developed in the form of biodegradable or non-biodegradable implants, which can be placed for long term in the vitreous [20, 27, 44].

The release and distribution of drug products administered intravitreally depend both on the properties of substances and on certain physiological parameters. Small molecules are able to diffuse rapidly in the vitreous, but the mobility of large molecules, particularly positively charged, is restricted [85]. It is known that low molecular weight (<500 Da) drug substances have a limited retention half-life of approximately 3 days and therefore require repeated administrations, while large molecules (greater than 500 Da) can persist in the vitreous humor for several days or even weeks [81]. The two main routes by which a drug is eliminated from the vitreous humor are either via the anterior chamber or across the retinal surface. The elimination and distribution kinetics are affected by the rate of drug diffusion through the vitreous and also by the geometry of the eye. The density of a given formulation might also affect the distribution within the vitreous, with high-density formulations depositing on the inferior retina causing localized adverse effects. The generalized elimination and distribution pathway is complicated in elderly patients with collapsed vitreous structure and in patients with vitrectomy [63, 70]. In addition, the long-term accumulation of intravitreal implants might significantly impact the vision of patients [86]. Still, intravitreal injection is currently the most acceptable and effective method to treat vitreoretinal disease. Intravitreal triamcinolone acetonide is being used to treat AMD and macular edema. Anti-VEGF drugs, such as pegaptanib, ranibizumab, and bevacizumab, are new intravitreal treatments for AMD and macular edema [95]. Present evidence suggests that, although the intravitreal route is useful in attaining high drug concentrations at the retina, it is not the ultimate strategy for drug delivery to the posterior ocular tissue. Sustained drug delivery devices offer an excellent alternative to multiple intravitreal injections: this approach increases drug bioavailability, ensures controlled and long-term release of molecules, and avoids repeated intraocular procedures [10, 42].

Periocular Route or Transscleral Diffusion

Periocular or transscleral injections are a less traumatic route for patients and have minimal risk of adverse reactions compared with the intravitreal route. It enables the deposition of molecules against the external surface of the sclera, thereby minimizing the risk of endophthalmitis and retinal damage associated with the intravitreal route of administration [13, 88]. From a drug delivery perspective, the transscleral route offers quite a few advantages: (i) the sclera, with its large surface area (16.3 cm^2), which is less resistant to permeation of molecules than conjunctiva [40, 51, 80, 96]; (ii) relatively high permeability to macromolecules [5, 76];

(iii) high degree of hydration, which is conducive to the diffusion of hydrophilic molecules; (iv) metabolic inactivity, which can facilitate delivery of drugs sensitive to metabolic enzymes [4]; and (v) feasibility of administering controlled and sustained release formulations [8]. Additionally, Olsen et al. observed that scleral permeability did not change with the age of the patient. This is very significant as age is a major factor in the pathogenesis and progression of AMD and diabetic retinopathy [76]. Transscleral or periocular pathways used for drug delivery to the posterior tissues of the eye include the retrobulbar, peribulbar, subtenon, and subconjunctival routes. With subconjunctival injection, the formulation is placed beneath the conjunctival membrane that covers the sclera. This enables the drugs to bypass the conjunctiva–cornea barrier, giving direct access to the transscleral route. Subtenon injection involves the placement of a formulation between the sclera and Tenon's capsule, an avascular membrane. As such, the contact time between the applied drug and the sclera is prolonged. Consequently, the subtenon route is considered to be one of the most promising routes for targeting the posterior segment of the eye [34, 51].

Although transscleral route eliminates some of the side effects of intravitreal delivery, they in turn have their own limitations. Frequent administration of periocular injections may lead to nonspecific absorption and undesirable systemic toxicity, especially when low therapeutic index drugs are used [29]. Nevertheless, only minute concentrations of a drug administered via the transscleral route end up in the vitreous and, thus, require very high doses to be effective [58, 100]. It has been shown that use of a proper injection technique, volume, and formulation can minimize this loss and improve transscleral bioavailability [18, 36, 52].

Because the drug molecules must cross through several layers of tissue, the bioavailability of the drug at the target site can sometimes be drastically reduced. These barriers are categorized into three major groups: static, dynamic, and metabolic [95]. Static barriers include the tissues that must be penetrated. These include sclera, choroid, and retinal pigmented epithelium (RPE) which together constitute the physical barrier to transscleral diffusion. The scleral matrix, composed of proteoglycans and collagen fibers, can hinder the permeation of solute molecules. The choroid together with the underlying Bruch's membrane, composed of lipids and lipoproteins, can also restrict drug passage. The innermost physical barrier, the RPE, forms the cellular barrier, posing an obstacle to paracellular transport (with its tight junctions) and transcellular diffusion of drug molecules. Individual pharmacokinetic properties of the drug play a crucial role in transscleral diffusion. Drug diffusion across these barriers is dependent on the molecular dimensions, molecular weight, atomic charge, and chemical components of the drug. Molecules of up to 70,000 Da can readily penetrate the sclera [4, 5, 69, 83, 33]. An inverse relationship between transscleral permeability and solute molecular weight has been observed [1, 5, 76, 87]. However, Ambati et al. reported that the molecular radius is a better predictor of scleral permeability than molecular weight, with the permeability decreasing roughly exponentially with increasing molecular radius [5]. Molecular lipophilicity and charge also affects the transscleral diffusion, with lower permeability being observed for cationic and lipophilic solutes [72]. Furthermore, Cheruvu

and Kompella reported that hydrophilic molecules exhibited higher permeability than lipophilic molecules of similar molecular radii (0.53–0.57) which is consistent with reports from other researchers [5, 14, 53, 57]. However, a balance may be critical, since many lipophilic compounds can easily penetrate the RPE, but results in toxicity due to slow drug elimination. Drug permeation also depends on scleral hydration and intraocular pressure; however, the latter has negligible effects at normal intraocular pressures (15–20 mmHg) [35, 64]. Elevated intraocular pressure (15, 30, and 60 mm Hg), however, had a significant effect on the transscleral permeability of solute molecules [91].

Bruch's membrane is the innermost layer of the choroid and also acts as a barrier to the entry of solute molecules. Cheruvu and Kompella demonstrated that, compared to the sclera, the choroid–Bruch's layer preferentially binds lipophilic drugs and is thus a bigger barrier to the diffusion of lipophilic molecules than the sclera [14]. The choroid–Bruch's layer, however, offers little resistance to the penetration of hydrophilic and anionic molecules. RPE is a monolayer of highly specialized cells located between the neural retina and the choroid and forms a formidable barrier to the diffusion of hydrophilic molecules across the sclera to the retinal layers [27]. Only selected molecules are exchanged between the choroid and the retina, owing to the tight junctions of the RPE. As such, the flux of a compound across the RPE depends on its permeability and the concentration gradient [7, 22, 35, 59, 68, 90]. The Bruch's membrane and RPE thus together form a major barrier to the diffusion of both hydrophilic and hydrophobic molecules through transscleral route to posterior eye segment.

Dynamic barriers include blood flow, lymphatic drainage, transport proteins of the RPE, drug efflux pumps, and bulk fluid flow from intraocular drainage systems. Some of the drug is lost into the systemic circulation and lymphatics, due to hydrostatic and osmotic pressure differentials [6, 59]. Blood vessels and the lymphatic systems on the episclera are the main contributors for the clearance of drugs administered via the transscleral route. Once the drug has reached the interior tissues of the eye, it can be cleared rapidly by the choroidal blood flow. Choroid is a network of blood vessels located between the sclera and the retina which acts as a sink for drug molecules that diffuse through the sclera. The choroidal region of the eye has the highest per unit blood volume [6]. The low osmotic pressure of the vitreous in comparison with the choroid along with the high intraocular pressure also serves as a driving force for solute clearance via the choroidal blood flow. However, Robinson et al. demonstrated greater influence of lymphatic clearance on retinal bioavailability of a corticosteroid compared with the choroidal circulation [90]. Moreover the uveoscleral outflow pathway that functions to drain the aqueous humor contributes.

To the clearance of drugs from the posterior segment. A number of nutrient influx and efflux transporters have also been identified on the RPE [27]. Drug moieties that are substrates of efflux pumps (P-gp, MRP, and BCRP) expressed on the RPE may exhibit compromised ocular bioavailability [27, 68]. Influx transporter-targeted prodrugs may be an approach to overcome the barrier properties of these efflux transporters [49, 50, 68]. The binding of drugs to various proteins in ocular tissues, including melanin, also affects the transport of a drug into the vitreous [83, 89, 26, 84].

Metabolic barriers include enzyme systems such as cytochrome P450 and lysosomal enzymes, which have the ability to degrade or detoxify drugs. These enzymes have been reported in the ocular tissues, with especially high expression levels in the iris-ciliary, choroid, and RPE, and to a lesser extent in the cornea, lens, aqueous humor, and vitreous humor [3, 28]. Both phase I and phase II metabolic enzymes are expressed in the eye, with the cytochrome P450 family and lysosomal enzymes being the most predominant [3]. The evidence of metabolic activity in the RPE components suggests a possible role of metabolism in the ocular drug bioavailability process [28]. Thus for effective transscleral drug delivery, a thorough evaluation of the effect of physiological mechanisms, disease states, and drug characteristics needs to be undertaken and built into the design stage.

Transscleral Iontophoresis

Another transscleral method involves an electrodynamic process of drug delivery called iontophoresis. In this technique, charged molecules are delivered across the sclera and into the posterior chamber of the eye via a direct electric current. In most cases, an iontophoretic probe is placed over the pars plana, enabling a bypass of the lens–iris barrier. The drug is applied with a weak electric current that drives charged molecules across the sclera and into the choroid, retina, and vitreous body [75]. A ground electrode of the opposite charge is placed elsewhere on the body to complete the circuit. Ionized drug molecules serve as the conductor of the current through the tissue. The technique is noninvasive and therefore avoids the risks of surgical implantation or intravitreal injections, and it does not affect the half-life of drug [44]. Studies have shown that iontophoresis eliminates many of the unwanted side effects of intravitreal injections and may improve the efficacy of periocular injections by decreasing the risk of retinal detachment, endophthalmitis, globe perforation, and ptosis [75]. Sometimes a burning sensation was noted by a few subjects at the site of application at higher current levels. This arrangement permits the precise delivery of high quantities of drugs through changes in the intensity of the applied current, yielding improved control of constant and uniform drug delivery. Iontophoresis has been shown to increase the transscleral permeability of many drugs, including fluorescein, steroids, antibiotics, antivirals, anti-inflammatory agents, and immunosuppressants [75]. Transscleral iontophoresis delivers high concentrations of the applied drug to the choroid and the retina with minimal side effects [35, 94]. However, adverse effects of iontophoresis include epithelial edema, a decrease in endothelial cells, inflammation, and burns. At higher current densities, iontophoresis has been shown to damage the choroid and destroy retinal layers [78].

While using an iontophoretic device, the main elements impacting the amount of drug delivered are the amount of current, concentration of drug, pH of drug, the duration of treatment, and the permeability of the tissue [46]. Another major concern is that after repetitive applications of current and heat, the resistance in the tissue may change over time, altering the electric field and leading to changes in drug permeation, thus altering drug peaks and troughs. For example, the coulomb-

controlled iontophoresis unit allows an automatic adjustment in electric current based on the changes in resistance across the conjunctival epithelium [41, 75]. This unit also provides self-calibration and acts as an indicator for proper electrical probe contact [41, 75]. Other iontophoretic units include the mini-ion unit and the EyeGate® II iontophoresis device (EyeGate Pharma, MA, USA) [75]. The portable mini-ion unit provides a variable electric current for a preset amount of time. It uses a hydrogel probe to deliver charged drugs to the posterior eye [30, 31]. The EyeGate II device is a new, updated version of the original EyeGate iontophoresis device. The EyeGate II uses an electric current to hydrolyze water and, thus, increase ion mobility, allowing greater concentrations of drug to reach the posterior eye. As the current induces like-charged ions to repel each other, more drugs are delivered through tissues. Preliminary results of phase II studies of the EyeGate II Delivery System have shown it not only to be effective in delivering siRNAs but also in increasing the cellular uptake of oligonucleotides and drug delivery to the target site as a result of the applied electric current [95]. Other iontophoretic devices available include OcuPhor (Iomed) and Visulex (Aciont) delivery systems.

Ocular Implants

Intravitreal, transscleral, and iontophoretic routes of drug administration tend to achieve high drug levels in the posterior eye and are typically more efficacious than systemic and topical methods. However, these drugs are susceptible to rapid clearance and require frequent administration. The half-life of most drugs in the vitreous is limited to a few hours [11]. Ocular implants provide a platform for sustained release of drugs from either biodegradable or non-biodegradable polymeric systems over several months to years [98]. Subconjunctival, scleral, and intravitreal routes are considered the most suitable for the purpose of drug delivery to the posterior eye segment via implants as they offer direct drug delivery to the target site, with minimal systemic loss [101]. Intraocular administration of the implants always requires minor surgery. In general, ocular implants are placed intravitreally, at the pars plana of the eye (posterior to the lens and anterior to the retina) [25]. Non-biodegradable implants are not metabolized in vivo, and may require replacement or invasive surgical removal once the drug is depleted, but provide more accurate controlled release over longer release periods. Polymers commonly used for the fabrication of non-biodegradable implants are silicone, polyvinyl alcohol (PVA), and ethylene vinyl acetate (EVA) [12]. Drug release occurs either by diffusion across a permeable membrane or by degradation of the polymer block [38, 48]. Biodegradable implants involve biodegradable vehicles that would allow the implants to be slowly converted to soluble forms via enzymatic and nonenzymatic reactions in the eye [8]; therefore they do not require posttreatment removal but can cause more erratic drug release profiles. This, in turn, eliminates the need for the implants to be removed and replaced in different locations and, thus, decreases side effects from multiple invasive procedures. Polymeric materials commonly used to fabricate biodegradable implants include poly(lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL),

polyanhydride, and poly(orthoester) (POE) [98]. Although this is an invasive technique, the implants have some benefits: bypassing the blood–ocular barriers to deliver constant therapeutic levels of drug directly to the site of action, avoidance of the side effects associated with frequent systemic and intravitreal injections, smaller quantity of drug needed during the treatment, prolonged activity, etc. [81].

Marketed ocular implants include Vitrasert® (Bausch & Lomb), Retisert® (Bausch & Lomb), and Ozurdex® (Allergan), approved by the FDA. Vitrasert was the first non-biodegradable polymer implant, containing ganciclovir approved in 1996 for the treatment of cytomegalovirus (CMV) retinitis in patients with AIDS. This device, surgically inserted through the pars plana into the posterior eye, is made of a central pellet of ganciclovir (4.5 mg) that is placed in a coating of polyvinyl alcohol (PVA), which is a permeable, water-soluble polymer that serves as the framework of the device and provides regulation of drug release. The PVA is then surrounded by ethylene vinyl acetate (EVA), which is impermeable and restricts the surface area available for drug diffusion. The EVA is surrounded by yet another level of PVA to increase regulation of the rate of drug release. The implant delivers the drug at a rate of 1 μg/h and lasts for 6–8 months [74]. Vitrasert has been shown to produce a statistically significant improvement over iv. administration in delivering ganciclovir to the posterior eye for the treatment of CMV retinitis [74]. Retisert is also a non-biodegradable polymer implant containing fluocinolone acetonide approved in 2005 for the treatment of chronic noninfectious posterior uveitis and releases the drug for up to 2.5 years. Another non-biodegradable implant, Iluvien®, is approved in some EU countries [39, 48, 74, 105, 115]. It is injected into the vitreous with a 25 gauge needle rather than being sutured into the sclera. It contains fluocinolone acetonide for the treatment of diabetic macular edema (DME). Although the safety and efficacy of several non-biodegradable implants have been demonstrated, the process of surgical implantation and removal has many potential deleterious side effects, including endophthalmitis, vitreous hemorrhage, and retinal detachment. In addition, once the drug is depleted, the implant needs to be removed and a new device implanted. To eliminate this, more research is focusing on the development of biodegradable implants, which are soluble and, thus, do not need to be removed or reimplanted when the drug is depleted. These implants have been manufactured in a variety of shapes including rods, sheets, discs, pellets, and plugs. Also they allow implantation through smaller incisions than the non-biodegradable counterparts [44]. Ozurdex was the first marketed biodegradable polymer ocular implant consisting of a PLGA-based rod containing dexamethasone approved in 2009 for the treatment of DME, retinal vein occlusion, and posterior uveitis. It is placed into the vitreous via a 22 gauge needle to release the drug up to 6 months. However, while these systems can deliver the drug over long periods of time, the release rate cannot be altered. Thus, stimuli-responsive drug delivery systems (DDS) suitable for ocular implantation are of interest as they have the potential to provide tunable drug delivery to the affected areas [24, 73]. Such systems offer fine control over drug release, helping to optimize therapeutic outcomes in individual patients [61], and could become particularly attractive for delivery of macromolecules in the treatment of ocular diseases [60].

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Chapter 4
Penetration Routes to Retina and Posterior Segment

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Abstract Many factors affect the selection of an appropriate penetration route for drug delivery to the posterior segment of the eye. Therefore, the molecular penetration routes need to be understood and considered thoroughly while designing a drug delivery system. In this chapter, four primary administration routes, these being (1) systemic, (2) topical, (3) periocular and suprachoroidal, and (4) intraocular, are discussed with unique advantages as well as their own challenges. To achieve a balance of the desired therapeutic outcomes without compromising safety and patient's adherence to therapy, a number of formulations/actives have been investigated so far and are discussed in the various sections of this chapter.

Introduction

In order to effectively deliver actives to the posterior segment of the eye, it is essential to understand and consider molecular penetration routes. Four primary administration routes may be employed for drug delivery to the posterior segment of the eye, and these are discussed in detail in this chapter. Each route brings a unique set of advantages and disadvantages [1]. Selection of the route may be based on a number of factors such as the target tissue, the physicochemical properties of the drug being administered, the potency and toxicity of the drug, as well as the required duration of action [2]. For the purpose of drug delivery to the retina, penetration routes have been be divided into (1) systemic, (2) topical, (3) periocular and suprachoroidal, and (4) intraocular.

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The systemic route works by delivering drugs through the bloodstream. With treatments administered via oral dosage forms or intravenous injection, this may seem like an easily accessible route; however, tight junctions found in the blood-retinal barrier make it almost impossible to deliver therapeutic levels of drug without causing any systemic side effects [3, 4]. The topical route, on the other hand, causes fewer systemic effects and offers a more direct route as drugs are applied directly onto the eye surface; however, the need to penetrate various ocular barriers such as the cornea and sclera renders this route inefficient in delivering therapeutic drug levels to the retina [3]. The periocular route involves injection or implantation into tissues in close proximity to the eyeball such as the subtenon, subconjunctival, peribulbar, and retrobulbar spaces [1, 5]. The suprachoroidal route, on the other hand, is used to bypass the sclera; however, drugs administered through this route still need to permeate through the choroid to reach the retina [6]. Intraocular administration involves injection of drugs directly into the vitreous humor or within the subretinal space, and this is the most effective route for drug delivery to the retina [2]. The periocular, suprachoroidal, and intraocular delivery routes can all result in therapeutic levels of drug in the retina; however, periocular and suprachoroidal routes are deemed less efficient compared to the intraocular route [7].

Many conditions affecting the posterior segment of the eye are chronic and require long-term treatment; therefore, patient's convenience and adherence to treatment have become the most important challenges when designing suitable drug delivery systems [8]. Important factors affecting the selection of an appropriate

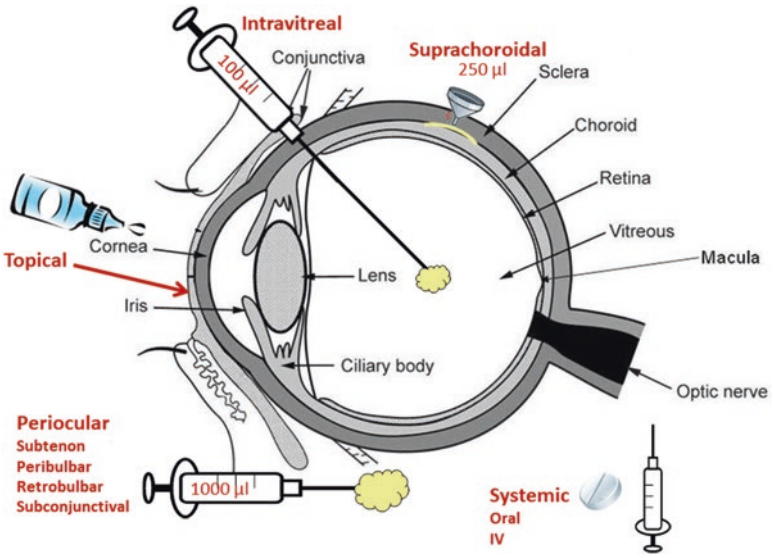

Fig. 4.1 Penetration routes for drug delivery to posterior segment of the eye where delivery routes and subclassifications have been listed in red bold font, with maximum administration volumes provided. Anatomical structures of the eye have been identified in black font. (Adapted with permission from [9])

penetration route for the purpose of drug delivery to the posterior segment of the eye need to be understood and considered thoroughly while designing a drug delivery system. Figure 4.1 gives an overview of the various penetration routes for delivering drugs to the retina and posterior eye segment.

Systemic Route

Although it is the most popular route for the treatment of general conditions affecting the human body, systemic administration has seen very limited use to treat eye conditions. Actives can be administered orally using tablets, capsules, or liquid dosage forms. In addition, intravenous injection or infusion is also used for systemic administration of various ophthalmic drug molecules. Large amounts of drug can be administered via this route; however, the percentage reaching the site of action in the eye is very limited [1].

The physiology and anatomy of the posterior segment of the eye have been discussed in detail in Chap. 1. The blood-retinal barrier (BRB), which consists of tightly connected retinal capillary endothelial cells and retinal pigment epithelial cells, restricts free movement of drug molecules from the blood to the retina and vitreous humor. This makes it almost impossible for large hydrophilic molecules such as bevacizumab (Avastin®) to cross this barrier [10]. On the other hand, small molecules such as timolol may be delivered across BRB to the retina [11], although large continuous doses of these drugs are required to achieve a therapeutic level in the eye. The vitreous humor usually measures 4–5 mL, while the entire blood volume is reported to be 5 L in a healthy human being; thus this creates a significant dilution effect for drugs being delivered via the systemic route. Due to the large dose requirements, poor safety, and the limitation to small molecules, this route is rarely used for the treatment of conditions affecting the posterior eye only [12]. One notable exception is Visudyne® (verteporfin), which is administered intravenously before being activated by light exposure once it has reached the retina. In addition, for conditions such as cytomegalovirus (CMV) infections, which affect numerous organs around the body, the systemic route may be used to treat its ocular complications due to the large systemic concentrations simultaneously treating non-ocular issues. However, even here systemic administration is often unable to produce a therapeutic effect in the eye. Therefore, these drugs are often co-administered via the intravitreal route to reach effective therapeutic concentrations [13].

Despite all of the difficulties encountered when treating retinal conditions via the systemic route, several attempts have been made to improve systemic drug delivery to the eye with the use of penetration enhancers and targeting strategies [14, 15]. Campbell et al. [14] reported the use of a small interfering (si)RNA technology which was able to disrupt the BRB in rabbits and allow influx of small molecular weight drugs for up to 2 days. Similarly, Thurman et al. [15] reported the use of inflammation inhibitors targeting the inflammatory pathway activated during age-related macular degeneration (AMD) in the retina. Systemic administration of such

anti-inflammatory drugs reduced damage to the retina and halted the progression of the disease. In another report, Thurman et al. [16] showed that systemic administration of an antioxidant could reduce oxidative stress in the retina using in vitro retinal pigment epithelial (RPE) cells (exposed to oxidative stress), thereby reducing the risk of developing AMD.

Interestingly, certain ocular conditions such as AMD and diabetic retinopathy (DR) can affect the integrity of the BRB rendering this route more accessible for large hydrophilic molecules [17]. This could increase the amounts of systemically administered active delivered to the retina. Indeed, Visudyne is a good example where the drug molecules reach to the site of action due to the compromised integrity of the BRB. The drug is then activated by laser light with its active form damaging the endothelial cells of abnormal blood vessels ultimately blocking them [18]. Furthermore, there are studies reporting the efficacy of bevacizumab administered via the intravenous route to treat AMD. One clinical study has shown that the systemic route may be used to treat AMD and DR improving visual acuity without significant systemic side effects in patients with no previous history of hypertension and thrombosis [19]. This could provide an alternative route of administration for patients who are non-compliant with intravitreal administration of such molecules.

Topical Route

Topical administration is very popular and used extensively for the treatment of anterior segment conditions. Approximately 90% of all ophthalmic formulations available in the market are administered via the topical route [12]. This route provides an easy application site where actives can be administered as simple solution, suspensions, ointments, or gels [20]. However, for this route to effectively deliver drugs to the retina, numerous static and dynamic barriers need to be overcome [21]. Low retention time on the ocular surface, various hydrophilic and hydrophobic layers of the eyeball, outward flow of the aqueous humor, and rapid clearance through the blood-aqueous barrier restrict the use of the topical route to treat retinal conditions [20, 21]. Despite these limitations, the topical route offers a very convenient way to treat anterior segment conditions.

Topically applied dosage forms are cleared rapidly by the lacrimal fluid (flow rate of 1 μL/min); therefore, excessive amounts of the active are removed through this mechanism via the nasolacrimal duct with the conjunctival sack only capable of holding up to 30 μL of any applied volume [22]. The remaining portion of the applied dosage form permeates either through the conjunctiva or cornea. Conjunctival absorption is associated with less barriers, with the drug then following scleral permeation routes. In the case of the corneal route, drug first needs to traverse the hydrophobic epithelium and hydrophilic stroma of this tissue, before finally traversing the hydrophobic corneal endothelium to reach the aqueous humor. It is estimated due to the numerous barriers that usually less than 10% of the applied drug absorbs through the cornea and reaches to the anterior chamber. Other factors that may impact drug absorption include the osmolarity, pH, and viscosity of the dosage

form [22]. From the anterior chamber, the applied drug either permeates through the trans-vitreous route by traversing the vitreous humor or via the uveal route by permeating across the choroid [23].

Physicochemical properties of an active play a significant role for permeation as only nonpolar lipophilic molecules can permeate freely through the anterior and posterior layers of the eye [24]. For polar hydrophilic molecules, transporters play a significant role to improve corneal penetration. Efflux transporters play a critical role in eliminating drug molecules and thus lower their bioavailability, while influx transporters such as amino acid, peptide, glucose, or lactate transporters facilitate the movement of nutrients and drug molecules into the eye. Both types of transporters have been studied extensively for the purpose of enhancing drug delivery to the retina via the topical route [25]. In addition, a number of prodrug molecules (e.g., prostaglandin analogues) have been developed and studied to increase the bioavailability, being recognized by the influx transporters as substrates [26]. Once inside the cell, these prodrug molecules break down into the actual drug to avoid elimination by the efflux transporters [25]. Figure 4.2 depicts the prodrug mechanism of action

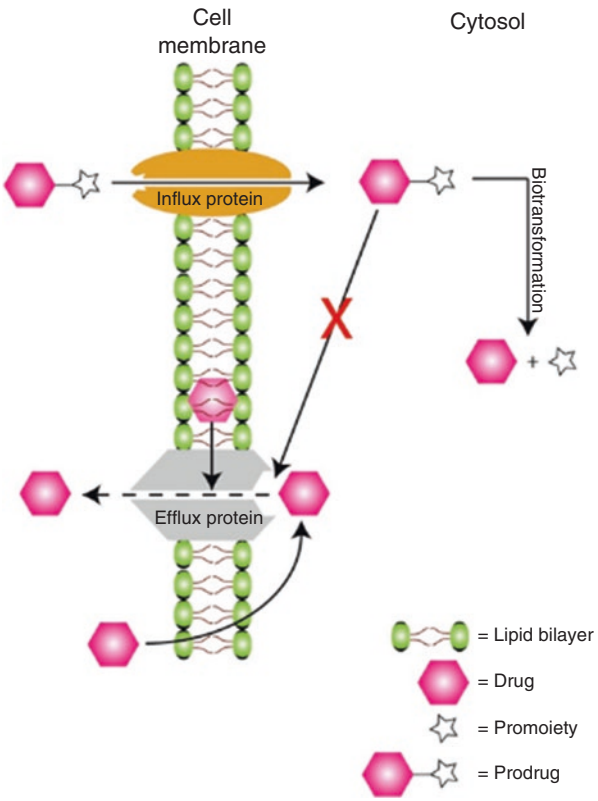

Fig. 4.2 Prodrug approach to increase drug bioavailability showing that the prodrug approach decreases drug efflux. (Adapted with permission from [25])

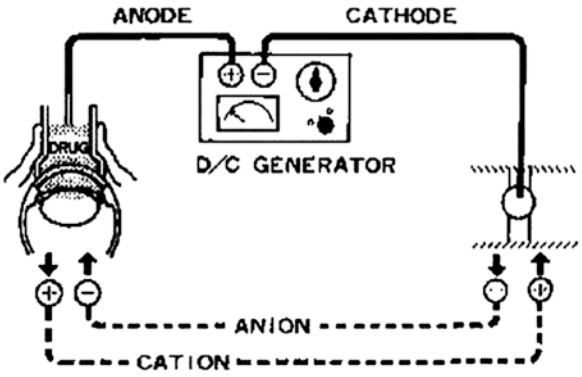

Fig. 4.3 Ocular iontophoresis diagram showing transscleral delivery of a positively charged drug by electrostatic repulsion. (Adapted with permission from [31])

in order to achieve higher bioavailability. The use of cell-penetrating peptides (CPP) is another novel way to improve ocular bioavailability of topically administered drugs. CPP work by penetrating the cell membrane either via active or passive pathways [27]. De Cogan et al. [28] studied the use of CPP in vivo using a rat choroidal neovascularization (CNV) model to deliver bevacizumab to the posterior segment of the eye following topical administration. They were able to detect clinically relevant concentrations of bevacizumab at the site of action with minimal toxicity or side effects. This study demonstrated the potential use of transporters/penetrators to achieve therapeutic drug levels in the retina using topical route.

Bioavailability of topically administered ionic drugs may further be improved using techniques such as ocular iontophoresis. In this technique, the donor electrode carrying the drug has the same charge as the drug and is placed on the ocular surface, where the drug works as a conductor. The other electrode is placed somewhere else on the body, and the drug is delivered due to the electrostatic repulsion between the donor electrode and the ionic drug (Fig. 4.3) [29–31]. Voigt et al. [32] found that the application of a drug using iontophoresis can increase the concentration of the applied drug up to eight times in the rabbit vitreous in vivo when compared to normal topical administration. This reflects the potential use of ocular iontophoresis to achieve therapeutic concentrations of actives in the posterior segment of the eye.

Periocular and Suprachoroidal Routes

At present, the periocular and suprachoroidal routes are comparatively less popular for the treatment of ocular conditions affecting the posterior segment of the eye. With the periocular route, actives are usually administered by injecting a drug solution or suspension in the subtenon, peribulbar, retrobulbar, or subconjunctival

spaces surrounding the eyeball. When compared to intraocular or suprachoroidal routes, a larger amount of the drug solution/suspension can be injected into these regions (up to 1 mL) [33]. However, the robust clearance mechanism in the subconjunctival space near the limbus and the outward flow of the vitreous humor through the layers of the eye limit the ocular bioavailability of actives injected via this route [34, 35]. In contrast, a relatively smaller volume of solution/suspension containing actives can be administered into the suprachoroidal space (maximum volume 250 μL) [36, 37]; however, as it is able to bypass the sclera, this route offers significantly higher ocular bioavailability at the site of action when compared to the periocular route [38]. In comparison to the systemic and topical routes of administration, the periocular and suprachoroidal routes offer less patient compliance due to the invasive mode of administration. However, in spite of this, these routes offer a superior safety profile and less complications when compared to the intraocular route [39].

Retention of actives in the periocular space following administration is a challenge due to their rapid clearance [34]. Orbital and conjunctival blood vessels as well as lymphatic supply to this area are considered the main routes of elimination for drugs administered via the periocular route [40]. Subtenon injection seems to be an exception from this mode of elimination; therefore, it yields a higher ocular bioavailability at the site of action and less systemic side effects. Subtenon injections bypass the conjunctival and orbital vessels and lymphatic supply [33]. Ghate et al. [33] showed that a posterior subtenon injection could yield five times higher concentrations of active at the site of action in a sustained pattern when compared to other periocular routes of administration. Consequently, posterior subtenon injection is considered a good alternative to the intraocular route and in certain cases has shown comparable efficacy and treatment outcomes [41].

Several novel strategies have been attempted in order to improve ocular bioavailability by prolonging formulation retention using the periocular route. Adding an impermeable backing layer on the collagen film containing dexamethasone and using it as a subtenon insert resulted in an increased duration of action from 2 weeks (as a topical rapidly dissolving collagen films) to approximately 6 months [12, 42]. This is an impressive improvement in the duration of action which makes it comparable to the marketed dexamethasone containing intraocular implant (e.g., Ozurdex®). Pontes de Carvalho et al. [43] investigated an episcleral exoplant sutured to the sclera in an in vivo rabbit eye. This approach increased intravitreal drug bioavailability by 30- to 40-folds when compared to a normal subtenon injection of the same compound. It also increased the duration of action up to 21-folds (from an average of 6 h to an average of 126 h) in comparison to a normal subtenon injection.

The suprachoroidal route is considered one of the most effective and safest administration routes for the treatment of posterior eye conditions [39]. The space between sclera and choroid (suprachoroidal space) is reversibly expandable and can hold a small volume of drug solution/suspension to act as a reservoir delivering drug directly to the retina through the choroid [44]. The use of this route avoids complications such as retinal detachment, vitreous hemorrhage, and endophthalmitis,

usually associated with intraocular administration [45]. The choroid is a highly perfused layer of the eye and the main route of elimination for drugs administered into the suprachoroidal space. Interestingly, the clearance of large hydrophilic molecules such as bevacizumab is faster when compared to small hydrophilic molecules [46]. This makes the suprachoroidal route less effective for the treatment of diseases where macromolecular anti-vascular endothelial growth factor (VEGF) antibodies are used. The administration of drugs is performed into the suprachoroidal space mainly via microneedles that are typically 1000 μm in length [47].

Novel formulations to increase drug penetration and ocular bioavailability as well as to achieve longer duration of action have been investigated for suprachoroidal use. ClEARSIDE® Biomedical has developed a promising microneedle-based suprachoroidal technology which delivers small drug molecules like corticosteroid for the treatment of uveitis (in phase 3 clinical trials) as well as anti-VEGF drugs for the treatment of retinal vein occlusion (also in phase 3 clinical trials) [48, 49]. Another promising suprachoroidal technology by Gilger et al. [50] developed a suprachoroidal implant of cyclosporine for the treatment of uveitis using an in vivo horse eye model with promising clinical outcomes for up to 24 months. The use of microcannulation to improve drug delivery via the suprachoroidal route has also been studied by Olsen et al. [51], who were able to detect therapeutic concentrations of triamcinolone acetonide using an in vivo pig eye model for up to 4 months after administration. Interestingly, this route has also been investigated to deliver bevacizumab for the treatment of AMD and DR. However, suprachoroidal administration of bevacizumab via a microcannula was deemed less effective with shorter half-life and inferior posterior bioavailability when compared to the intraocular route [46].

Intraocular Routes

Intravitreal administration is the most efficacious route for the treatment of retinal conditions [52]. Drugs are administered directly into the vitreous humor; however, the delivered drug may be readily eliminated or broken down by metabolic enzymes. Therefore, repetitive administration of high doses of drug is generally required to achieve effective long-term treatment [53]. Patient compliance for this route of administration is low, and intravitreal injection may be associated with many complications including vitreous hemorrhage, cataract formation, retinal detachment, endophthalmitis, and increased intraocular pressure [54]. To reduce the frequency of administration and overcome the problems associated with intravitreal injection, implants and slow drug-eluting devices have been developed which are injected or surgically placed directly into the vitreous [55, 56].

Intravitreal drug distribution is impacted by various physiological factors, namely, the vitreous structure, convective flow patterns, and saccadic eyeball movement [57–59]. The extent to which each of these impacts drug distribution is largely influenced by the size and polarity of the drug molecules or the delivery system [57, 60]. For example, studies in bovine vitreous have demonstrated unrestricted diffusion of particles with a diameter <510 nm, whereas the gel structure proved a

substantial barrier for larger (>1190 nm diameter) particles [61]. The same study also demonstrated the importance of charge on migration, with anionic vitreal collagen and glycosaminoglycans hindering the migration of cationic particles. Separately, liquefaction, which is an age-dependent loss in vitreous gel integrity, has shown to notably increase the rate of diffusion/sedimentation of molecules in this tissue irrespective of their size [62, 63].

Nomoto et al. [64] demonstrated the superiority of intravitreal injections when compared to the topical and periocular routes. In this study, 1.25 mg bevacizumab administered to rabbit eyes via the intravitreal route achieved 300-fold larger C_{max} in the retina and choroid than when the molecule was administered via subconjunctival injection with drug barely detectable after topical administration. In addition, drug remained above therapeutic concentrations for substantially longer durations following intravitreal rather than subconjunctival administration. Given its superior depot capacity to periocular routes and the proximity to retinal tissues, the intravitreal route also remains the most common site for implant administration [38].

Following intraocular administration, penetration into the retinal layers is essential to achieve therapeutic efficacy. Here, drugs or particles have to first penetrate the inner limiting membrane (ILM) which is a basement membrane separating the vitreous humor and retina [65]. Being composed of anionic glycosaminoglycans and collagen, penetration through this layer is again very difficult for cationic drugs and particles while being more straightforward for anionic or uncharged molecules and drug delivery systems [66, 67]. Additionally, the ILM serves as a significant barrier to penetration of viral vectors [68]. In such cases, the subretinal route, which bypasses the ILM and involves injection directly into the retina, has been considered the only route suitable for gene therapy, although this is a high-risk process and requires substantial training [69].

Recently, physical methods have been investigated to improve intraocular particle penetration. One such method gaining widespread interest in this realm is ultrasound. Studies have demonstrated an ability of ultrasound waves to increase the permeability of molecules in numerous tissues and cells of the body [70]. These findings were further confirmed in ocular models, where the administration of ultrasound to stimuli-responsive nanobubbles significantly increased the penetration of a co-administered macromolecule in vitro into different retinal cells [71]. Separately, ultrasound was also shown to impact particle migration within the vitreous humor. In contrast to the typical convection and saccade governed by particle migration, transscleral ultrasound was able to noninvasively direct hyaluronic acid-human serum albumin-based nanoparticles away from the site of impulse administration without causing significant tissue disruption or temperature increase [72].

There are two major elimination pathways for drugs administered via this route as shown in Fig. 4.4 [73, 74]. The anterior elimination route involves drug diffusion from the vitreous to the aqueous humor and further elimination via aqueous humor turnover and uveal blood flow. This is the main route of elimination for large hydrophilic drug molecules such as bevacizumab [75, 76]. On the other hand, the posterior elimination route involves drug permeation across the retina and choroid and subsequently the BRB; therefore, this is the main route of elimination for small hydrophobic drug molecules which can easily cross the BRB [35, 77].

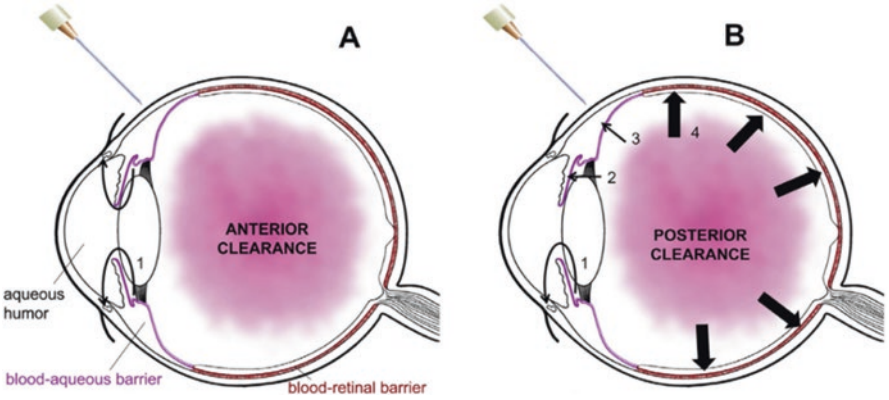

Fig. 4.4 The routes of drug elimination following intravitreal administration (**a**) anterior and (**b**) posterior clearance. (Adapted with permission from [74])

Conclusion

Various penetration routes discussed in this chapter offer unique advantages as well as present their own challenges. An ideal dosage form should achieve the desired therapeutic outcomes without compromising safety and patient adherence to the therapy. To achieve such a balance, a number of formulations/actives have been investigated as discussed in the various sections of this chapter. Furthermore, the physiological or pathological variability between patients may affect the selection of the route as well as require personalized/tailored treatment. Intelligent drug delivery systems which can be controlled by the user or the healthcare professional may offer superior therapeutic outcomes in such circumstances. In addition, physical methods such as ultrasound may be employed to further enhance the penetration of actives at the site of action. We envisage that in the future, drug delivery to the posterior eye could benefit greatly from such personalized drug delivery systems with enhanced penetration.

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Chapter 5
Diabetic Retinopathy: Pathogenesis, Treatment, and Complications

Samhitha Gudla, Divya Tenneti, Makrand Pande, and Srinivas M. Tipparaju

Abstract Diabetic retinopathy is a complication of diabetes. Majority of diabetic patients with high blood glucose face the challenge of dealing with retinopathy and macular edema as the disease progresses. Although treatment choices and care are available to manage and symptomatically treat Diabetic retinopathy the current understanding is limited and lacks options for treatment and rescue strategies. Pharmacological options include anti-VEGF treatment strategies and surgical procedures. The present review provides insights in to type of diabetic retinopathy, along with different stages of the disease. In addition, the roles for health care providers, importance of pharmacists for treatment and management of diabetic retinopathy patient care are discussed.

Keywords Diabetic retinopathy · Drug delivery · VEGF · Glucose

Introduction

Diabetic retinopathy (DR) is a complication of diabetes that affects the eyes. Diabetes affects insulin production and sensitivity and therefore the ability to absorb glucose, leading to high blood sugar levels. When blood sugar levels are high, damage can occur to the blood vessels of the light-sensitive retina, which allows for vision. For instance, high blood sugar levels can cause a narrowing or blockage of

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the retinal arteries and lead to reduced or no blood flow to the retina. As a result, endogenous processes trigger angiogenesis allowing growth of new blood vessels, but this leads to further complications. These changes to the retina affect vision and can cause blindness in diabetics.

Significance

DR holds medical, social, and economic significance. The disease affects up to 80% of people who have had diabetes for more than 20 years and accounts for 12% of all new cases of blindness. Globally, it is the leading cause of vision loss, affecting an estimated 285 million people worldwide. Recent studies have determined that about one in three people with diabetes have DR. Since it is such a prevalent health issue, the economic significance of DR is also high. In the United States alone, it is estimated that $500 million is spent on diabetes-related blindness costs. Worldwide, it is estimated that $232 billion is spent on diabetes and its complications. Given the enormous cost and financial burden that DR brings, it is essential to take measures to prevent DR, possibly by keeping good control of blood sugar levels and by early detection of eye diseases. Given the increasing prevalence of diabetes, the worldwide costs associated with it are projected to rise even more.

Studies have also been conducted to find correlations between DR and socioeconomic status. However, clear, strong relations could not be found. This weak or absent correlation can be attributed to a number of competing influences, including lifestyle, health behaviors, attitude, mortality rate, and health-care systems. For instance, a higher socioeconomic group, which receives benefits of good diabetes care and treatment, may counter those effects with a sedentary lifestyle and the consumption of western foods. This lack of correlation does not negate the importance.

There are different classifications of DR, including nonproliferative diabetic retinopathy (NPDR), proliferative DR, diabetic maculopathy, and advanced diabetic eye disease.

Patient Care Overview

Although with recent studies diabetic retinopathy (DR) and diabetes macular edema (DME) are two common ophthalmic complications for the diabetic patients, it can be kept under control through patient awareness and by adopting simple changes in lifestyle. Early detection, treatment, and improved glycemic control can limit the onset or progression of DR and DME.

The primary management for DR and DME includes three main therapies:

- Laser photocoagulation
- Intravitreal vascular endothelial growth factor (VEGF) inhibitors
- Intravitreal corticosteroid implants

DR and DME have multifactorial etiology due to the fact that combination therapy is gaining more popularity. Even though the change in lifestyle and regular screening can prevent and/or reduce the effect of DR and DME, health-care providers and patient's adherence is poor and needs to be regulated with proper management. Screening and prevention goes hand in hand, and it is observed that 40% of the patients with DM and DME can prevent further ocular complications presented with their routine ocular screening. Diabetic eye exam compliance in a US Medicaid population increased from 46% to 64% between 2010 and 2012. The economic cost for treating vision complication due to diabetes mellitus is estimated to be about $490 million each year indicating the burden for patients and managed care system imposed by DR and DME.

Though treatments are available to manage complications due to DR and DME, the length of the treatment causes additional burden for patients and managed care system due to necessity of longer duration of the treatment. Health plans, accountable care organizations, and other providers have more interest in investing time and proper education in ensuring their patients with diabetes (DM) receive proper vision screening and maintain adequate disease control to avoid complication due to DM which includes DR and DME. It is crucial to manage cost-effectiveness of currently available treatments of DR or DME as well as identify opportunities to improve patient adherence to treatment.

Health-Care Providers and Pharmacist's Role

Encourage adherence to eye exam visits in patients with diabetes and for managing DR or DME.

Focus on Preventative Strategies

- Glycemic control
- Blood pressure control
- Lipid control
- Proteinuria and BUN/creatinine ratio

DR and DME Patient Education

- Increase awareness, describe risk of vision loss, explain how to prevent by addressing barriers to effective diabetes care, and use motivational interviewing.
- Lack of education – speak in layperson terms and provide reminders for routine eye exams.

- Explain therapy requirements (frequent visits), cost, and possible adverse effects.
- Monitor therapy safety and efficacy; describe what to expect with therapy, stopping vision loss, vision improvement expectations, etc.

The following simple chart can help the patient to manage DR and DME during different disease stages

Disease stage	Presents with one of the following	Management
Early	Multiple small drusen Few medium-sized drusen Mild retinal pigment epithelial (RPE) abnormalities	Quite smoking Control body mass index (BM) and blood pressure Increase dietary intake of antioxidants
Intermediate	Numerous medium-sized drusen At least one large druse Geographic atrophy	Keep lifestyle the same as the early disease stage Antioxidant supplements like AREDS and AREDS2
Advanced "dry" stage	Drusen with atrophy in the center of the macula	Keep lifestyle the same as the early disease stage Antioxidant supplements like AREDS and AREDS2
Advanced "wet "stage	Neovascularization with hemorrhage Lipid deposits Swelling and damage to the macula capillaries	Keep lifestyle the same as the intermediate disease stage Vascular endothelial growth factor(VEGF) inhibitors Anti-angiogenic therapy Laser therapy

Study on Different VEGF Inhibitor Treatment Costs

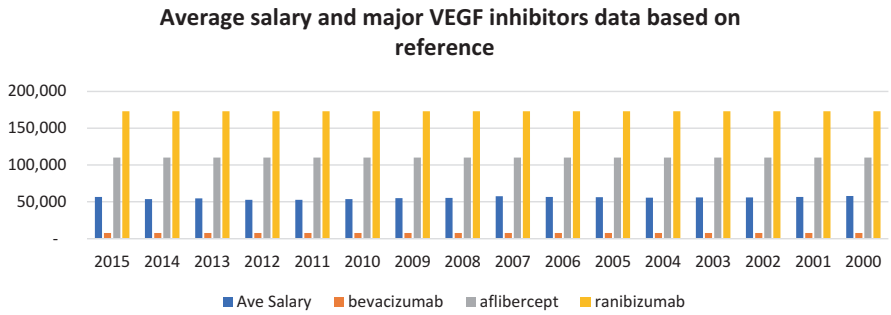
In a previously published report [1], the researchers calculated the incremental cost-effectiveness ratios (ICERs) of the three drugs. One-year trial data were used to calculate cost-effectiveness for 1 year for the three anti-VEGF drugs. In addition, the researchers used mathematical modeling to project 10-year cost-effectiveness. In the patients with worse vision, 20/50 or more, aflibercept improves vision to a greater extent than bevacizumab or ranibizumab. At vision levels better than this, all drugs perform equally well.

The current study found that the more expensive agent (aflibercept) performs better in patients with worse vision. The study results also underscore the possibility of effective as-needed treatment [2]. Fixed-interval dosing would be superior to as-needed dosing. They randomly assigned participants to receive intravitreous aflibercept (2.0 mg), bevacizumab (1.25 mg), or ranibizumab (0.3 mg). Patients were treated on a needed basis as often as every 4 weeks, as opposed to on fixed dose intervals. From baseline to 1 year, the mean visual acuity letter score improved by

13.3 with aflibercept, 9.7 with bevacizumab, and 11.2 with ranibizumab. A closer examination of the data, however, revealed that the difference between the drugs was driven by improvements in eyes with worse visual acuity at baseline.

Laser photocoagulation therapy reduces risk of vision loss in patients with high-risk proliferative diabetic retinopathy and, in some cases, severe nonproliferative diabetic retinopathy.

Per Year Cost Comparison



Gaps in DR and DME Care

- Studies involving anti-VEGF therapies need to better translate to clinical practice and results be clinically significant.
- Nine injections during the first year of treatment are impractical and lead to noncompliance.
- Ability to read one additional line on an eye chart may not have meaningful functional value.
- Lack of evidence for treatment non-responders.
- Necessary DME-related services such as screening, diagnosis, treatment, and ongoing care may not be covered by insurance providers.
- Precise data on DME financial impact to individual and society are needed to justify costs advocating for improved treatment and outcomes for diabetic macular edema.

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https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3627413/
Medscape Medical News from the Source: U.S. Census Bureau, Current Population Survey, 1968 through 2016 Annual Social and Economic Supplements.

Pathogenesis

The pathogenesis or development of DR can occur in various ways. Due to the high blood sugar levels, arterial walls of diabetics can thicken and become narrow. In the eyes, this leads to less blood flow, therefore causing DR [3]. The vascular and hematological changes of diabetic patients also lead to thickening of the capillary basement membrane, causing capillary endothelial cell damage. Red blood cells become deformed, leading to increased stickiness of platelets and increase plasma viscosity [3]. All these symptoms can in turn lead to microvascular occlusions, in which the artery leading to the eyes, the ophthalmic artery, is clamped off. This prevents bleeding and rupture. In order to bypass this occluded artery, new arteries could grow and branch off as seen in proliferative DR. Occlusion then leads to retinal ischemia, which is the state in which blood supply to the eye is cut off [4]. The blood in the artery before the occlusion pools up, causing the artery to enlarge. This ballooning and weakened area in the artery, referred to as a microaneurysm, could rupture, leading to a hemorrhage and retinal edema. This can lead to neovascularization [4].

Factors Affecting DR

Many factors influence the likelihood and onset of DR. For instance, age and puberty significantly affect DR because of the hormonal factors responsible for growth that are involved. High levels of IGF1 and IGF2, smoking, anemia, obesity, and hyperlipidemia can all lead to the progression of DR [5]. Poor metabolic control, specifically hyperglycemia, can accentuate the progress of DR, along with ocular factors such as glaucoma, hypertension, and pregnancy [5]. DR can also be genetic, leading to increased risk of proliferative retinopathy in people with HLA DR4 and DR3 genes [5]. Whereas all of these factors increase the prevalence and likelihood of DR, myopia can decrease the prevalence and severity of retinopathy.

Different Types of DR

Nonproliferative Diabetic Retinopathy

There are two main types of DR, namely, nonproliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy. NPDR is an early stage of DR in which tiny blood vessels within the retina leak blood or fluid, causing the retina to swell. NPDR can be characterized by microaneurysms, retinal hemorrhages, edema, hard exudates (yellowish waxy patches arranged in circinate pattern), and venous abnormalities such as beading, looping, and dilation [6]. Apart from these, NPDR can also be characterized by cotton wool spots that are small, white, superficial

areas which represent areas of nerve fiber infarcts. These are a sign that the eye is not getting enough oxygen. Intraretinal microvascular abnormalities (IRMA), which are fine, irregular red lines connecting arterioles with venues representing AV shunts, are also characteristic of NPDR. NPDR can be classified into different stages, namely, mild, moderate, severe, and very severe [6].

Proliferative Diabetic Retinopathy

As the conditions of NPDR approach the severe stage, the eye may be forced to form new arteries through a process called neovascularization. This then becomes known as proliferative DR, in which new arteries form in order to bring oxygen to the hypoxic retina. Proliferative DR affects around 5–10% of the total population and develops in more than 50% of DR cases 25 years after the onset of diabetes [7]. The primary feature of PDR is neovascularization, which is caused by the angiogenic factors elaborated by the retinal tissue in an attempt to neovascularize the hypoxic retina. The angiogenic factors are most commonly the vascular endothelial growth factors(VEGF) with isoforms like VEGF-A, VEGF-B, VEGF-C, and VEGF-D, placental growth factors, pigment epithelium-derived factors, etc. Similarly, there are several endogenous inhibitors of angiogenesis such as endostatin, platelet factor 4, and angiostatin [7]. It is hypothesized that the net balance between the VEGF and endostatin is associated with retinopathy. About one quarter of the retina has to be non-perfused before PDR develops [7].

There are two main types of proliferative DR, including PDR without high-risk characteristics and PDR with high-risk characteristics, which is also known as advanced PDR. The high-risk characteristics include neovascularization of the disc (NVD) to one fourth of the disc area, less than one fourth of the disc area, or more than half of the disc area with vitreous hemorrhage (VH) or preretinal hemorrhage (PRH) [7].

Further Side Effects

Diabetic Maculopathy

DR can lead to further complications of the patient, such as diabetic maculopathy and advanced diabetic eye disease. Diabetic maculopathy is a condition that arises from retinopathy [8]. It is concerned with damage to a specific part of the retina, the macula. When this swelling occurs in the central part of the retina (the macula), it is known as macular edema. Since the macula is the region of keenest vision, swelling of the macula could lead to reduced or blurred vision, whereas leakage or swelling elsewhere in the retina will usually not have too severe of an effect on vision. This swelling, or edema, occurs due to increased permeability of the retinal capillaries.

Symptoms for diabetic maculopathy include trouble reading and recognizing faces in the center of your vision. There are also four different classifications of maculopathy, namely, focal exudative maculopathy, diffuse exudative maculopathy, ischemic maculopathy, and mixed maculopathy [8].

The diagnoses for clinically significant macular edema (CSME) can be made if one of the three criteria is present on slit-lamp examination within a 90D lens: thickening of hard exudates at or within 500 microns of the center of the fovea associated with adjacent retinal thickening, the retina at or within 500 microns of the center of the fovea, and development of zone of retinal thickening 1 disc diameter or larger in size at least a part of which is within 1 disc diameter of foveal center.

Treatment for diabetic maculopathy is most commonly done using laser photocoagulation [9]. One specific type of laser treatment is the focal treatment, in which burns are applied to microaneurysms and microvascular lesions located 500–3000 μm from the center of the macula. The spot size is 50–100 μm, and the exposure time is 0.1 s, with sufficient power to obtain a gentle whitening or darkening of the lesion. In another laser treatment, known as the grid treatment, burns are applied to areas of diffuse retinal thickening of more than 500 μm from the macula. The spot size is again 100 μm, and the exposure time is 0.1 s, resulting in a high-intensity burn [9].

Another treatment option apart from the laser photocoagulation is a pars plana vitrectomy [10]. Vitrectomy is a surgery to remove the vitreous gel containing retinal detachment or blood. This procedure can give better access to the retina of the eye and can get rid of the edema. The pars plana vitrectomy is named as such since the instruments used to do the procedure go through the pars plana, or the flat portion of the ciliary body located near the point where the iris and sclera touch. This procedure is done only if severe persistent vitreous hemorrhage is present or if there is a premacular subhyaloid hemorrhage [10].

Advanced Diabetic Eye Disease

Another side effect of DR is advanced diabetic eye disease. This is characterized by vision-threatening complications in patients whose laser photocoagulation treatments have been unsuccessful or inadequate. There are many methods of diagnosis for this disease. Some characteristics used for diagnosis are persistent vitreous hemorrhage, tractional retinal detachment (caused by progressive contraction of fibrovascular membranes over areas of vitreoretinal attachment), tractional retinoschisis, and rubeosis iridis (caused by retinal ischemia) [11]. This disease can be preretinal, intragel, or both. Intragel hemorrhages take longer to clear than preretinal hemorrhages because the former result in more extensive bleeding. Patients should be warned that bleeding might be precipitated by severe exertion or straining, hypoglycemia, or direct ocular trauma. The treatment for this disease again is usually pars plana vitrectomy [10].

Treatment

Screening

Patients with DR should be screened frequently to monitor the condition of their disease. Typically, diabetics should be screened every year for symptoms of NPDR. Patients who already have moderate NPDR should be screened every 6 months to ensure that it is under control and not worsening. Patients with severe NPDR should be screened every 3 months, and patients with PDR should be screened every 2 months. Frequent screenings can be advantageous for the prevention and control of DR in diabetic patients [12].

Drug Delivery

Medical drugs can be taken to help treat DR. Delivery of drugs to the posterior eye is challenging, owing to anatomical and physiological constrains of the eye [13]. There is an increasing need for managing rapidly progressing posterior eye diseases, such as age-related macular degeneration, diabetic retinopathy, and retinitis pigmentosa. Drug delivery to the posterior segment of the eye is therefore compounded by the increasing number of new therapeutic entities (e.g., oligonucleotides, aptamers, and antibodies) and the need for chronic therapy. Currently, the intravitreal route is widely used to deliver therapeutic entities to the retina. However, frequent administration of drugs via this route can lead to endophthalmitis, increased intraocular pressure, and retinal detachment. Various controlled delivery systems, such as biodegradable and non-biodegradable implants, liposomes, and nanoparticles, have been developed to overcome such adverse effects, with some success [13]. The periocular route is a promising alternative, owing to the large surface area and the relatively high permeability of the sclera. Yet, the blood–retinal barrier and efflux transporters hamper the transport of therapeutic entities to the retina. As such, the efficient delivery of drugs to the posterior eye remains a major challenge facing the pharmaceutical scientist [13].

The first line of drugs in the treatment of DR are anti-VEGF (anti-vascular endothelial growth factors). These drugs work by stopping a protein called vascular endothelial growth factor (VEGF), which is produced by the cells in the retina, from working. The overproduction of VEGFs has been connected to hypoxia, growth of new blood vessels, and consequently blindness. The two most widely used anti-VEGF drugs to counter this problem are bevacizumab (Avastin) and ranibizumab (Lucentis) [14, 15].

Specific Anti-VEGF Drugs

Bevacizumab, commercially known as Avastin, is a full-length, recombinant, humanized monoclonal antibody that works against all VEGF isoforms. It is used to treat eye diseases as well as a number of different cancers. It works by binding to all isoforms of VEGF-A and inhibiting their activity. The typical dose taken is 1–1.25 mg or 0.05 mL [14].

Ranibizumab, commercially known as Lucentis, is a genetically manipulated version of bevacizumab. It is a monoclonal antibody fragment (Fab) that is anti-angiogenic and has been approved to treat macular diseases and vision loss. Because it is genetically manipulated from the same parent mouse antibody as bevacizumab, its effectiveness is also similar to that of bevacizumab. The antibody works by inhibiting VEGF-A. All isoforms are also bound, including VEGF-110, a plasmin-cleaved form of VEGF165. The normal dosage of ranibizumab is 0.3–0.5 mg. Although ranibizumab has been proven to be relatively safe, some side effects may include conjunctival hemorrhage, eye pain, or intraocular inflammation [16].

Another anti-VEGF drug that is used is pegaptanib sodium, commercially known as Macugen. This is another anti-angiogenic drug, used to treat neovascular macular degeneration. It acts by binding specifically to the pathological 165 isoform of VEGF, which is the most important in angiogenesis, and blocking its actions, therefore reducing the growth of blood vessels and working to control leakage and swelling. An advantage of this drug is that it spares the normal vasculature, therefore giving it a dual mechanism of anti-angiogenesis and anti-permeability. The normal dosage is 0.3 mg or 90 μL [17, 18].

Anecortave acetate, commercially known as Retaane, is also known as an angiogenic steroid because of its functions. It inhibits the remodeling of basement membranes and extracellular matrix components in angiogenesis, as well as the expression of VEGF in smooth muscles. It can also be used to treat age-related macular degeneration and to reduce intraocular pressure. Anecortave acetate is delivered via the posterior juxtascleral depot (PJD) that delivers the drug onto the sclera near the macula. This method allows for decreased intraocular infection and retinal detachment. Retaane is typically delivered once every 6 months. Possible complications of this drug include endophthalmitis, vitreous hemorrhage, persistent floaters, rise in IOP, retinal pigment epithelial tear, and retinal detachment [19].

Other Methods of Treatment

Although anti-VEGF drugs are the most common method of treatment for DR, there are other options as well. For example, protein kinase C is an intracellular signaling molecule, whose activation plays an important role in the development of ocular complications. PKC inhibitors can diminish blood flow related to hyperglycemia and therefore has potential use as a therapy for DR. Other methods can include the use of aldose reductase and ACE inhibitors, antioxidants such as vitamin E, and

intravitreal steroids such as fluocinolone acetonide implants and intravitreal injections of triamcinolone at a 2–4 mg dosage [17].

Routes of Drug Delivery

Systemic, topical, periocular, and intravitreal routes are used to deliver pharmaceuticals to the posterior segment of the eye. The topical route has a lower bioavailability due to rapid drainage through the nasolacrimal ducts, a hydrophobic corneal epithelium, the blood–aqueous barrier, and the systemic absorption. Conversely the blood–retinal barrier (BRB) hinders the diffusion of systemically administered drugs to the posterior segment of the eye. Thus, the ideal routes of drug delivery are the periocular and the intravitreal routes.

Intravitreous injection of anti-VEGF, antibiotics, and steroids is the currently accepted route of administration to treat posterior segment diseases, such as diabetic retinopathy, age-related macular degeneration (AMD), vascular occlusions, cystoid macular edema, uveitis, viral retinitis, endophthalmitis, and retinal detachment. This enables direct application of the drug eliminating the barriers which are common with topical and systemic administration. A higher intraocular bioavailability yields more efficacious treatment of posterior segment diseases. Intravitreal injections are typically given at pars plana 3.5–4 mm posterior to the limbs. The availability of infusion devices such as insulin pumps (Fornia, Zhuhai, Guangdong, China) has also added to improve treatment modalities [17].

Periocular routes comprising of the retrobulbar, peribulbar, subtenon, and subconjunctival administration of drugs enable the molecules to be deposited on the external surface of the sclera, thus minimizing the risk of endophthalmitis and toxic retinal reactions. Of these, the subtenon route is considered to be the most effective method to treat posterior segment diseases of the eye [17].

Conclusion

DR is a widely prevalent disease and a common cause of visual loss. It can progress in the absence of symptoms, producing irreversible damage to the retina. The key to managing this ailment is realizing that prevention is better than treatment and a repeated follow-up of these patients to detect the earliest sign of diabetic retinopathy. Interventions are most efficacious when started early in the disease, when retinal damage is minimal and clinical findings are few or absent. Periodic ophthalmoscopic examinations are essential in detecting the progression of retinopathy and development of disease characteristics which indicate a need for treatment. Regular screening examinations along with intensive control of hyperglycemia, serum lipid levels, and blood pressure not only retard the progression of DR but also contribute to reducing cardiovascular mortality.

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Part II
Nanotechnology Based Formulations for Retina and Posterior Segment Disease

Chapter 6
Liposomes for Retina and Posterior Segment Disease

Kathleen Halasz and Yashwant V. Pathak

Abstract Liposomes are bilayer vesicles composed of phospholipids, which can contain the non-lipophilic drugs to increase its bioavailability and to enter the posterior segment of the eye. These liposomes can be formulated in different sizes via these four basic steps: drying lipids from the organic solvent, dispersing the lipid within an aqueous media, purification, and analyzing the product. Majority of the liposome formulations are generally composed of poly(ethylene glycol) (PEG)-modified lipids, which have been proven to increase circulation time. This drug delivery method renders both active and passive mechanism, which are specific to the targeted area. Liposomes act as barriers when they come in contact with ocular surfaces and protect therapeutic agents from the metabolic enzymes found at the tear junction. However, there are some challenges that still have to be tackled, for instance, sterilization, short shelf life, and also other unknown consequences of their long-term in vivo use. Even with the above issues, liposomes offer a sustained drug release, which reduces the frequency of the intravitreal injections and thus their side effects.

Introduction

The eye is the most essential sensory organ within the body due to its unique ability to allow us to visually perceive the world around us as well as to maintain balance [1]. Ocular diseases in the posterior segment are the most predominant cause of visual damage and/or deficiency today. However, most of today's treatments and therapies may only be applied to the anterior segment of the eye through topical administration methods. There are a variety of diseases that are encompassed within the posterior segment, such as diabetic retinopathy, endophthalmitis, cytomegalovirus retinitis, posterior uveitis, and age-related macular degeneration (AMD) [2].

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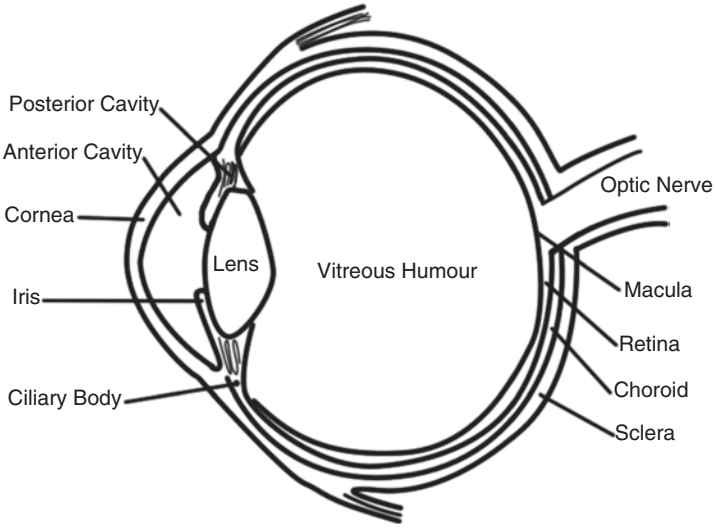

Fig. 6.1 Schematic representation of the human eye

Table 6.1 Characteristics of the various routes of administration to the posterior segment of the eye

Administration route	Characteristics
Intravitreal injection (IVT)	Drug is injected directly into the vitreous humor Drug is eliminated through the retina or anterior chamber High required injection frequency pay leads to cataracts, retinal detachment, etc.
Subconjunctival (SC) route	Drug is delivered to the conjunctival membrane around the cornea Enters posterior segment through the sclera Less invasive than IVT

The three main goals of ocular drug targeting are to control the release of drugs, to target the drugs to a specific site, and to enhance the permeation of the therapeutic agents throughout the eye [3].

The posterior segment of the eye contains the sclera, choroid, macula, vitreous humor, retina, and optical nerve (Fig. 6.1). There are several methods for ocular drug administration, such as topical, systemic, and periocular. Transient diffusion models have demonstrated that approximately 5% of the desired concentration of hydrophilic drugs will reach the anterior chamber [4]. Accessing the posterior segment through the sclera is also difficult due to cationic and lipophilic solutes, making the utilization of liposomes beneficial in circumventing this type of barrier. Systemic administration requires higher doses of drug in order to obtain the appropriate therapeutic concentration in the posterior segment, as it would need to pass through the retinal pigment epithelium (RPE) [4]. The most promising administration routes to this segment are either subconjunctival (SC) or intravitreal administration (Table 6.1) [5].

However, it is difficult to achieve sufficient drug concentration in this area, regardless of the administration method, due to several protective mechanisms, such as the sclera, blood-retinal barrier, Bruch's membrane-choroid (BC), and conjunctiva [5]. The blood-retinal barrier consists of retinal capillary endothelial cells as well as retinal pigment epithelium cells, which function to protect the eye from any foreign substances. These barriers, in addition to tight junctions, tear fluid excretion, compartmentalization, and avascularization, result in poor bioavailability of drugs within the desired region of the eye [6].

Liposomes

Liposomes were first described by Alec Bangham, who experimented to prove that phospholipids spontaneously morph into multilamellar concentric bilayer vesicles if left in suspension within an aqueous solution [7]. They were first introduced as drug delivery vessels in 1965 and have since had a major impact on many fields of biomedicine [8]. There are several liposome formulations that have been approved by the FDA such as Doxil, Caelyx, verteporfin, vincristine, Myocet, etc. [9, 10].

Liposomes are approximately 50 nm–100 μm and are composed of amphiphilic phospholipids. Their structure resembles a hollow sphere, which is made up of an aqueous, hydrophilic compartment encompassed by lipid bilayer [6]. The biphasic nature of these vessels allows them to encapsulate both hydrophilic and lipophilic drugs. Liposomes may be classified as small unilamellar vesicles (SUVs) (10–100 nm), large unilamellar vesicles (LUVs) (100–3000 nm), giant unilamellar vesicles (GUVs), or multilamellar vesicles (MLVs) (Fig. 6.1, Table 6.2) [6, 9]. Most liposome formulations are composed of poly(ethylene glycol) (PEG)-modified lipids, which have been proven to not syndicate post-intravenous administration, thus increasing circulation time [10]. When used to encapsulate therapeutic agents, they have been proven beneficial in their ability to overcome barriers and cellular uptake, enhancing biodistribution, increasing intravitreal half-life, and targeting and stabilizing therapeutic compounds through their unique and various physicochemical properties [8, 9].

Regardless of the exact method of liposome formulation, there are four basic steps that are uniform: drying lipids from the organic solvent, dispersing the lipid within an aqueous media, purification, and analyzing the product [11]. The most commonly used methods of liposome formulation are solvent evaporation,

Table 6.2 Classification of liposomes [8]

Classification	Size
Small unilamellar vesicles (SUVs)	20–200 nm
Large unilamellar vesicles (LUVs)	200 nm–1 μm
Giant unilamellar vesicles (GUVs)	>1 μm
Multilamellar vesicles (MLVs)	>0.5 μm

reverse-phase evaporation, detergent dialysis, and lipid film hydration [8, 12]. Once the vesicle is formed, the liposome is characterized through photomicroscopic observation, paper chromatography, and/or transmission electron microscopy observation (TIFR). Photomicroscopic observation determines physical morphology, while TIFR determines size through utilization of a 0.1% Formvar film 300-mesh copper grid [12].

Drug Loading

The utilization of liposomes can be applied to both hydrophilic and hydrophobic drugs. Once loaded, hydrophilic therapeutic agents become encapsulated in the aqueous layer. However, hydrophobic drugs will become bound within the lipid bilayers rather than the aqueous layer. Liposomes may also be utilized as carriers for ionic molecules through the preparation use of either cationic or anionic lipids [8]. Drugs can be encapsulated within liposomes through either passive or active loading techniques. Passive techniques are utilized to add drug during the formation of the liposome (Table 6.3), and active loading is utilized to add drug after the liposome has already been formed [11]. More specifically, passive techniques primarily involve hydration of the lipid within an aqueous solution of the desired therapeutic agent. This method results in a co-dispersion of liposome and drug due to the ionic, hydrophilic, and hydrophobic interactions. Although this method may be seen as convenient, the maximum encapsulation efficiency obtained is approximately 80%.

Table 6.3 Passive liposome drug-loading techniques [11]

Passive loading techniques			
Method	Types	Advantages	Disadvantages
Mechanical dispersion	Sonication	Most commonly used	Low internal volume: Encapsulation efficacy Potential degradation
	French pressure cell: Extrusion	Proteins not exaggerated like in sonication method	Difficult to obtain appropriate high temperature
	Freeze-thawed liposomes	Convenient and simple	Can only obtain encapsulation efficacies between 20% and 30%
Solvent dispersion	Ether injection	Simple	Results in heterogeneous population between 70 and 200 nm
	Ethanol injection	Simple	Results in heterogeneous population between 30 and 110 nm Difficult to remove ethanol
	Reverse-phase evaporation	Results in high encapsulation efficacy (~80%)	Contact of encapsulated materials may lead to protein denature

Table 6.4 Characteristics of different liposomal drug delivery systems [9]

Liposomal drug delivery system	Description
Conventional liposomes	First-generation liposomes Can be cationic, anionic, or neutral Reduces toxicity Rapidly cleared from bloodstream
Ligand-targeted liposomes	Targeted drug delivery Can add multiple ligands (i.e., antibodies, peptides/proteins, carbohydrates)
Sterically stabilized liposomes	Improved circulation time Decreases drug side effects

The method of active loading requires the drug to be added to the liposome post ion gradient formation. Once added, the drug passes the bilayer(s) and becomes trapped within the liposome's aqueous core [10]. The most common types of liposomal drug delivery systems utilized today are conventional liposomes, sterically stabilized liposomes, and/or ligand-targeted liposomes (Table 6.4) [9].

The capacity of liposomal drug loading is dependent on the size of the liposome, the type of lipid utilized in formulation, as well as the physicochemical properties of the drug being encapsulated. For instance, the drug-loading capacity of MLVs is far greater than the drug-loading capacity of SUVs due to their size. However, LUVs have been found to be the optimal size for most therapeutic agents [8]. Once the drug is encapsulated within the liposome, encapsulation efficiency is determined.

$$\text{Encapsulation Efficiency} = \frac{\text{Encapsulated Drug Amount}}{\text{Total Drug Amount}} - \times 100$$

Drug Delivery

Administered drug-loaded liposomes may release drugs through passive diffusion or vesicle erosion [8]. Through passive diffusion, therapeutic agents are able to penetrate the lipid layers of the liposome for desired administration, where the rate of diffusion depends on composition, side, and physicochemical properties [8]. Therefore, if the desired delivery is through passive diffusion, unilamellar vesicles will have greater success than multilamellar vesicles. The drug delivery mechanism of vesicle erosion involves both phospholipase and high-density lipoproteins, which are both present in blood plasma. Upon contact with the liposomal drug carrier, the phospholipid layers are damaged resulting in the release of drug. In this method, the rate of release is dependent on the rate of membrane disruption [8]. The way a liposome interacts with a cell for drug delivery in vivo is dependent on the surface charge, size, composition, and ligands present on the liposome as well as its

microenvironment. Once the liposome comes in contact with the cell, the drugs may be delivered through adsorption, fusion, or endocytosis [8].

Targeted Drug Delivery

Targeting the drug-liposome complex can be done through either active or passive mechanisms [13]. Passive targeting involves diffusion through less permeable membranes without any ligand conjugations. In active targeting, the liposome would have specific ligands conjugated to its coating that will bind to receptors on the target cells [13].

For example, Smith et al. studied the expression of folate receptor alpha within the retinal pigment epithelium of mice via assays, immunohistochemistry, reverse transcription-polymerase chain reaction (RT-PCR), and laser scanning confocal microscopy. The results showed that the receptor is present and functions within the RPE [14]. Therefore, one may assume that there is potential to utilize folic acid ligands on the surface of drug-encapsulated liposomes to ensure a targeted drug delivery to the posterior segment.

Application in Ocular Drug Delivery

The main benefits of utilizing liposomes as drug delivery carriers are as follows [8, 11, 15]:

- Sustained drug release.
- Site-specific targeting and passive targeting.
- Improved solubility of amphiphilic and lipophilic drugs.
- Increased penetration into desired cell/tissue/organ.
- Biodegradable and biocompatible.
- Enhances therapeutic effect.
- Improves pharmacokinetic profile.
- Reduces toxicity that is associated with higher dosages.

When looking specifically at ocular drug delivery, liposomes have the ability to become barriers while in contact with ocular surfaces and, therefore, are able to protect therapeutic agents from the metabolic enzymes found at the corneal epithelium – tear junction [16].

In addition to their unique ability to protect their encapsulated drugs, the liposomes behavior with the eye is also dependent on its surface charge. Specifically, there is an electrostatic interaction between the negatively charged surface of structures within the eye and the positively charged liposomes. This allows for an increased viscosity as well as a decrease in drug elimination rate within the site of interest [3].

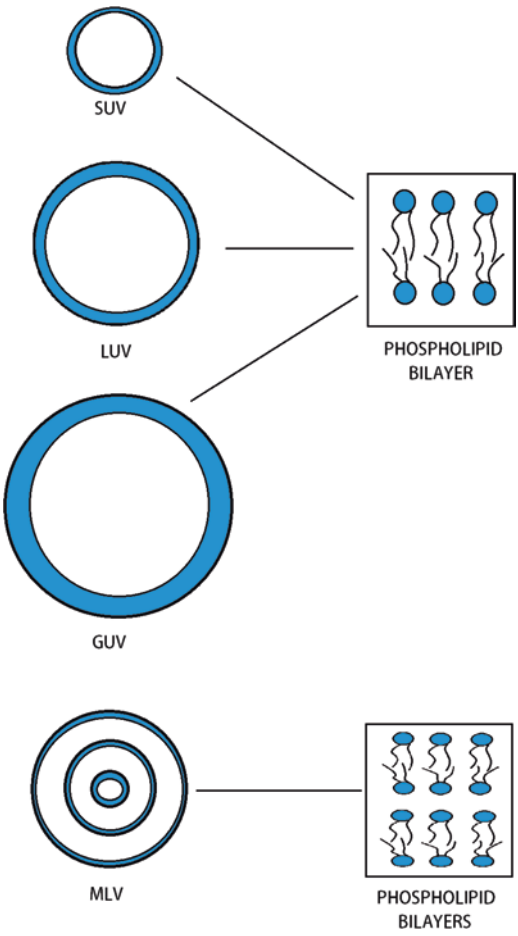

Fig. 6.2 Visual representation of various liposome structures

Role of Surface Charge and Vesicle Type in Liposome-Corneal Interaction

Schaeffer and Krohn utilized rabbits to investigate the role of vesicle type in transcorneal permeation by preparing 3.0×10^{-5} M penicillin G encapsulated with liposomes and observed fourfold higher corneal flux in vitro [17]. The results of the study found corneal permeation in the order of SUV(+) > MLV(−) > SUV(−) > SUV > MLV free drug. Therefore, the negatively charged corneal cells had the highest affinity for the positively charged SUV liposome. The intravitreal method of administration of drug-loaded liposomes in this manner has also resulted in vitreal condensation/clouding and other retinal abnormalities [8]. However, compared to conventional treatments, the sustained release of drug-loaded liposomes reduces the frequency of the injections resulting in a decrease in these side effects (Fig. 6.2).

Passive Targeting Drug-Encapsulated Liposomes

It has been known that cancers possess a general imbalance in angiogenic factors. For example, tumors demonstrate an overexpression of vascular endothelial growth factor (VEGF). This increase results in a more permeability extracellular matrix, allowing for higher, more localized drug concentrations in the tumor [13]. Several liposome-based formulations of drugs in the treatment of cancers have already been approved for human use such as Doxil™ [13]. This liposome-encapsulated form of doxorubicin shows greater efficacy with lower toxicity due to its passive targeting mechanism via increase tumor permeation and the enhanced permeability and retention (EPR) effect.

Similarly, posterior segment diseases such as AMD also display an increase in VEGF, and conventional treatments are used to suppress this factor [1]. Through the utilization of similar methods taken for anticancer drugs, it is possible that this same treatment may also be utilized to treat posterior segment diseases with an increased efficacy and lower toxicity. Therefore, there will be far greater patient compliance due to an annual decrease in intravitreal injection frequency.

Low Molecular Weight Chitosan-Coated Liposomes

Li et al. coated liposomes with low molecular weight chitosan (LCH) to be evaluated as possible candidates for ocular drug delivery. Chitosan is a cationic polymer that is water-insoluble under physiological pH value. However, when the molecular weight of chitosan is decreased through the use of physical, enzymatic, or chemical depolymerization, water solubility may be achieved due to the decrease in hydrogen bonds. The liposomes were loaded with diclofenac sodium (DS) via injection method. The liposome was then coated with 5% LCH, and encapsulation efficiency was determined (Table 6.5). When researchers utilized chitosan during liposome formulation, the results showed a significant improvement in the precorneal residence time due to the mucoadhesive nature of the chitosan in addition to the unique sustained release abilities provided by the liposome carrier. The biodegradability is also advantageous as the degradation products of both the liposome and the chitosan are nontoxic [15].

Table 6.5 Encapsulation efficiency of low molecular weight chitosan (LCH) liposomes [15]

Formulation	Encapsulation efficiency (%)
Non-coated liposome	99.6 ± 4
LCH-coated liposome	99.8 ± 0.5

Challenges and Limitations in the Utilization of Liposomes as Drug Carriers

Although the potential advantages of using liposomes as drug carriers are promising, there are several challenges that researchers must learn to overcome. For example, formulation methods vary and sterilization may be difficult, storage of drug-loaded liposomes may be problematic due to a short shelf life, and long-term in vivo side effects are currently unknown [3, 8].

Conclusions and Future Prospective

The utilization of liposomal drug carriers has shown decreased toxicity as well as clearance for some drugs while maintaining an increased half-life. The drug release can be sustained with optimal drug concentrations in the site of interest and does have the ability to decrease the frequency of intravitreal injections in posterior segment diseases such as AMD [6].

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Chapter 7
Nano/Microparticles for Retina and Posterior Diseases

Anita Patel, Jayvadan K. Patel, and Elie Beit-Yannai

Abstract Treatment and management of diseases of the posterior segment of the eye such as age-related macular degeneration, cytomegalovirus retinitis, diabetic retinopathy, posterior uveitis, retinoblastoma, retinitis pigmentosa, and choroidal neovascularization is a challenging task due to the anatomy and physiology of ocular barriers. For instance, traditional routes of drug delivery for therapeutic treatment are hindered by poor intraocular penetration and/or rapid ocular elimination. One possible approach to improve ocular therapy is to employ nanotechnology. In this chapter, the focus will be on the products of nanotechnology, having at least one dimension in the nanoscale including nano/microparticles with and without targeting ligands, which are making a significant impact in the fields of ocular drug delivery and gene delivery. Additionally, the use of nano/micro-carriers, such as cyclodextrin nanoparticle, polymeric nanoparticle, and functionalized nanoparticle for the treatment of retinal and posterior diseases, has been discussed. Although the above nano/microparticles may be administered by various routes including topical, intravenous, intravitreal, and periocular, each nano/microparticles should be tailored for the disease, drug, and site of administration. In addition, recent advances in the research and development of drug delivery methods of the posterior chamber of the eye, with an emphasis on the use of nano/microparticles, have been summarized.

Keywords Posterior segment · Ocular barrier · Nano/micro-carriers · Biodegradable

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Implications of Nanotechnology in Posterior Segment Ocular Drug Delivery

Nanotechnology is a recent hot topic issue because of its prospective to appreciably impact a number of fields related to biology, chemistry, engineering, as well as medicine. It has gained momentous research attention over the past decade. Nanoscience refers to the study of material characteristics at the nanometer scale. The use of nanotechnology is being investigated for numerous different ophthalmic applications for back of the eye diseases. These applications comprise enhanced drug delivery to the target tissue for the management of posterior segment disorders, enhancing diagnostics and retinal prosthesis. A variety of drug delivery devices in the nanoscale including microparticles, nanoparticles, and nanoconjugates have been explored for improved drug delivery [1, 2].

The eye is a relatively isolated organ divided into anterior and posterior segments with numerous avascular structures [3]. In this regard, the effectiveness of topical drug delivery via eye drops is only restricted to the treatment of anterior segment eye diseases. Drugs can enter the posterior segment of the eye via three distinguishing noninvasive routes:

- After topical application through the conjunctiva/sclera.
- After topical application from the cornea and aqueous humor.
- After topical, parenteral, oral, or other administration routes that deliver drug to the blood circulation from the systemic circulation.

The eye, principally the posterior segment, is composed of tissues that are complicated for drugs to penetrate owing to structural peculiarities such as the barrier function. As a result, many research studies on nano/micro-sized drug carriers have been conducted in the field of ophthalmology [4, 5]. Current treatments for posterior segment diseases suffer from significant disadvantages including frequent administration and potential for injection-related adverse events, which threaten patient quality of life. Nanomedicines can be designed to significantly improve patient compliance and ultimately, therapeutic outcome. The use of micro- and nanoparticles for treating ocular disorders brings some important advantages such as [6]:

- Ensure the localized drug delivery.
- Diminish the peak concentration resulting in minimizing the burst effect in drug release kinetic having as a main consequence the decrease of the system toxicity.
- Decrease the frequency of administration by increasing the drug's half-life.
- Improve patient compliance.
- Increased sensitivity and selectivity as compared to conventional diagnostic agents.

Barriers that Restrict Intraocular Drug Transport

There are a plethora of ocular diseases that may be vision threatening. The responsiveness toward characteristically developed drugs is limited, and the majority fails to correct the underlying problem. Therefore, there is a lack of truly curative treatments for most eye diseases. The main reasons for these limitations are biopharmaceutical problems correlated to the unique characteristic of the eye that limits the permeability of the pharmacological agents. The eye is to some extent isolated from the rest of the body by several types of barriers that hinder the effective passage of many drugs (Fig. 7.1), leading to minimum dose absorption [7]. These barriers consist of the following:

(i) Muco-aqueous layer of the tear film to protect the anterior surface of the eye.
(ii) Corneal epithelium with abundant tight junctions and desmosomes.
(iii) Iris blood vessels that lack fenestrations.
(iv) Nonpigmented layer of the ciliary epithelium that constitutes the blood-aqueous barrier and restricts the passage of molecules from the blood to the inner part of the eye.
(v) Retinal pigment epithelium (RPE), together with the endothelium of the retinal vessels, which makes up the inner and the outer blood-retina barriers, in that order, that restrict the passage of molecules from the blood to the retina and vitreous cavity.

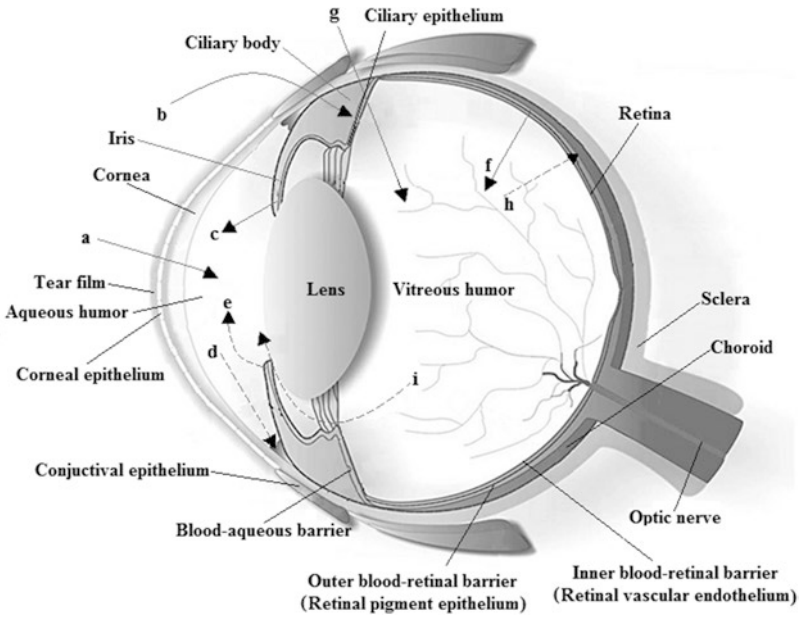

Fig. 7.1 Schematic presentation of the ocular structure showing a summary of ocular pharmacokinetics

In Fig. 7.1 the alphabets refer to following processes:

(a) Transcorneal permeation of drug from the lachrymal fluid into the anterior chamber.
(b) Non-corneal permeation of drug across the conjunctiva and sclera into the anterior uvea.
(c) Distribution of drug from the bloodstream via the blood-aqueous barrier into the anterior chamber.
(d) Elimination of drug from the anterior chamber by aqueous humor passage into the trabecular meshwork and Schlemm's canal.
(e) Elimination of drug from the aqueous humor into the systemic circulation across the blood-aqueous barrier.
(f) Distribution of drug from the blood into the posterior eye across the blood-retina barrier.
(g) Intravitreal drug administration.
(h) Elimination of drug from the vitreous via the posterior route across the blood-retina barrier.
(i) Elimination of drug from the vitreous via the anterior route to the posterior chamber.

Drugs penetrate the epithelium either transcellularly or paracellularly. The transcellular route predominates for lipophilic drug molecules, while the paracellular route predominates for hydrophilic molecules as well as small ions. The pore size has been predictable to be about 1 nm (permeable for drugs with molecular weight less than about 700 dalton) even though studies have specified that some pores could be up to 5 nm in diameter [8, 9]. It is accepted that the majority of drugs permeate the epithelium through passive diffusion, and even if drug transporters have been located in the epithelium, their consequence is still indistinct [10]. Consequently, less than 5% of the drug can permeate the corneal barrier and gain access to the inner eye [11–13].

The endothelium is a single cell layer membrane with large intracellular junctions. It can be believed as a permeable lipophilic barrier that offers no penetration resistance toward hydrophilic drugs but may propose a little resistance toward lipophilic drugs [14]. The conjunctiva is approximately 15–25 times more porous than the sclera, and the sclera is approximately 10 times more porous than the cornea [15]. The choroidal vasculature can contribute to drug clearance from the eye and, therefore, make up a permeation barrier during drug penetration from the eye surface to the retina and vitreous. While systematically administered drugs can reach the choroid membrane, drug delivery into the retina or the vitreous body is complicated to achieve through conventional methods due to the presence of the blood-aqueous barrier in addition to the inner and outer blood-retina barriers in those structures [16]. Direct intravitreal injection of drugs into the vitreous cavity is used to attain higher drug concentrations in the vitreous and the retina [17–20]. Nonetheless, repetitive injections are necessary to maintain drug concentrations at an effectual therapeutic level over a certain period of time for the reason that the half-life of drugs in the vitreous is relatively small.

Furthermore, certain physiological processes put into the poor effectiveness of conventional drug formulations. For example, blinking and tear drainage through the lachrymal drainage system take steps to diminish the residence time of topically applied molecules. Eye drops placed onto the ocular surface are washed away in less than 30 s [21]. The upholding of corneal transparency depends on several strategies, one of them being the sealing of the corneal epithelium using specialized structures, such as tight junctions and desmosomes. The corneal epithelium is then roughly resistant to any substance larger than 500 Da [15]. The majority of frequently used topical drugs are larger than that and, accordingly, do not permeate the cornea. In its place, they permeate throughout the conjunctiva and the underlying sclera in what is known as the "nonproductive passage." Overall, less than 5% of topically administered drugs reach the intraocular tissues [22]. So, the existence of numerous ocular tissue and cell barriers along with the physiological processes obstructs the efficient passage of many drugs, leading to the smallest amount of dose absorption into the eye.

What Is a Nano/Microscale?

Nanotechnology refers to a field of science whose unifying materials are about molecular scale on dimensions between 1 and 1000 nm**.** The macroscopic classification of ophthalmic dosage forms is not related to their microscopic structure, and they cannot per se be defined as micro/nanotechnology such as gel-forming solutions, powders for solutions, ophthalmic suspensions, ophthalmic ointments, ophthalmic emulsions (e.g., creams), ophthalmic gels, and ocular inserts [23].

Nano/microtechnological drug formulations are classified according to the diameter of their particulates. Microtechnology usually refers to technological devices with dimensions near 0.1–100 μm and particles between 0.1 and 100 μm, which are called microparticles. Microparticles can comprise micronized drug particles for intravitreal injection, but more often such particles consist of micronized polymer/drug matrix-like structures, i.e., microspheres or polymer-coated microparticles.

Nanoparticles are particles with at least 1 dimension less than 100 nm. Nanoparticles can refer to nanospheres in which case the drug molecules may either be incorporated in the matrix (e.g., drug/polymer matrix) or nanocapsules in which the drug is entrapped in a cavity enclosed by a polymeric shell (e.g., polymer-coated drug particles). Examples of nanoparticles for intravitreal drug delivery include albumin nanoparticles for delivery of ganciclovir and a fomivirsen analog [24] and tamoxifen-loaded nanoparticles [25].

Different Kinds of Nano/Microparticles for Ocular Delivery

Drugs can be incorporated into biodegradable polymers to form either a matrix system or a reservoir system [26]. In a matrix system, the drugs and polymer are combined, and the drug is released through diffusion from the polymer matrix with simultaneous polymer degradation. Nano/microspheres are matrical-type nanostructures that entrap or adsorb the biologically active molecule onto the surface. The reservoir-type system involves encapsulating drugs within polymeric shells and is the system used for biodegradable nano/microcapsules in which surrounding polymeric wall contains an oil core where the active molecule is dissolved [26]. These nanostructures can be coated with a hydrophilic polymer or even functionalized with antibodies attached to the coating.

There has been advancement in the design of nanoparticles, and Sánchez and Alonso [27] extensively reviewed the features of polymers used to prepare the carriers. The general term "nanoparticle" will be used in this chapter for the sake of simplification. Though, this term can refer to nanospheres or nanocapsules, all of which are more properly designated as colloidal systems represented in Fig. 7.2.

The first generation of colloidal systems consisted of polymerized matrices known as nanoparticles and nanocapsules composed of a polymeric wall containing an oil core. Second-generation systems were identical to nanocapsules but with better hydrophilicity associated with a coating polymer. Third-generation systems are functionalized by the attachment of antibodies or lectins, among other targeting moieties to the surface structure.

Many techniques have been developed for nano/microparticle preparation in respect to the predicted ocular application. The methods are physical, chemical, or else mechanical taking into account the nature of the polymer; polymeric nanoparticles can be made from natural or synthetic polymers. The most commonly used natural polymers are polysaccharides such as chitosan, alginate, hyaluronic acid and dextran and polypeptides like gelatin and other collagen materials and synthetic polymers are polyacrylates, polylactides (PLA), poly(lactide-co-glycolide) (PLGA)

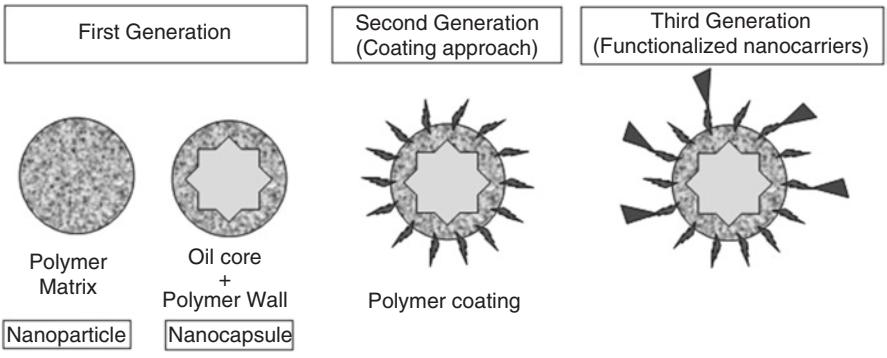

Fig. 7.2 Progression of colloidal systems

copolymers, poly(ε-caprolactone), poly(methyl methacrylate), poly orthoesthers, etc. frequently taken into consideration by researchers for nano/micro carriers preparation. The potential for their design is almost inestimable. The intended application influences the type of material used for their preparation [28–30]. These polymers are suitable for controlled-release applications because they are harmless and non-immunogenic and degrade through enzymatic reactions and hydrolysis to natural metabolic products over a period of months to years [31, 32]. Drug release profiles can be personalized through variations in polymer molecular weights and copolymer formulations [30].

What's more is that nano/microparticles can be prepared in different sizes, charges, and other physicochemical features. This presents vast adaptability upon them. The physicochemical characteristics of nano/microparticles not only bestow flexibility in terms of the kind of drug to be loaded, but they also influence the cellular uptake and intracellular trafficking. In addition, the physicochemical characteristics are critical for other properties, such as interaction with plasma proteins, other blood components [33], and with immune cells [34], all of which are pertinent to the organ distribution.

Disposition of the Nano/Microparticles from the Back of the Eye

Disposition or elimination of nano/microparticles from the site of administration depends on the physiological and pathological surroundings of the system, in addition to the properties of the nanoparticle and materials composing the system. Size, charge, recognition by inborn clearance mechanisms/cells/proteins, and rate of biodegradation are a few important parameters that can persuade nanoparticle disposition, although other properties may also have an effect on disposition.

For example, as the size of a macromolecule increases, its removal from the vitreous is reduced, at least to a point [35]. Likewise, relative to a small drug molecule in solution, particulate systems, including nanoparticles, extend the residence time of the drug in the vitreous, as well as other parts of the back of the eye [35, 36]. Given the negative charge of vitreal matrix components, it is projected that positively charged nano/microparticles may attach to vitreous matrix and be retained for longer periods relative to other types of particles [37].

If nano/microparticles are administered by routes other than the intravitreal route, the half-life will be different. Recent facts indicate that small nanoparticles of 20 nm in diameter are cleared more quickly from the periocular space than the suprachoroidal space [38–40]. Binding of proteins and other biological materials to the particles and changes in the properties of nanoparticles at the site of administration may change particle disposition [37].

The different materials used in preparing nano/microparticles may erode or degrade at different rates, resulting in particle disintegration and taking away of the smaller components thus formed. Understanding the disposition of a nano/micro-

system is one of the vital factors in determining the frequency of dosing, as well as selecting the most suitable route of administration for drug therapy. As well, understanding the disintegration and disposition of the nano/microsystem components and by-products would be helpful in establishing the safety of nano/microsystems.

Routes of Drug Delivery to the Posterior Eye

Achieving the therapeutic drug concentrations in the posterior segment of the eye continues to be a challenge in medical research, although in recent years important progress in this area has been made. Currently for targeting the posterior segment of the eye, four different types of drug administration routes are used: topical, systemic, intravitreal, and periocular administration (Fig. 7.3).

Topical Administration

Topical administration has the highest compliance with patients, but the therapeutic results of targeting the posterior segment are minimal on this route. Mainly, because of the anatomical characteristics such as the low permeability of the cornea, lachrymal secretion, and nasolachrymal drainage, transcorneal drug absorption is very low [41].

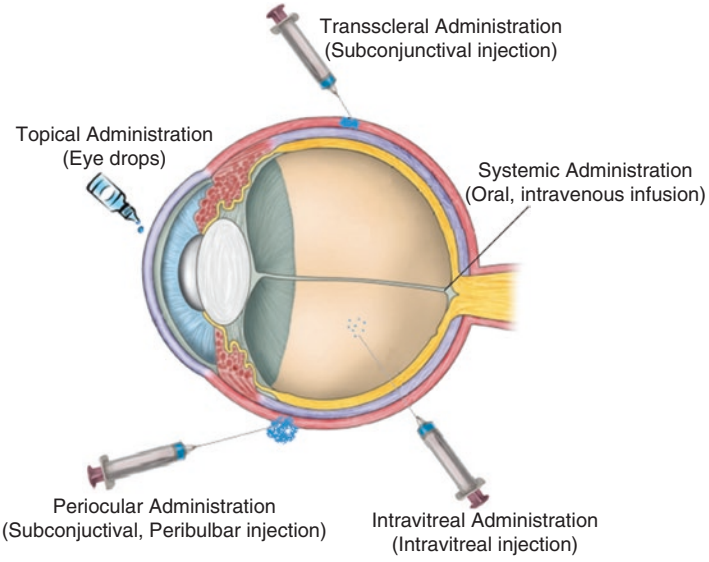

Fig. 7.3 Drug delivery routes to the posterior eye

Systemic Route

Systemic route has a slight role in delivering therapeutics to the posterior eye as it required administration of high concentrations of a drug to attain a therapeutic concentration in the posterior eye, because of the tight barrier properties of the retinal pigment epithelium. Such administration leads to adverse effects as a result of nonspecific tissue uptake of the drug [42]. The reduced bioavailability of 1–2% drugs across the blood-retina barrier demands the administration of high doses of drugs, and that can cause serious side effects. Still, several drugs with lipophilic properties (chloramphenicol, minocycline, cephalosporins) that can permeate the barrier are administered systemically in the treatment of serious ocular infections. Similarly, mannitol and carbonic anhydrase inhibitors, such as acetazolamide, are administered intravenously to reduce intraocular pressure [43].

Intravitreal Delivery

Intravitreal injection, an intraocular delivery mode, provides the highest bioavailability of the drug to the retina because of the proximity of the vitreous to the retina. Intravitreal delivery involves injecting the drug directly into the vitreous humor using a 30-G needle [44]. Today, a range of pharmaceutical forms are administered by intravitreal injection, such as aqueous solutions, viscous solutions prepared with hydrophilic polymers, suspensions, and nano/microparticulate systems [45]. Though, the release and distribution of medicinal products administered intravitreally depend both on the properties of substances and on certain physiological parameters. It is known that low molecular weight drug substances diffuse quickly and require frequent administrations, while large molecules (greater than 500 Da) can persevere in the vitreous humor for several days or even weeks. However, noteworthy side effects have been reported, including retinal detachment caused by repeated injections, retinal hemorrhage, endophthalmitis, uveitis, etc., because of high concentrations upon bolus dose administration [46].

Periocular Injections

Periocular routes of delivery include subconjunctival, sub-Tenon, retrobulbar, peribulbar, and posterior juxtascleral routes, which involve injecting outside the globe of the eye and in the proximity of the sclera to exploit high scleral permeability for retinal drug delivery. The delivery of the drug to the retina is most likely achieved by the diffusion of the drug through the sclera, choroid, and the retinal pigment epithelium. This mode of delivery to the retina is aptly known as the transscleral route of delivery of drugs to the eye [47]. Periocular injections are a less traumatic

route for patients and have negligible risk of adverse reactions compared with the intravitreal route. The accumulation of a drug "deposit" on the surface of the sclera can take place following the subconjunctival administration of a drug delivery system. The sclera has a much higher permeability to drug substances than the conjunctiva, which permits a facile transscleral permeation and diffusion of the drug substances [48, 49]. Periocular route is also a potential route for gene delivery since intravitreal injections may result in undesired transfection of cells important for vision [50]. However, repeated administration of periocular injections may bring about nonspecific absorption and unwanted systemic toxicity, particularly when low therapeutic index drugs are used [51].

Nano/Microparticles and the Posterior Segment of the Eye

Although the cornea constitutes one of the most selective barriers to foreign molecules for the eye, transcorneal permeation of topically administered ophthalmic drugs intended for the posterior segment is indefatigably required. It is because the greatest way to treat intraocular inflammation, either infectious or noninfectious, is by injecting drugs into the vitreous. The vitreous is a gelatinous, cell-free structure with the aim of retaining molecules and also delivering them to nearby structures, such as the ciliary body or the retina pigment epithelium, a vital component of the retina [52].

Repeated intraocular injections are required to treat retinal disorders. These injections turn up potential undesired side effects, higher risk of infections, and poor patient compliance. The view of frequent intravitreal injections to treat serious intraocular diseases affecting the choroid and retina has moved researchers to come across for healthier solutions derived from the use of nanoparticles as drug carriers [52].

As a result, much attempt has been invested in the last decade to optimize drug delivery systems for intraocular treatment. However, the capability to attain long-term drug delivery in the retina and nearby tissues while reducing the number of intraocular injections to just one seems practicable at this time. Numerous kinds of nanoparticles carrying different active molecules, including genetic material, are currently in preclinical studies using the abovementioned approaches [53–55]. The primary idea is to get benefit of the vitreous capacity for retaining and delivering molecules to tissues with which it is in direct contact and to use it like a biological reservoir once the nanoparticles are placed inside.

Uniqueness of Nano/Microparticles Which Make Them Ideal Candidate for the Treatment of Retinal and Posterior Segment Diseases

Bioadhesion and/or Rapid Internalization Capability

Topical route of administration can principally advantage from nanomedicines that quickly adhere to and/or internalize in the eye surface tissues including corneal and conjunctival epithelia. Several approaches have been assessed to get better ocular surface adhesion including the use of polyethylene glycol, poly(acrylic acid), and other mucoadhesive polymers to enhance corneal or conjunctival adhesion and hence retention [56–59]. On the other hand, these approaches permitted restricted prolongation in precorneal residence. Positively charged polymeric materials conversely may permit more prolonged retention on the eye surface. One study performed in rabbits indicated that when timolol- and brimonidine-loaded nanoparticles are formulated with the dendrimer hydrogel, single topical application sustained eye surface retention of nanoparticles and drug delivery in various eye tissues for up to 7 days, without inducing any disgusting toxicity to the cornea or conjunctiva [60]. In one more study, cyclosporin A was used as a model drug to show improved drug delivery to the eye surface, in the treatment of dry eye syndrome. De Campos and colleagues studied the ocular disposition of cyclosporine A-loaded chitosan nanoparticles, following topical instillation in rabbits [61]. Thus, nanoparticles can increase ocular surface tissue drug delivery, probably through mucoadhesion and/or enhanced paracellular delivery.

Enhanced Target Recognition and/or Cell Entry by Surface Modifications

Despite the fact that plasma membranes of cellular barriers let the passive diffusion of small molecule drugs, they are more limiting for the entry of hydrophilic small molecules and macromolecules, which are classically hydrophilic. For the entry of such poorly penetrable molecules, specific mechanisms of cell entry may subsist. For instance, solute transporters may aid the entry of small molecules, while proteins and large peptides may gain entry into cells via receptor-mediated endocytosis. The use of receptor-mediated endocytosis is a striking approach to increase cellular uptake of nanoparticles. It can be predicted that a small number of protein or peptide ligands coated on the surface of a nanoparticle might facilitate receptor interaction followed by internalization of the nanoparticle. Nanoparticles customized on their surface with inimitable features for improved delivery are referred to as functionalized nanoparticles. Such an approach can permit cellular entry of a larger amount of drug encapsulated in the nanoparticle, which may otherwise not enter the cells.

Transferrin receptor is renowned to undergo internalization and recycling [62], and respiratory epithelial cells internalize deslorelin, a peptide agonist of luteinizing hormone-releasing hormone (LHRH) receptor [63]. To augment nanoparticle cell affinity and uptake in ocular tissues, researchers investigated the possible value of conjugating transferrin or deslorelin on nanoparticle surface (nanoparticle functionalization). Nanoparticles (20 nm, polystyrene) functionalized with transferrin or deslorelin showed rapid (within 5 min) entry into the bovine cornea and conjunctiva and exhibited better uptake as well as trans-tissue transport in these tissues, when compared to the non-functionalized nanoparticles [64]. Such improved uptake and transport in conjunctiva are expected to make easy transscleral drug delivery to the back of the eye from nanoparticles.

Sustained-Release Capability

A number of diseases afflicting the posterior segment of the eye counting diabetic retinopathy and age-related macular degeneration require chronic treatment for periods of quite a few years. Intravitreal and periocular routes are the two major routes that can potentially deliver effective drug levels to the retina. Repeated injections by either route can lead to serious complications, raising safety concerns and decreased patient compliance. To prevent or lessen injection frequency, sustained drug delivery systems can be employed to deliver drug to the retina for prolonged periods. Several nano−/microparticle-based drug delivery systems have been investigated for transscleral sustained delivery of drugs to the retina. These systems comprise particulate systems like nano/microparticles [1, 65–68].

Responsive to Stimuli Including Light, Heat, Electrical Signals, pH, and Oxidative Stress

If triggered release of a high dose is desired in a localized manner, stimuli-responsive nanoparticles would be practical. Triggers for drug release can include light, heat, electrical signals, pH, and oxidative stress among others. Additionally, induction of a phase change such as a transition from a gel to a solution state or solid to a gel state can be used to trigger release by reason of enhanced diffusion in the transformed phase.

Eye, being a receptacle for light, is particularly right for light-responsive nanoparticles [69]. Light can induce changes in liposomal membranes such as an elevation in temperature, leading to disruption of the lipid bilayer followed by drug release. Additionally light-sensitive polymeric systems can be predicted for back of the eye applications. Photodynamic therapy, approved for treating subfoveal choroi-

dal neovascularization, makes use of nanoparticle-encapsulated photosensitizer for localization in the neovascular lesion following intravenous administration. Following accumulation, a non-thermal laser is used to activate the photosensitizer, subsequently leading to occlusion of the neovasculature. pH-sensitive nanoparticles can be triggered to release their content in endosomal compartments that are acidic. Iontophoresis can possibly be employed for triggered release of solutes from nanosystems, by driving appropriate ions into the environs of the drug delivery system in the eye.

Fujii and colleagues prepared magnetite and green fluorescent protein plasmid-loaded cationic liposomes and demonstrated the ability of magnetic forces to enhance the cellular expression of green fluorescent protein (GFP) when compared to Lipofectamine [70].

An alternative to triggered release is the formation of delivery systems including controlled-release systems in situ. It can be envisioned that some of the above triggers can be used to form delivery systems in situ. For instance, UV light-sensitive polymers [71] can potentially be coadministered with a drug to the eye, followed by UV cross-linking to form controlled-release systems in situ.

A novel approach for scavenging reactive oxygen species well-known in retinal degenerative diseases was presented by Chen et al. [72] and reviewed by Edelhauser et al. [73]. Cerium oxide nanoparticles, which are nontoxic, non-immunogenic, and defensive at a very low dosage, provided defense in vivo using a light-damage animal model.

Application of Nano/Microparticles for Retinal and Posterior Segment Diseases

The benefits of nanoparticle-based delivery comprise improved topical passage of large, poorly soluble and/or poorly permeable molecules such as glucocorticoid drugs or cyclosporine for immune-related, vision-threatening diseases. Other large and unstable molecules, such as nucleic acids, delivered using nanoparticles present hopeful results for gene transfer therapy in severe retinal diseases. As well, nanoparticle-mediated drug delivery increases the contact time of the administered drug with its target tissue, such as in the case of brimonidine, one of the standard treatments for glaucoma, or corticosteroids used to treat autoimmune uveitis, a severe intraocular inflammatory process. In addition, nanocarriers permit the non-steroidal anti-inflammatory drug indomethacin to reach inner eye structures using the transmucosal route. It seems that polymer-based nano/microparticles because of their diversity and reliability stand for an alternative solution for drug prolongation of the posterior segment drug delivery [52].

Natural Polymer-Based Nano/Microparticles for the Treatment of Posterior Eye Disorders

Polysaccharides from human (hyaluronic acid) and nonhuman (chitosan, alginic acid) sources are perhaps the most commonly used natural material for ocular drug delivery [74]. Chitosan is a cationic polysaccharide which presents lots of exceptional biological properties such as biodegradability, nontoxicity, biocompatibility, and mucoadhesiveness [75]. Ketorolac-loaded chitosan nanoparticles have been developed by Aşık et al. [76] as a possible alternative to repetitive intravitreal injections for treating ocular diseases such as pseudophakic cystoid macular edema.

Chitosan nanoparticles obtained by ionotropic gelation realized by Rajendran and coworkers had revealed an outstanding capacity for the association of acyclovir used for treating a wide ocular infection [77]. The results showed that chitosan nanoparticles can improve the corneal permeation, contact time, and bioavailability of acyclovir owing to the mucoadhesive properties of chitosan as a result of the electrostatic interaction between the positively charged chitosan nanoparticles and the negatively charged corneal cells. The same method, ionotropic gelation, has been used for preparing aptomycin-encapsulated chitosan nanoparticles as a new formulation for the treatment of bacterial ocular endophthalmitis [78]. Loaded nanoparticles were found to interact with mucin (a component of ocular fluids), and it seems that they are able to release the antibiotic directly to the site of infection and increase at the same time the residence time in the eye. The group of Motwani developed nanoparticles based on chitosan-alginate polyionic complexes again by ionotropic gelation [79]. The formed complex played the role of limiting the release of encapsulated drug in respect to each polysaccharide alone. The nanoparticles were loaded with gatifloxacin, an antibacterial agent used in the treatment of ocular infections; the release results are heartening as the drug is sustained released over by non-Fickian diffusion.

An association of oppositely charged polysaccharides, chitosan and alginate, has been utilized by Nagarwal et al. [80] for obtaining nanoparticles loaded with 5-fluororuracil (5-FU) designed for topical chemotherapy of conjunctival/corneal squamous cell carcinoma. They have found nanoparticles loaded with 5-FU formulation could be used for ocular application equally in the anterior as well as posterior segments. Wadhwa et al. [81] have developed another polysaccharide combination between chitosan and hyaluronic acid obtaining nanoparticles loaded with antiglaucoma drugs: dorzolamide hydrochloride and timolol maléate. It seems that hyaluronic acid augments the mucoadhesiveness and effectiveness of nanoparticles, and measuring the intraocular pressure for this formulation in comparison with a commercial product, diminish of this parameter, was by much more effectual in the case of chitosan-hyaluronic acid nanoparticles.

Synthetic Polymer-Based Nano/Microparticles for the Treatment of Posterior Eye Disorders

Natural polymers offer variable purity, and it is not easy to get reproducible particles and sometimes controlled-release profiles for the encapsulated drug. Quite the reverse, synthetic polymers are usually provided with excellent purity, and the drug release kinetic from synthetic polymeric nanoparticles can be also be modulated. Commonly used synthetic polymers for drug delivery applications include biodegradable aliphatic polyesters such as polylactides (PLA), poly(lactide-co-glycolide) (PLGA) copolymers, and poly(ε-caprolactone) as well as nondegradable polymers such as poly(methyl methacrylate) and polyacrylates.

Core-shell chitosan surface-modified PLGA nanoparticles for targeting glioma have been developed; PLGA was used as the core of nanoparticles, while chitosan was used as a coating shell [82]. Carmustine and O^6-benzylguanine were at the same time loaded in the nanoparticles and tested in vitro; the results revealed that nanoparticles enhance the plasma stability of carmustine and improve the cellular uptake of nanoparticles. Dexamethasone entrapped in biodegradable PLGA nanoparticles was developed by the solvent evaporation process for the treatment of acute and chronic posterior segment eye diseases such as uveitis [83]. The results proved their applicative prospective as the amount of dexamethasone encapsulated within nanoparticles seems to be enough for obtaining a therapeutic effect; the purpose of this study was to get better the encapsulation ability of PLGA nanoparticles to encapsulate dexamethasone.

Nanoparticles have been used to target the RPE for sustained drug delivery. Bourges et al. [54] explained the possibility of targeting the retina and the RPE using a single intravitreal injection of polylactide nanoparticles loaded with the dye, rhodamine 6G and Nile Red, which rapidly accessed the retina and were observed for 4 months postinjection. Kim et al. [37] used human serum albumin nanoparticles to track the movement of intravitreally injected nanoparticles as a function of surface charge and retinal injury. Anionic nanoparticles traversed the collagen fibrils of the vitreous more readily than the cationic nanoparticles, presenting prospective as drug delivery vehicles for the subretinal space and the RPE. Gaudana et al. [12] reported that ligands, such as folate and biotin, attached to the surface of steroidal nanoparticles, can increase uptake by the RPE.

Steroids such as budesonide and dexamethasone have been tested in polymeric nano/microparticles for sustained drug delivery. Kompella et al. [67] concluded that nano/microparticles containing budesonide, a corticosteroid, could inhibit vascular endothelial growth factor (VEGF) expression in vitro in a RPE cell line ARPE-19. PLA nanoparticles (345 nm) and microparticles (3.6 μm) containing budesonide were subconjunctivally injected in rats and were capable to sustain retinal levels of budesonide compared with the steroid solution alone. Loftsson et al. [84] estimated delivering steroids to the retina in rabbits by topical application of a low-viscosity aqueous suspension, which contained dexamethasone/γ-cyclodextrin microparticles (20.4 μm) and attained vitreal as well as retinal steroid concentrations equivalent to

levels observed 1 month after an intravitreal injection. Cortesi et al. [85] used spray-drying technique to encapsulate acyclovir in polyacrylic microparticles for ophthalmic administration that demonstrated a controlled drug release profile.

de Kozak et al. [25] examined the effectiveness of incorporating tamoxifen, a nonsteroidal estrogen receptor modulator, into polyethylene glycol-coated nanoparticles for the treatment of experimental autoimmune uveoretinitis. Intravitreal injection in a rat model performed 1–2 days before expected disease onset in controls considerably inhibited the disease because of a shift in the immune response from a Th1- to a Th2-type response. Sakai et al. [86] investigated the intravenous administration of PLA nanoparticles loaded with β-methasone phosphate and tagged with rhodamine to target experimental autoimmune uveoretinitis induced with S antigen peptide in a rat model. The nanoparticles accumulated in the retina and choroid within 3 h and remained for 7 days postinjection, ensuing a decline in the ocular infiltration of activated T cells and macrophages, as well as reduced hypertrophy of Müller cells.

Amrite and Kompella [39] determined that subconjunctivally administered nanoparticles and microparticles, of 200 nm and larger, could be retained at the injection site in rats for at least 2 months. Amrite et al. [38] demonstrated that periocular blood and lymphatic circulation affected the clearance rate of 20 nm particles administered through periocular injection in dead and living rats, observing only minor transport across the sclera and insignificant transport across the sclera-choroid-RPE. In an attempt to sustain retinal celecoxib delivery, Ayalasomayajula and Kompella [87] then incorporated celecoxib into PLGA (85:15) microparticles and administered them subconjunctivally in rats. Retinal drug levels were maintained for a 2-week period and inhibited diabetes-induced retinal oxidative stress. Amrite et al. [65] were capable of inhibiting diabetes-induced elevations in prostaglandin E2, VEGF, and blood-retina barrier leakage using a posterior subconjunctival (periocular) injection of celecoxib-loaded PLGA microparticles in a streptozotocin diabetic rat model. Therapeutic concentrations of celecoxib were maintained in the retina in vivo for 60 days and result in no damage to the retinal tissues.

Nano/Microparticles in Gene Therapy

Nano/microparticles have prospective in the field of gene therapy by functioning as nonviral vectors to facilitate cellular penetration, shield against degradation, and preserve sustained delivery. Panyam et al. [88] revealed that PLGA nanoparticles could flee the endo-lysosomal compartment and stop the degradation by lysosomal nucleases, a superiority essential for a drug delivery vehicle. The mechanism of rapid escape is by selective reversal of the surface charge of nanoparticles from anionic to cationic in the acidic endo-lysosomal compartment, which causes the nanoparticles to interact with the endo-lysosomal membrane and escape into the cytosol. Endo-lysosomal escape makes PLGA nanoparticles a smart delivery

vehicle for macromolecules, such as DNA, and drugs like dexamethasone. Bejjani et al. [53] investigated the use of PLA and PLGA nanoparticles as vectors for gene transfer to a bovine and a human ARPE-19 cell line. The plasmids used were green fluorescent protein for expression within the cytoplasm or red nuclear fluorescent protein for expression within the nucleus. Intravitreal injections in vivo in a rat model concluded that PLGA could successfully sequester and internalize plasmids, resulting in gene expression in RPE detectable 48 h postinjection and maintained for 8 days. Mo et al. [89] used human serum albumin nanoparticles loaded with the Cu, Zn superoxide dismutase (SOD1) gene for in vitro transfection studies using human ARPE-19 cells. The gene-loaded nanoparticles had a transfection efficiency of 80%, a fivefold increase in SOD1 expression over untreated cells and no cytotoxicity. Singh et al. [90] presented a novel application using an intravenous injection of surface-functionalized PLGA nanoparticles to target neovascular tissue for gene delivery of anti-VEGF intraceptor, an intracellular VEGF inhibitor, in a laser-induced, rodent model of choroidal neovascularization. Anti-VEGF intraceptor expression was increased in retinal vascular endothelial cells, photoreceptor outer segments, and RPE cells using intravenous administration of nanoparticles functionalized with either transferrin, arginine-glycine-aspartic acid peptide, or both, thereby inhibiting the progression of neovascularization.

Nanoparticle Safety: Toxicity and Interaction with the Immune System

Not just ocular but any biomedical application of nanoparticles as a therapeutic agent needs biocompatibility with living tissues by not producing toxic, injurious, or immunological responses in them. Key features influencing the biocompatibility of nanoparticles are the physicochemical characteristics such as charge, size, shape, solubility, and chemical groups on the surface that provide particle charge and lipo- or hydrophobic features [91]. Nanomaterials are receiving increasing attention for their promise as biomedical miracles, but unfortunately, their use may ultimately be limited because of concerns about toxicity. For instance, smaller size nanoparticles are favored for superior interactions at the cellular level. Nevertheless, smaller nanoparticles have larger surface area per unit mass, which may signify higher reactivity and thus, cell or tissue toxicity [92].

Risks posed by both organic and inorganic nanoparticles include aggregation, tissue accumulation, and adsorption of plasma proteins onto the surface. This latter consideration is particularly important when thinking about an intravenous nanoparticle administration or a potential access of the blood system using other non-systemic administration routes, e.g., intraocular administration to treat posterior segment diseases that involve blood-retina barrier impairment. Nanoparticles aggregation may block cell metabolism or even impair tissue function. For instance, aggregation of topically applied nanoparticles onto the ocular surface may block the

lachrymal drainage punctum and impair tear film recycling. Additionally, indiscriminate nanoparticle accumulation in ocular tissues may distort tissue architecture and, consequently, alter function. Finally, there may be toxic effects due to the presence of high levels of the loaded drug in a nontarget tissue.

Potential cytotoxic activity of nanoparticles may include alterations of cell membranes such as membrane disruption. For instance, Kiang et al. [93] used poly(propyl acrylic acid) to formulate chitosan-DNA nanoparticles with improved in vitro transfection efficiency. That polymer was specifically designed to interrupt the lipid bilayer in cell membranes. By changes in the pH, it triggered the release of DNA from the endosomal compartment. Very recently, Akagi et al. [94] evaluated the relationship of different physicochemical characteristics of 200-nm-size nanoparticles composed of poly(gamma-glutamic acid). The protein-loaded nanoparticles had remarkable hemolytic activity in erythrocytes, depending on nanoparticle hydrophobicity and pH, with the greatest activity present at pH 7–5.5 and absent at physiological pH.

Toxic effects of diverse kinds of nanoparticles have been reported in several organ systems. One of the main mechanisms described by which nanoparticles may damage cells and tissues is oxidative stress generation, which sequentially, may initiate the activation of different transcription factors [95]. In general, nanoparticles can be taken up by lymphatic nodes and distributed through the lymphatic system in parallel with the blood vascular system. The ocular mucosa possesses lymphoid tissue that drains to different face and neck lymphatic ganglia.

An additional consideration is the potential inflammatory, immunostimulatory, and immunosuppressive properties described for different kinds of nanoparticles [34, 96]. There are limited data available about this topic [96], but it is known that some nanoparticles are antigenic themselves. The antigenicity depends on particle size, especially those of ultra-small size (25 nm or smaller) that improves lymphatic uptake, and surface charge [97, 98].

At present, the most important issue related to the development of nanoparticle-based novel therapies is the scrupulous evaluation of the possible immunotoxic effects. Researchers in the field of nanomedicine agree that the possible environmental and health-related risks should be carefully analyzed. There certainly are regulations in Europe, the United States, and Japan intended to assess the immunotoxic potential of recently developed pharmaceuticals [99, 100]. However, there are no specific protocols for those nanotechnology-based tests because the properties of the nanoparticles may interfere with the established tests [101], the first step toward that purpose has been taken by the Nanotechnology Characterization Laboratory (NCL), US National Cancer Institute [102], whose mission is to carry out and standardize the preclinical characterization of nanomaterials intended for cancer therapeutics and diagnostics developed by researchers from academia, government, and industry. The NCL serves as a national resource and knowledge base for cancer researchers and facilitates the development and translation of nanoscale particles and devices for clinical applications.

Summary and Conclusions

Nanoparticles and other products of nanotechnology are made of a range of materials that are biocompatible to improve, target, or sustain drug and gene delivery. Even though nano/microparticles are administered through various routes, each nano/microparticle is tailored for the target disease, drug, and site of administration. Despite the fact that many therapeutic molecules may benefit from nanoparticles, nanoparticle technologies are readily applicable to molecules that are poorly soluble and/or permeable to improve their delivery. Nano/microparticles have shown great potential for growing the armory of drug delivery systems available for treating posterior segment disease attributable to their aptitude to provide sustained delivery and reduce complications that result from treatments requiring multiple injections. Intravenous administration of nanoparticles with surface modifications can target the retina. Transscleral delivery of anti-VEGF drugs loaded in PLA or PLGA nano/microparticles is gaining much attention as a feasible and effective method of administration for the treatment of posterior segment disease. It is currently feasible to design nano/microparticles with specific delivery requirements for ocular administration. Those nano/microparticles are able to safely deliver the loaded therapeutic molecule while preventing damage or deactivation of it. The loaded agents can act more efficiently and with fewer side effects when compared to the same agents administered without the nano/microparticles. Nevertheless, many questions still remain. It is a critical matter to resolve them so that new and more efficient drug formulations based on nanoparticle technology for ocular therapy can be made available for patient care. As nanoparticle technologies go forward for ophthalmic use, the safety, scalability, and core value of these delivery systems will be better established.

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Chapter 8
Nonviral Delivery Systems for Gene Therapy for Retina and Posterior Segment Disease

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Abstract Gene therapy is a hopeful strategy for the treatment of retinal disorders with no effective treatment. Gene replacement therapy is the most widely used strategy to modulate the gene expression in clinical research of inherited or acquired ocular diseases. Viral vectors are at the forefront of translational gene therapy mainly due to their high efficacy; nevertheless, concerns regarding safety have fostered the progress of nonviral therapy. Nonviral systems are non-immunogenic and avoid the risk of insertional mutagenesis. Moreover, they can be easily produced at large scale and have the potential to deliver larger genetic payloads. However, vector engineering to attain tissue-selective targeting and/or regulate the extent of gene expression is a challenging issue of nonviral gene therapy. Subretinal or intravitreal injections are the best option for the success of gene delivery to the posterior segment of the eye, regardless of the type of vector used. Preclinical studies with nonviral vectors have shown encouraging results for the treatment of macular degeneration and some inherited retinal disorders such as X-linked retinoschisis, Stargardt disease, retinitis pigmentosa, and Leber congenital amaurosis. These recent advances point to nonviral gene therapy as a feasible therapeutic tool for retinal disorders.

Keywords Nonviral vectors · Gene therapy · Ocular diseases · Solid lipid nanoparticles · Liposomes · Polymeric nanoparticles

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Introduction

Ocular gene therapy is a hopeful approach to treat, cure, or prevent diseases changing the gene expression in the eyes. According to the European Medicines Agency (EMA), a gene therapy medicinal product means a biological medicinal product which fulfills the following two characteristics: (a) it contains an active substance which contains or consists of a recombinant nucleic acid used in or administered to human beings with a view to regulating, repairing, replacing, adding, or deleting a genetic sequence; (b) its therapeutic, prophylactic, or diagnostic effect relates directly to the recombinant nucleic acid sequence it contains or to the product of genetic expression of this sequence [44]. The modulation of the gene expression to treat inherited or acquired pathological conditions can be addressed by introducing DNA, messenger RNA (mRNA), small interfering RNA (siRNA), microRNA, or oligonucleotides. Gene therapy based on the administration of DNA and mRNA acts by means of therapeutic protein supplementation, whereas the use of siRNA and microRNA provides a posttranslational gene silencing. An emerging field in the treatment of monogenic disorders is the genome editing, which corrects the disease by replacing a sequence of a defective gene by a healthy copy in order to restore the "wild-type" DNA, enabling the cell to produce what is needed to have optimal phenotypic outcome [32]. In vivo approach aimed at treating the mutations directly implies the use of sequence-specific endonucleases, such as meganucleases, zinc-finger nucleases (ZFNs), transcription activator-like effectors (TALENs), and CRISPR/Cas (clustered regularly interspaced short palindromic repeat (CRISPR)-associated) systems [104].

Among the organs targeted by gene therapy, the eye has been at the forefront of translational gene therapy largely due to appropriate disease targets and its suitable anatomic features. The main advantages of the eye when treated with gene therapy include the following: it has a well-defined anatomy, it is accessible, it is relatively immune privileged, it can be easily examined, and in the same subject, one eye can be used as the experimental target and the other one as a control [93]. These advantages have fostered research that has culminated in various gene therapy clinical trials for ocular diseases, most of them related to disorders of the retina, a major cause of severe vision impairment.

Various strategies can be applied in retinal gene therapy depending on the underlying disease. Gene replacement is employed for the treatment of disorders that are due to loss-of-function mutations, and it is based on the delivery of a correct copy of the defective gene without removal of the endogenous mutant one. Gene silencing inhibits the expression of a mutated gene via modification of mRNA, and it is applied to disorders caused by gain-of-function mutations [96]. In vivo retinal genome editing is under preclinical approach at the moment, although it is a very active field and advances at a rapid pace. Currently, in the database of Gene Therapy Clinical Trials Worldwide [50], 34 clinical trials restricted to "ocular diseases" are reported, with gene replacement therapy being the most widely used strategy.

The clinical application of gene therapy has been limited owing to many technical barriers. Among them, the development of safe and effective delivery vectors is a key challenge. Although viral vectors have substantially advanced the field of gene therapy thanks mainly to their high efficacy, concerns regarding safety are bringing interest in the progress of nonviral therapy. Viral vectors, apart from the immunological and oncogenic risk, present other disadvantages such as the limited gene size packaging capacity and production difficulties. Nonviral systems have the potential to overcome many of these drawbacks; they show generally a very low immunogenicity and avoid the risk of insertional mutagenesis. Moreover, they have also the potential to deliver larger genetic payloads, and their production is simpler, cheaper, and more reproducible than viral vectors [39, 89]. Nevertheless, despite the development of a wide variety of nonviral vectors, low transfection efficacy remains the main obstacle for the progress of these systems toward the clinic. In fact, 23 from the 34 clinical trials reported for ocular diseases use viruses as vectors. Different strategies and efforts are still ongoing in the field of nonviral vectors thanks to the advances in material sciences, including the design of new lipid and polymers useful for gene delivery, the rapid progress of nanotechnology, and the progress in nucleic acid chemistry [105].

Nonviral Vectors for Retinal Gene Therapy

Nonviral strategies based on physical methods (iontophoresis, electroporation, gene gun, nucleofection) have achieved considerable progress, but gene expression efficiency is still a limitation [20, 30, 79]. Chemical nonviral vectors are the most widely studied, including the nanoparticulated systems. Nanoparticles are exceedingly suitable for gene therapy because of their small size, ability to access the intracellular compartment, incredible surface-area-to-volume ratio, capacity to carry large payload, and minimal damage to cell membranes and cellular environment [77]. Another important advantage of nanoparticles is the capacity to transport different ligands such as antibodies, peptides, molecular sensors, and probes, among others, to target cells with high precision and specificity. Nonviral particles for gene therapy can be broadly divided into two groups depending on the material employed: lipidic systems, named lipoplexes, and polymeric systems, called polyplexes [108].

Lipidic Delivery Systems

The assembly of cationic lipids and nucleic acids through electrostatic interactions results in complexes named lipoplexes. However, cationic lipids confer excessive positive surface charge which has been shown to enable increased protein interactions and compromised distribution kinetics through rapid blood clearance as well as immune stimulation [47]. In order to minimize immunotoxicity of cationic lipids,

lipid nanoparticles (LNP) have provided remarkable results in recent clinical trials. Among LNP, liposomes and solid lipid nanoparticles (SLNs) are the preferred ones to deliver nucleic acids.

Liposomes are spherical vesicles composed of an aqueous compartment surrounded by a phospholipid bilayer of natural or synthetic origin, with size that can range from 20 nm to a few microns. Due to their resemblance to biological membranes, liposomes show higher biocompatibility than polymeric vehicles, which contribute to better delivery systems. Technological factors, such as the lipid-to-nucleic acid ratio or total lipid concentration in the final complex, are determinant for efficient gene delivery. Liposomal encapsulation of nucleic acids has shown to be an effective method to transfect corneal cells, inner retinal layer, and retinal pigment epithelia (RPE) [74]. In order to improve the efficacy and site specificity, significant effort has been dedicated to modify the composition and chemical structure of liposomes [1]. Different compounds have been incorporated to their structure, such as protamine sulfate [69, 88], poly-ethylene glycol (PEG) [85], or Arg(R)-Gly(G)-Asp(D) motif peptides [25].

SLNs are considered one of the most effective lipid-based colloidal vehicles [93]. SLNs consist of an aqueous dispersion of a layer of surfactants surrounding a solid lipid core, with particle sizes ranging from 50 to 1000 nm. Like liposomes, SLNs are composed of well-tolerated physiological lipids, often approved in pharmaceutical preparations for human use. Moreover, they have demonstrated good stability and can be sterilized and lyophilized. To improve the capacity of transfection, a variety of ligands can be incorporated on the SLNs surface, including dextran [41], protamine [40], cell penetration peptides [37], chitosan [43], or hyaluronic acid (HA) [5]. These components provide a higher protection to the genetic material, favor the cell internalization, and/or improve the trafficking of the nucleic acids inside the cell [93]. SLNs loaded with different plasmids have been shown to transfect retinal cells after intraocular administration by different routes in rats [42] and in mice [6, 7]. Other lipid-based systems studied for gene delivery to the retina include niosomes [73, 80] and span-polyarginine nanoparticles [86].

Polymeric Delivery Systems

Several polymers have been assayed to prepare polymeric nanoparticles, either of synthetic nature, such as poly-lactic-co-glycolic acid (PLGA), poly-L-lysine (PLL), or polyethylenimine (PEI), or being readily available in nature, such as chitosan or cyclodextrins [81]. These materials have a great potential for gene delivery because they generally have good biocompatibility and biodegradability, both properties related to their chemical structure. Another advantage is that polymers allow for adequate vector size as well as structural modifications, which is an important strategy to increase the efficiency of the delivery process. PEI nanoparticles have emerged as a powerful tool for nonviral transfection mainly because PEI promotes the endosomal escape; this system has demonstrated capacity to deliver antisense

oligonucleotides in vitro in rat retinal Müller glial cells and also in vivo after intravitreal administration [52, 59]. However, the cationic pDNA/PEI complexes have shown cytotoxicity on human RPE culture cells (ARPE-19) and strong aggregation in the vitreous body; high gene expression in the retina without such cytotoxicity after intravitreous administration was achieved by coating the PEI complexes with anionic polymers [65].

Polyesters, including poly(lactic) acid (PLA), poly(glycolic) acid (PGA), and their copolymer PLGA, have been also used for retinal nucleic acid delivery due to their ability to bind plasmids, their nontoxic features, and rapid internalization capacity [12, 23].

Polysaccharide-based nanoparticles are well suited for ocular gene delivery. HA and chitosan have been combined to obtain gene delivery nanoparticles (HA-CS-NP) for ocular applications [35]. The combination of HA-CS-NPs with cationic lipids has also been proposed as an effective nonviral vector for application in eye diseases [49].

Albumin [8], dendrimers [71], and PLL [70] are polymeric compounds also proposed as nucleic acid delivery systems for ocular applications. These compounds are able to protect the genetic material and to internalize them into the cell cytoplasm, increasing their presence in the nucleus.

Poly(2-(N,N-dimethylamino)ethyl methacrylate) (PDMAEMA) has been described as a very interesting polymer for gene therapy, and it is less toxic than PEI [106]. Recently, PDMAEMA, synthesized by reversible addition-fragmentation chain transfer in a defined-size polymer, has been able to direct gene expression in the RPE cell line D407 [15].

Barriers for Successful Nonviral Retinal Gene Therapy

The success of a treatment for a retinal disease based on gene therapy is greatly dependent on the selection of the most appropriate route of administration and the availability of a system efficiently internalized by the target cells. The ideal administration route will be the one that leads to the highest transfection rate for the targeted retinal cell type and the least risk of side effects. Topical administration is not currently an effective route to reach therapeutic concentrations of drugs in the back of the eye, especially in the case of large molecules such as the nucleic acids. Different periocular routes may be suitable for ocular administration including peribulbar, retrobulbar, posterior juxtascleral, sub-tenon, and subconjunctival, the most studied for gene delivery [60]. In a previous study in mice, after subconjunctival injection of RNA-loaded particles, most of them migrated toward the cornea, the targeted cells, although appreciable uptake by retinal cells was also observed [45]. In spite of the utility of the periocular routes, for a successful delivery of active molecules to the posterior segment of the eye, intravenous or intraocular administrations have been shown to be better options [11, 38]. The main barrier for drugs or genes injected systemically is the blood-retinal barrier, limited to large systemic

doses of lipophilic molecules. Targeting strategies have led to gene expression in the inner retina and the RPE after intravenous administration in mice, but bioavailability is still a major limitation [107].

Injection of the vectors into the subretinal space allows the contact of the nucleic acids with photoreceptors (PR), outer retinal layers, and RPE cells. Studies in mice have shown that subretinal delivery is the most effective to transfect PR and RPE cells; in fact, this route is the most used for inherited retinal diseases [108]. However, subretinal administration is an invasive method, and there is a high risk of ocular damage, i.e., lesions in RPE, hemorrhages, retinal tears, sub- or preretinal fibrosis, and retinal detachment [16]. Drug delivery by intravitreal injection is relatively easy, high doses are possible, and it is already routinely used. Although intravitreal delivery is less invasive than subretinal administration, adverse events such as retinal detachment or endophthalmitis may occur [66]. Suprachoroidal administration, below the sclera and above choroid, is safer than subretinal route and delivers the drug close to RPE, although it has shown to be little effective. Touchard et al. [97] have developed a transfection method called suprachoroidal electrotransfer, which combines the administration of a nonviral plasmid DNA with the application of an electrical field. However, from a practical point of view, only subretinal and intravitreal administration provide a significant concentration of the therapeutic compound in the target tissue.

Once the vector reaches the retinal cells, it has to overcome several physical barriers, including cellular internalization, escape from endocytic vesicles, diffusion through the cytoplasm, and, in the case of DNA-based systems, the transport into the nucleus [39]. Fig. 8.1 shows a scheme with the cellular barriers that a nonviral vector has to overcome for a successful delivery of the genetic material.

Modifications on the particle and genetic materials are designed to overcome all these obstacles.

Cell Membrane

Once the nucleic acid delivery system reaches and binds to the surface of the target cell, the entrance into the cell is initiated. In the absence of any specific ligand on the surface of the vector, the attachment to the cell surface occurs through electrostatic interactions between the charges of the vector and the cell membrane [36], with endocytosis being the most frequent pathway for internalization. Multiple mechanisms of endocytosis have been described: phagocytosis, macropinocytosis, clathrin-dependent endocytosis, or clathrin-independent endocytosis, which include caveolae-mediated endocytosis, flotillin-dependent endocytosis, GRAF1-dependent endocytosis, Arf6-dependent endocytosis, or RhoA-dependent endocytosis [36, 90]. The predominant entry mechanism depends on the target cell and on the composition of the vector.

Internalization of the vectors may be improved by using cell-penetrating peptides (CPP). For instance, in a previous study, SLNs were decorated with SAP, a

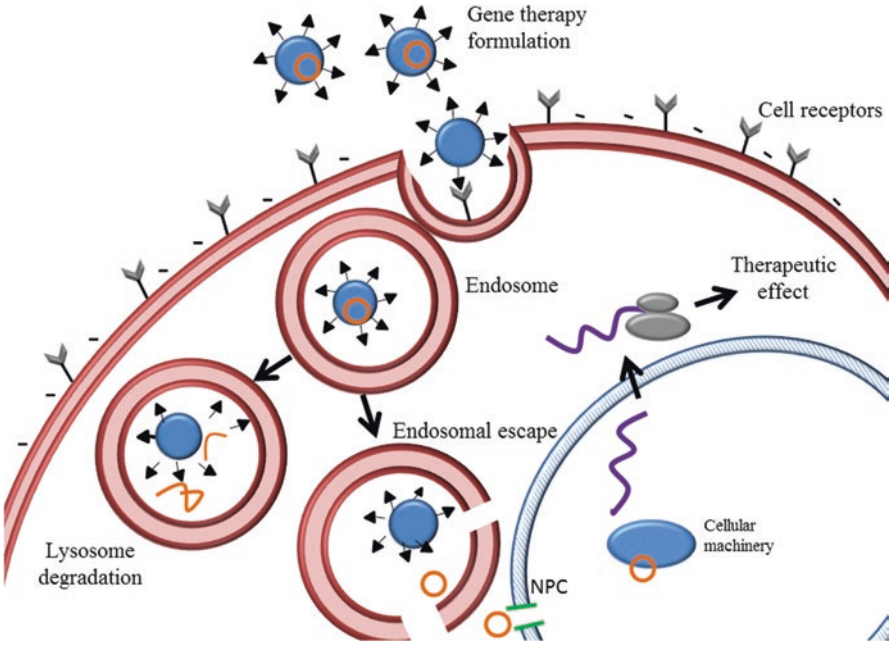

Fig. 8.1 Barriers that nonviral vectors need to overcome as nucleic acid delivery systems. (1) Binding to plasma membrane through electrostatic interactions between the cationic vectors and the negative charges of the plasma membrane or through interaction of ligands with specific receptors on the cell membrane. (2) Entry into the cell, mainly by endocytosis, which as ultimate step finishes in the degradative endolysosomes. (3) Escape of nucleic acids from the endosomes before degradation in lysosomal vesicles is crucial for effective transfection. Once released in the cytoplasm, RNA molecules will reach the intracellular target, whereas DNA molecules still have to bypass the nuclear membrane (4). In mitotic cells, DNA enters during interruption of the nuclear membrane, but in postmitotic cells, nuclear localization signals are needed to lead to active translocation by means of interaction with nuclear pore complexes (NPC)

proline-rich peptide whose sequence is $(VRLPPP)_3$ (made with three repetitive VRLPPP units; V = Val, R = Arg, L = Leu, and P = Pro) and that has demonstrated good translocation properties and is non-cytotoxic. SAP was able to improve the transfection efficacy of the SLNs in ARPE-19 cells because it induced a change in the dominant internalization mechanism, from clathrin endocytosis to caveolae/raft-dependent endocytosis, thereby decreasing the lysosomal pathway and consequently, reducing vector degradation [37]. In another study, a complex prepared with liposomes, protamine, and DNA was modified with the TAT protein of human immunodeficiency virus 1 (HIV-1), a cell-permeable peptide. This system resulted in efficient cell-specific delivery and a long-term expression of *Rpe65* gene to mice lacking this gene; as a result, in vivo correction of blindness was detected [88].

The use of ligands for targeting to specific cells has also shown to improve the transfection effectiveness of nonviral vectors by means of the binding to cell surface receptors. For instance, the polysaccharide HA binds to the CD44 receptor, which

is widely expressed in RPE cells, and it has been used to enhance the in vivo transfection efficiency of nanoparticles [7, 49, 103]. Cell internalization through the CD44 receptor avoids the degradation of the vector that occurs when other uptake mechanisms are involved, such as the lysosomal degradation after clathrin-mediated endocytosis.

Escape from Endosomes

Inside the cytoplasm, the release of the vector from either endosomes or lysosomes has been reported to be a major limitation for transfection. In the case of polyplexes, cationic protonable polymers can induce endosomal escape through the proton sponge effect, as mentioned above for PEI/DNA complexes. Vesicle-disturbing peptides conjugated to polyplexes may also facilitate the endosomal escape. Another strategy is the use of lysosomotropic agents, such as chloroquine, procaine, and spermidine, that promote pH buffering in endosomal vesicles [2]. Regarding lipoplexes, fusion of the cationic lipids has been proposed to facilitate not only the endosomal escape but also the DNA release [101].

Nuclear Envelope

Upon release in the cytoplasm, RNA molecules are available to reach the target and initiate the effect, but DNA has to bypass another important barrier, the nuclear membrane. When polyplexes are used as nonviral vectors, an incomplete polyplex dissociation in the nucleus has been proposed as a limiting step for efficient transfection [29]; in fact, polyplexes have been detected intact inside the nucleus, where they presumably undergo dissociation [17].

The entry into the nucleus is an important limiting step for transfection with DNA, as the nuclear membrane is a selective barrier to molecules bigger than 40 kDa, such as plasmids. In actively mitotic cells, the disruption of the nuclear membrane allows plasmids to enter into the nucleus; however, in postmitotic cells, like PR, the entry of large molecules depends on nuclear localization signals (NLS). NLS sequences lead to active translocation through the nuclear envelope. The peptide protamine presents NLS sequences of six consecutive arginine residues, and it is frequently used as a component of nonviral vectors. In retinal cells, this peptide has shown to be able to significantly improve the transfection efficacy of nonviral vectors [40] and also in vivo after ocular administration [42, 88].

Decreasing the size of genetic material may help to increase nuclear internalization and transfection efficacy. In this sense, the minicircles, compact DNA vectors that lack a bacterial backbone, have led to superior levels and longer duration of gene expression with respect to full-length DNA plasmids [61].

Gene Expression

Plasmids delivered via nonviral vectors can be maintained episomally, thus avoiding the risk of insertional mutagenesis, although transient instead of stable transfection is usually achieved. Vector engineering to attain selective tissue targeting and/or regulation of the extent of gene expression is a challenging issue of retinal gene therapy that demands active research. Mammalian gene expression can be regulated by several elements such as enhancers, locus control regions, boundary elements, insulators, scaffold/matrix attachment regions (S/MARs), and CpG depletion [62, 82]. In this sense, the high load capacity of nonviral systems features an important advantage, allowing the inclusion of additional regulatory elements in order to target and to improve the level and long-term expression.

S/MAR sequences, which anchor chromatin to the nuclear matrix proteins during the interphase, were included in a plasmid containing the *RPE65* gene [63]. The plasmid, administered encapsulated in PEGylated-PLL nanoparticles to the subretinal space of *rpe65*$^{-/-}$ mice, led to a long expression of the transgene related to the stability of the expression cassette, which was isolated intact 1 year postinjection [64]. Various systems for transgene integration have been developed to promote long-term expression, such as transposition systems based on the recombinases FC31 and Sleeping Beauty. Integrase from bacteriophage FC31 has also conferred genomic integration of plasmid DNA and has led to long-term expression in rat RPE cells after subretinal injection followed by electrotransfer [24]. A nonviral strategy based on the Sleeping Beauty transposon system also resulted in stable expression of pigment epithelium-derived factor (PEDF) in ARPE19 cells [57]. These findings have conducted to the evaluation of this strategy as a possible treatment of age-related macular degeneration associated to neovascularization [58].

Tissue-specific promoters for retinal cells have been used as a strategy to circumvent the lack of cell specificity of nonviral vectors. For instance, structural improvement of the Rs1h-deficient mice retina has been shown after successful delivery of a plasmid containing the gene *RS1* under the control of a specific promotor for PR (murin opsin promoter, mOPS) formulated in SLNs [7]. Wang et al. used liposome-based vectors and different promoters that were able to achieve cell specificity for a variety of cell types: RPE cell specificity with vitelliform macular dystrophy (VMD2), rod cell specificity with mouse rhodopsin, cone cell specificity with red/green opsin, and ganglion cell specificity with thymocyte antigen promoters [100]. PEGylated liposomes containing an expression plasmid encoding bacterial galactosidase under the influence of either the simian virus (SV)40 promoter or the glial fibrillary acidic protein (GFAP) gene promoter have been used to target the cornea after intravenous administration [107].

The induction of the delivered gene expression only when it is needed is also a challenge. It can be achieved including inducible regulatory sequences in the promoter, which will be only active in the presence of specific environmental signals. In other cases, gene expression is regulated by drugs. For instance, an autogenous transgene regulatory system (ARES) is inducible by isopropyl β-d-1-thiogalactopyranoside

(IPTG), which has no off-target effects in mammals. Sochor et al. used this system to control reversibly the luciferase expression in the murine retina after oral delivery of IPTG [92].

Nonviral Vectors in Gene Therapy for Retina and Posterior Segment Diseases

Many studies in animals have demonstrated the potential utility of gene therapy for the treatment of ocular diseases. As a result, translational clinical research has started. Nevertheless, up to date, the clinical trials reported for ocular diseases use viral vectors or naked genetic material, and nonviral vectors are still restricted to preclinical phases.

X-Linked Retinoschisis (XLRS)

XLRS is a retinal degenerative disease affecting young males, caused by mutations in the gene *RS1* that encodes the secreted protein retinoschisin, with a prevalence of approximately 1:5000 to 1:25000 [91]. Disorganization of retinal layers and distinct abnormalities in the electroretinogram are hallmarks of the disease [94]. The splitting of retinal layers, with bilateral foveal schisis, is observed at early stages of the disease, and it results in cystic degeneration of the central retina. The progression and severity of XLRS is very variable leading to mild to severe loss in central vision. Currently, there is no cure for the schisis formation, and the treatment is focused on preserving the low vision.

Gene augmentation therapy may be an excellent therapeutic approach, due to the well-understood monogenic origin of this disease. In addition, retinoschisin is a secreted protein, and not only the transfected cells benefit from the replacement of the gene, since once secreted, the protein spreads from the site of expression. Novel therapies may be addressed in retinoschisin-deficient mice, which show close resemblance of the retinal phenotype with XLRS patients and represent an excellent disease model [18, 102]. Studies in the mouse model with viral vectors as delivery systems of *RS1* emphasize the potential of gene therapy for XLRS and highlight the importance of careful design and optimization for specific, minimally invasive, and long-lasting gene therapy [56, 83, 84]. Recently, a preclinical dose escalation study of intravitreal *RS1* gene delivery with viral vectors has been carried out [19]. Structural improvement was shown by reduction of retinal cavities 3–4 months after injection, and electroretinogram values were normalized at 3–4 months and 6–9 months postinjection, even when the production levels of retinoschisin were lower than in wild-type mice. A fully normal level of the protein expression seems not to be necessary for a therapeutic effect.

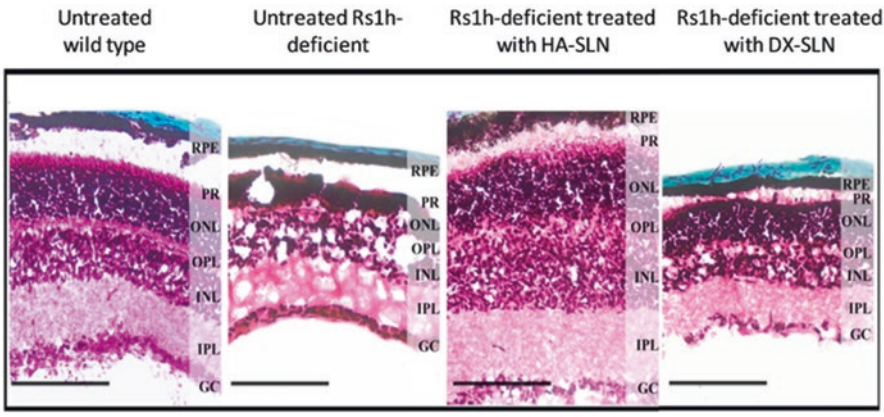

Fig. 8.2 Microscopic images of retinas stained by Masson's trichrome technique. Photographs show the differences in the retina structures of wild-type mice and retinoschisin-deficient mice untreated and treated with SLN-based vectors (HA-SLN and DX-SLN) 2 weeks after intravitreal administration. Green, connective tissue; dull green, muscle; dark blue, nuclei; pink, cytoplasm and muscle fibers. Images were captured at 20× magnification. Scale bar: 100 μm. (Reprinted from "Biomaterials, 90, Apaolaza et al., Copyright [7]," with permission from Elsevier). RPE Retinal Pigment Epithelium, PR Photoreceptors, ONL Outer Nuclear Layer, OPL Outer Plexiform Layer; INL Inner Nuclear Layer, IPL Inner Plexiform Layer, GC Ganglion Cell Layer

Nonviral vectors are a promising alternative to viral vectors for attempting the treatment of XLRS with gene therapy. Our research group has demonstrated the capacity of SLN loaded with a plasmid containing the *RS1* gene to transfect a number of retinal layers after ocular injection to the mice model of XLRS and to induce the production of the therapeutic protein retinoschisin. As it is shown in Fig. 8.2, the production of retinoschisin led to a partial recovery of the structure of the retina [6, 7].

These studies showed successful gene transfer using lipid-based nanocarriers, with promising results that point to nonviral gene therapy as a feasible future therapeutic tool for posterior segment disorders.

Stargardt Disease

Stargardt disease is the most common inherited juvenile macular degeneration in humans, with a pattern of autosomal recessive inheritance [14]. The gene involved in Stargardt disease is named *ABCA4*, which encodes for a PR-specific all-trans-retinal transporter [4]. Due to a defective ABCA4 protein, vitamin A aldehyde forms deposits in RPE cells during the process of disk shedding and phagocytosis. Consequently, abnormal high levels of lipofuscin pigments accumulate in the RPE, triggering RPE cell death and causing secondary PR degeneration [78]. The

impairment and loss of vision in Stargardt patients can be due to hundreds of mutations in the *ABCA4* gene. The mutations in this gene are also responsible for other visual diseases such as cone-rod dystrophy and autosomal recessive retinitis pigmentosa [33, 72]. Heterozygous mutations in *ABCA4* may lead to the development of age-related macular degeneration. At present, there is no cure for ABCA4-associated disease, and gene therapy has been proposed for Stargardt disease. Currently, a phase I/II clinical trial based on viral vectors for subretinal administration is underway [28]. The large size of the ABCA4 cDNA, a limitation for viral delivery, makes nonviral vectors as a suitable alternative. In a recent study [55], compacted DNA nanoparticles (8–10 nm in diameter) formulated with PEG-substituted PLL (CK30PEG) were used to inject *ABCA4* to *ABCA4*-deficient mice by subretinal route. After administration, the expression of the transgene was detected for up to 8 months, and a significant correction of functional and structural Stargardt phenotypes was observed, including improved recovery of adaptation to darkness and decrease of lipofuscin granules.

Retinitis Pigmentosa

Retinitis pigmentosa is the most common subtype of retinal degeneration, responsible for loss of vision in one in 4000 people worldwide [87]. Defects in more than 60 genes have been identified in patients with retinitis pigmentosa, as autosomal dominant (30–40% of cases), autosomal recessive (50–60%), or X-linked (5–15%) forms. Notwithstanding, mutation in 30–35% of patients cannot be identified [87]. The features of the disease and its progression vary significantly among patients, but night blindness due to loss of rod PR in the early phase of the disease is very frequent. Over time, cone PR are also affected resulting in decreased central visual acuity. As a consequence, patients describe tunnel vision, which may result on complete blindness [13, 54].

Gene therapy for retinitis pigmentosa aims to slow down or stop the progress of retinal degeneration. Preclinical evaluation in animal models provides expectations for future clinical application. Due to the variety of genes involved in the disease, several animal models have been developed. One strategy to generate animal models that mimics the human autosomal recessive retinitis pigmentosa is directed to mutations in the genes encoding the two rod cyclic nucleotide-gated (CNG) channel subunits. Knockout of *CNGB1* in mice results in a phenotype that recapitulates the principal pathology of retinitis pigmentosa patients [75]. Another animal model of retinitis pigmentosa is a dog deficient in a GTPase regulator-interacting protein 1 (*RPGRIP1*) [67]. Rhodopsin gene (*RHO*), which encodes the photosensitive pigment in rod PR, is another gene whose mutations (>100) have been identified in individuals with retinitis pigmentosa [87]. There has been an attempt to treat *RHO*-linked retinitis pigmentosa by means of a new strategy named "mutation-independent suppression and replacement," which comprises both gene suppression and gene replacement [76]. The Royal College of Surgeons (RCS) rat is a widely studied

animal model of retinal degeneration with a mutation in the MER proto-oncogene tyrosine kinase (*Mertk*) gene, and it serves as a model of an autosomal recessive form of retinal degeneration [34]. Both functional and structural retina preservations were achieved with gene replacement therapy in this model of *Mertk*-related retinitis pigmentosa. Based on this proof of concept, a phase I clinical trial of subretinally administered *Mertk* in a viral vector was conducted. Peak gains of greater than three lines of vision were observed in two of the six patients recruited in the trial [51].

The gene *RDS*, which encodes the retinal degeneration slow protein, is frequently associated to retinitis pigmentosa. A well-characterized animal model for *RDS*-associated retinitis pigmentosa (the $Rds^{+/-}$mouse) is available to provide a valuable and readily accessible in vivo system for developing and testing gene therapy [31, 98]. *RDS* gene replacement mediated by nonviral vectors has been assayed in this model after subretinal administration. Cai et al. [21] developed DNA nanoparticles consisting of single molecules of DNA compacted with PEG-substituted lysine 30-mer peptide (CK30PEG10K). Vectors were administered by subretinal route, and *RDS* mRNA levels peaked at postinjection day 2–7 and remained elevated at the latest time point examined, 120 days after administration. A significant improvement in the outer segment structures was observed, rod function (measured by electroretinography) showed statistically significant improvement compared with controls, and cone function in nanoparticle-injected eyes reached the wild-type levels. More recently, span-polyarginine (SP-PA) nanoparticles were developed to mediate gene transfer in the subretinal space of a mouse model of retinitis pigmentosa carrying a point mutation (A216P) in the *Prpf31* gene. SP-PA nanoparticles were able to efficiently transfect mice retinas with GFP and *Prpf31* plasmid. Statistically significant improvement in visual acuity and retinal thickness was found in mice treated with the SP-PA- *Prpf31* nanoplatform [86].

These findings confirm the potential of nonviral vector-mediated gene replacement as treatment of retinitis pigmentosa.

Age-Related Macular Degeneration

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in people aged 50 years or older in the developed world. More than nine million Americans have AMD, and cases are expected to nearly double by 2050 to 17.8 million [26]. Depending on the histopathological characteristics, AMD can be classified into several categories: early, intermediate, and advanced AMD [99]. In early and intermediate AMD, only minimal visual acuity impairment occurs, but advanced AMD is the leading cause of blindness worldwide. AMD may be avascular or may be characterized by the subretinal invasion of choroidal vessels. Whereas avascular AMD is a slow progressing disorder, in which PR degeneration follows RPE cell degeneration, neovascularization-related AMD progresses rapidly to blindness following RPE cell degeneration.

Among the multiple factors that play a role in AMD, the strong genetic contribution is well documented [48]. In fact, gene therapy is considered one of the improved treatments under study for AMD [46]. In a phase I clinical trial, 28 patients with advanced neovascular AMD were treated using adenoviral vector-mediated intravitreal gene transfer of PEDF, which is an antiangiogenic cytokine. This therapeutic strategy appears to help arrest the growth of neovascular AMD [22]. As mentioned above, currently a nonviral strategy based on the Sleeping Beauty transposon system is also under preclinical evaluation to achieve stable expression of PEDF as treatment of AMD associated to neovascularization [58].

Leber Congenital Amaurosis

Leber congenital amaurosis (LCA) is an autosomal recessive disease resulting from mutation in at least 15 genes [3]. Prevalence of LCA is 1 in 35,000 newborn of all blind children [95]. Patients with this severe retinal disease suffer from a marked impairment of the visual acuity at birth or during the first 6 months of life, sensory poorly reactive pupils, and severely diminished or non-detectable electroretinogram activity [95].

There is no successful treatment for LCA, but four independent clinical trials have been carried out for human RPE65-associated LCA [9, 10, 27, 68]. The *RPE65* gene encodes for all-trans-retinyl-ester hydrolase, which is a 65 KDa enzyme that in RPE is critical for the production of 11-cis-retinal. This compound is transported to the PR where it binds to apo-rhodopsin; the apo-rhodopsin-11-cis-retinal complex reacts with a photon to produce a change in membrane potential, which generates a nerve signal that travels to the visual cortex for image formation and recognition. Deficiency of all-trans-retinyl-hydrolase due to mutation in *RPE65* gene happens in about 6% of LCA cases in humans [95]. In the most recent clinical trial for RPE65-LCA, a phase III clinical trial, patients that received subretinally injections of the *RPE65* gene in a viral vector have shown successful improvement of the sensitivity to light and functional vision [53].

RPE65 gene has been also formulated in nonviral vectors composed of liposomes and protamine. Efficient cell-specific delivery and long-term expression of the *RPE65* gene in mice lacking RPE65 protein led to in vivo correction of blindness [88]. In order to obtain long expression of the *RPE65* gene with nonviral vectors, the S/MAR sequence was included in the corresponding plasmid. As mentioned above, this strategy led to detection of the expression cassette 1 year after subretinal injection to mice lacking the *RPE65* gene [63].

Conclusions

Ocular gene therapy is a hopeful approach to treat, cure, or prevent diseases by modulating gene expression in the retina and in the posterior segment of the eye. Human clinical trials are beginning to show encouraging results, although nonviral vector engineering to attain tissue-selective targeting and/or regulate the extent of gene expression is still a challenge. Preclinical studies with nonviral vectors have shown encouraging results for the treatment of some ocular diseases, such as macular degeneration, and some inherited retinal disorders including X-linked retinoschisis, Stargardt disease, retinitis pigmentosa, and Leber congenital amaurosis. These recent advances point to nonviral gene therapy as a feasible therapeutic tool for retinal disorders.

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Chapter 9
Oxidative Stress in Ocular Disorders: Exploring the Link to Pesticide Exposure and Potential for Using Nanotechnology for Antioxidant Delivery

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Abstract Ocular toxicity caused due to pesticide-induced oxidative stress is a topic of great interest in toxicological research in the recent past. The human eye is directly exposed to various toxins especially pesticides. Exposure to pesticides through various routes could lead to severe ocular disorders due to oxidative stress. Antioxidant biomolecules have a great potential to combat the effects of pesticides in ocular structures. Most of these antioxidant biomolecules lack bioavailability in ocular structures; hence developing a novel nanoparticle-based antioxidant formulation could solve this issue and can offer maximum therapeutic potential for antioxidant biomolecules in prevention/control of ocular toxicity induced by pesticide. This review gives a cumulated information on various reported studies on pesticide-induced oxidative stress and how it may cause ocular toxicity. Further in this review, we have discussed how nanotechnology product-based delivery of antioxidant biomolecules could reflect on their therapeutic potential in prevention or control of pesticide-induced oxidative stress and its further effect on ocular health.

Keywords Pesticides · Oxidative stress · Ocular toxicity · Antioxidants · Nanotechnology

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Introduction

Pesticides are chemicals or biological agents (such as a virus, bacterium, antimicrobial, or disinfectant) which are widely used in agriculture practices for enhancement of food production and to control disease vectors in public health [1–3]. The use of pesticide in our day-to-day life has drastically increased over the years; reports on pesticide use suggest that between 1996 and 2011 the estimated amount of pesticides used in the USA increased by 404 million pounds [4]. Though pesticides have some uses, they were demonstrated to have many harmful and potential toxic effects on human health like causing neurological disorders, cancers, and other health effects like irritation of skin and eyes and mimicking hormones causing reproductive problems [2, 5]. The World Health Organization and the UN Environment Programme in one of their reports estimated that in the developing countries each year, three million people are affected with severe poisoning from pesticides, and among them about 18,000 were reported dead [6]. Another study reported that, in developing countries, yearly around 25 million workers suffer from mild pesticide poisoning [5].

Exposure to pesticides could be through any of the common routes like inhalation, ingestion, and dermal contact [7]. Recent scientific reports including a case study have suggested that the ocular route could also be a route of pesticide entry [8]. The eye is the most sensitive organ which is directly exposed to various environmental toxins including various pesticides [9]. Pesticide exposure has been reported to cause disorders in various components of the eye (conjunctiva, cornea, iris, lens, and retina) [9]. The mechanism of pesticide-induced toxicity to the eye is still unclear; many studies suggest that the pesticide-induced oxidative stress could be a possibility for these disorders in the eye.

The increased use of pesticides in the recent past warrants the study of possible mechanisms that could lead to pesticide-induced ocular disorders. We have designed this review to aggregate and cumulate the information regarding pesticides' role in causing oxidative stress, and we further explained how oxidative stress could lead to various ocular disorders. In this article, we even described various studies depicting the potential role of natural antioxidant biomolecules that can control or prevent disorders due to pesticide-induced oxidative stress. Further in this review, we explained the possibility of nanobiotechnology interventions to improve the therapeutic potential of the natural antioxidant biomolecules in preventing ocular disorders due to pesticide-induced oxidative stress.

Oxidative Stress and Eye Disorders

The aerobic organisms produce the energy required to fuel biological functions in mitochondria via the electron transport chain [10]. In the process of energy production, the aerobic organisms also result in the formation of reactive oxygen species (ROS) which results in cell damage [10]. Apart from the biological path, ROS are

also produced due to exposure to environmental factors like UV radiation, smoking, alcohol consumption, and excessive drug intake [11]. Recent reports suggest that pesticide exposure is also a crucial factor in ROS production and modulating the antioxidant defense system [12]. This disturbance in antioxidant defense system and failure in its maintenance results in oxidative stress that is a leading cause of many ocular diseases.

Age-Related Macular Degeneration (AMD)

Age-related macular degeneration (AMD) results in damage to the macula (central retina), thus altering the central vision and further leading to blindness [13]. The retina of the eye is composed richly of polyunsaturated fatty acids. These fatty acids are known for high oxygen consumption, thus making the retina more susceptible to oxidative stress leading to AMD [14]. It has been speculated that the ability of retinal pigment epithelium (RPE) to digest rate of photoreceptor outer rod segments (POS) is decreased by the accumulation of melanin oligomers within lysosomes in RPE [15].

Retinopathy

Retinopathy is damage to the retina caused due to diabetes, arterial hypertension, and radiation [16, 17]. There is a prodigious amount of experimental evidence available suggesting that oxidative stress and the overproduction of ROS play a great role in diabetes-induced retinopathy [17, 18]. In diabetic conditions, the membrane lipid peroxidation and oxidative damage to DNA are elevated in the retina [19]. It has been well established that the antioxidant defense system which controls the ROS in the body is handicapped during the diabetic conditions due to overproduction of ROS and thus causes damage to retinal cells [20].

Cataract

Cataract is an eye disorder caused due to lens protein aggregation and is a leading cause of blindness [21]. Aggregation of lens protein induced by oxidative stress is a major causative factor for cataract [22]. ROS that are generated through metabolic and photochemical reactions trigger the oxidation-induced damage of lens and ultimately lead to cataract formation [23]. Oxidation of lens protein leads to formation of HMW aggregates which disturb homogeneity of the lens and causes scattering of light before reaching retina [24]. Cysteine residues in lens protein are oxidized by superoxide anion leading to formation of HMW aggregates and finally cataract [24].

Pesticide-Induced Oxidative Stress

Pesticides which are sprayed for destroying or mitigating any pest also contaminate various components of environment like soil, water, and food resulting in continued human exposure, thus causing chronic health effects [1, 8, 25]. There are strong evidences that pesticide exposure causes various cancers and neurological disorders [1, 12], and this is mainly attributed to the redox signaling [26, 27]. The pesticide-induced oxidative stress is mainly caused due to the accumulation of ROS which further leads to lipid peroxidation and DNA damage [28]. Pesticides are reported to adopt a multistep mechanism to induce oxidative stress. Firstly the inbuilt enzymes convert them to secondary reactive products and/or ROS which then results in damage to antioxidant defenses leading to impairment of antioxidant enzyme function [29].

Organophosphorus pesticides (OP) inhibit enzyme acetyl cholinesterase (AChE) and butyryl cholinesterase (BuChE) and cause damage in central nervous system (CNS) due to lack of cholinergic transmission. The same effect is also observed in autonomic ganglia, parasympathetic nerve endings, some sympathetic nerve endings, and neuromuscular junction [10]. Exposure to these pesticides was also reported to cause disorders in human through changes in antioxidative enzymatic activities [29]. OP generate ROS as a result of their metabolism by cytochrome P450s by an electron transport pathway [11]. Most commonly used OP like chlorpyrifos and diazinon caused oxidative stress to neuronal cells by increasing the levels of oxidized glutathione (GSSG). Thus, the toxicity of these pesticides was significantly higher in the neuronal cells having glutathione deficiency [30]. A study on OP chlorpyrifos ethyl (CE) suggested that CE induced erythrocyte lipid peroxidation and caused impairment of inbuilt antioxidant system. This suggests that ROS are involved in toxic effects of this insecticide [31]. Another insecticide, malathion, was reported to cause damage to the blood and liver of rats by inducing lipid peroxidation [32]. Methyl parathion and diazinon, most widely used organophosphate insecticides, induced oxidative stress in the liver, gills, and muscle tissues of *Oncorhynchus mykiss* fish. These insecticides significantly increased levels of malondialdehyde (MDA) and fluctuated the levels of antioxidant defense system [33]. Various studies also suggest organochlorine pesticide-induced oxidative stress [34]. Endosulfan was reported to cause immune toxicity in rats which was a result of oxidative stress-induced impairment of antioxidant defense system. [35]. In serum, the levels of thiobarbituric acid reactive substance (TBARS) increased in a dose-dependent manner on oral administration of DDT (100 and 200 ppm) and lindane (40 and 80 ppm). These compounds also increased the activity of SOD in red blood cells which clearly suggest the involvement of oxidative stress in organochlorine pesticide-induced immunotoxicity [36]. Tattoo, a carbamate pesticide having mancozeb as main chemical constituent, caused mild oxidative stress in the brain, liver, and kidney of goldfish while it was exposed to a dose of 3–10 mg/L; increased levels of SOD, catalase, and glutathione peroxidase in the treated organs support the fact [37]. Agriculture fungicide carbendazim was reported to modulate the antioxi-

dant defense system in Leydig cells of rats at very low doses [38]. A brief summary of various in vitro and in vivo studies on pesticide-induced toxicity due to oxidative stress is given in Table 9.1

Table 9.1 Summary of various in vitro and in vivo studies on pesticide-induced oxidative stress

Pesticide	Damage caused in	Dose	Mechanism	Ref
Chlorpyrifos	Neuronal cells	–	Increase in levels of oxidized glutathione (GSSG) and induce oxidative stress	[30]
	Erythrocytes	0, 0.01, 0.1 g/l and 0, 0.4, 2, 10, 50, 100 g/l	Induce lipid peroxidation and thus changes in antioxidant defense systems	[31]
Diazinon	Neuronal cells	–	Increase in levels of oxidized glutathione (GSSG) and induce oxidative stress	[30]
	The liver, gills, and muscle tissues of *Oncorhynchus mykiss* fish	–	Fluctuate the antioxidant defense system due to increase in levels of malondialdehyde (MDA)	[33]
Malathion	The blood and liver of rats	100, 316, 1000, and 1500 ppm	Induce lipid peroxidation	[32]
Methyl parathion	The liver, gills, and muscle tissues of *Oncorhynchus mykiss* fish	–	Fluctuate the antioxidant defense system due to increase in levels of malondialdehyde (MDA)	[33]
Endosulfan	Immunotoxicity	8 and 16 mg/kg body weight of rats	Impairment of antioxidant defense system by reducing the activities of superoxide dismutase and catalase leading to lipid peroxidation	[35]
DDT	Immunotoxicity	100 and 200 ppm	The levels of thiobarbituric acid reactive substance (TBARS) were increased in serum	[36]
Lindane	Immunotoxicity	40 and 80 ppm	The levels of thiobarbituric acid reactive substance (TBARS) were increased in serum	[36]
Tattoo	The brain, liver, and kidney of goldfish	3–10 mg/ L	Increase activities of SOD, catalase, and glutathione peroxidase and induce oxidative stress	[37]
Carbendazim	Leydig cells of rats	25 mg/(kg (body weight)/ day	Modulate the antioxidant systems like SOD, catalase, and GPx and cause oxidative stress	[38]

Pesticide-Induced Oxidative Stress in Ocular Disorders

Pesticide exposure to the eye involves direct entry of these toxic materials into eye tissues due to environmental exposure; sometimes the exposure may be through accidental splashes of these toxic materials which get absorbed on the eye tissue and further cause toxicity to the eye [39]. The accumulation of pesticide in the eye could lead to excessive production of ROS in the eye like what has been reported in various other tissues. The inbuilt antioxidant systems such as SOD, CAT, GPx, GR, and GSH protect the eye from oxidative damage [40]. The inability of the biological system to maintain a balance between excess productions of reactive oxygen species and combat the reactive intermediates could result in oxidative stress [40]. This oxidative stress in turn causes various eye disorders like cataract [22], age-related macular degeneration (AMD) [14], and retinopathy [18, 41]. Various epidemiology reports suggested the association of pesticide exposure to adverse ocular effects. Fungicides like maneb or mancozeb and ziram were reported to be the cause of retinal degeneration [42]. An epidemiology study revealed an association of exposure to various classes of pesticides like fungicides, herbicides, and insecticides to retinal damage [43]. A very recent comparative toxicological study on lens of fish (*Cyprinus carpio communis*) concluded that the organophosphate pesticide, monocrotophos, induces irreversible cataractous changes [44]. A similar study using the cornea of the same fish species suggested that on exposure to monocrotophos, the fish developed corneal necrosis due to shrinkage of microridges on corneal epithelium [45]. Chlorpyrifos (63 mg/kg, single oral treatment) caused cell apoptosis, lipid peroxidation, and DNA damage in the retina of mouse. A study reported that chlorpyrifos also altered substantially the activities of antioxidant enzymes such as SOD, CAT, and GPx in the retina of mouse [46]. Another recent study revealed that the chlorpyrifos resulted in oxidative stress in the ARPE19 cells. In this study a significant cell death was not observed, but increase in ROS was leading to decrease in glutathione (GSH) was reported [47]. A summary of few pesticides and their possible role in ocular disorders has been given in Table 9.2. A schematic representation of various routes of pesticide entry, induction of oxidative stress, and their adverse effects on the eye is given in Fig. 9.1.

Antioxidants and Their Role in Preventing Pesticide-Induced Oxidative Stress

Antioxidant biomolecules inhibit oxidation of other molecules and prevent ROS induced-damage by efficiently neutralizing ROS [49, 50]. The three ways by which the antioxidants act are (a) chain breaking by true antioxidants, where they stabilize ROS and decay it to harmless product; (b) preferential oxidation by reducing agents, where they prevent oxidation by reducing the rate of chain reaction; and (c) chelating mechanism by the antioxidant synergists [51]. The human body is equipped

Table 9.2 Summary of various epidemiological, in vitro, and in vivo studies on pesticide-induced ocular disorders

Pesticide	Damage caused in	Species	Dose	Observed change	Ref
Maneb or mancozeb	Retina	Human	N/A	Retinal degeneration	[42]
Ziram	Retina	Human	N/A	Retinal degeneration	
Monocrotophos	Cornea	*Cyprinus carpio communis* (Linn) (Fish)	0.038 and 0.126 ppm	Corneal necrosis attributed to the formation of crystalloid-like structures, thinning, and shrinkage of microridges on the corneal epithelium	[45]
	Lens	*Cyprinus carpio communis* (Linn) (Fish)	0.038, 0.062, and 0.126 ppm	Cataract development	[44]
Chlorpyrifos	Retina	Mouse	63 mg/kg, single treatment	Cell apoptosis, lipid peroxidation, and DNA damage	[46]
	Retinal cells	Retinal pigment epithelium (RPE) (ARPE19 cells)	1 nM–100 mM at 24 h and 9 days	Increase in oxidative stress in the retinal cells resulting decreased levels of glutathione (GSH)	[47]
Fenthion	Retina	Rat	50 mg/kg, twice a week for 1 year	Retinal degeneration	[48]

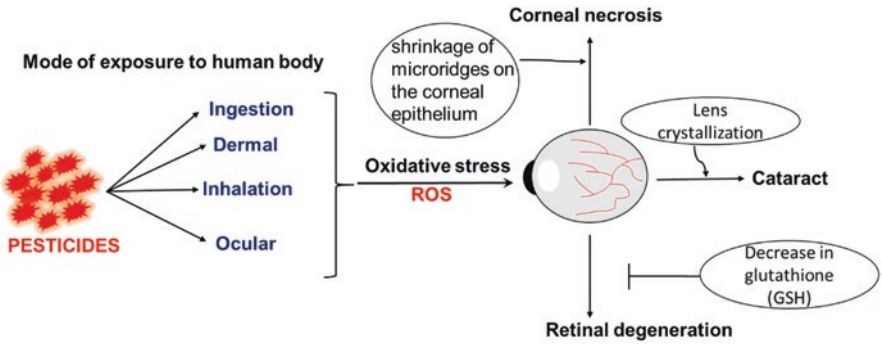

Fig. 9.1 Schematic representation of various routes of entry of pesticide into the human body and its toxicity to the eye. The pesticides enter the human circulatory system through various routes shown and induce oxidative stress by excess production of ROS. The excess ROS in the eye leads to severe ocular diseases like age-related macular degeneration, retinopathy, and cataract which are the leading causes of blindness in the world. This image was made using the PowerPoint Drawing Toolkit from MOTIFOLIO

with an antioxidant defense system of its own which include enzymes like superoxide dismutase (SOD), catalase, and glutathione which protect from oxidative damage [52]. Apart from the inbuilt antioxidant defense systems, many dietary antioxidants were also reported to have a great role in controlling oxidative stress caused due to exposure to pesticides [53]. Though the exact mechanism of action of these dietary antioxidants on pesticide-induced oxidative stress is unclear, various studies reported that they control the adverse effects of pesticides, thus reducing the burden on inbuilt antioxidant systems [53]. Figure 9.2 explains how antioxidants prevent/control pesticide-induced ocular toxicity.

The role of natural antioxidant biomolecules in controlling/preventing pesticide-induced oxidative stress in various tissues like brain, liver, testis, and kidney was well reported in the past [54–56]. Hypodermic injections of vitamin C (250 mg/kg) and vitamin E (150 mg/kg) administered in combination once daily for 6 days effectively reduced chlorpyrifos-induced inhibition of activity of acetylcholinesterase and increase in calcium ion levels in retinal cells of mouse. This mixture of antioxidants also effectively maintained the levels of antioxidant enzymes [46]. Intramuscular administration of the combination of vitamin C (250 mg/kg) and vitamin E (150 mg/kg) to Sprague-Dawley rats ameliorated increase in MDA and expression of Fas receptor and soluble Fas ligand (sFasL) concentrations and

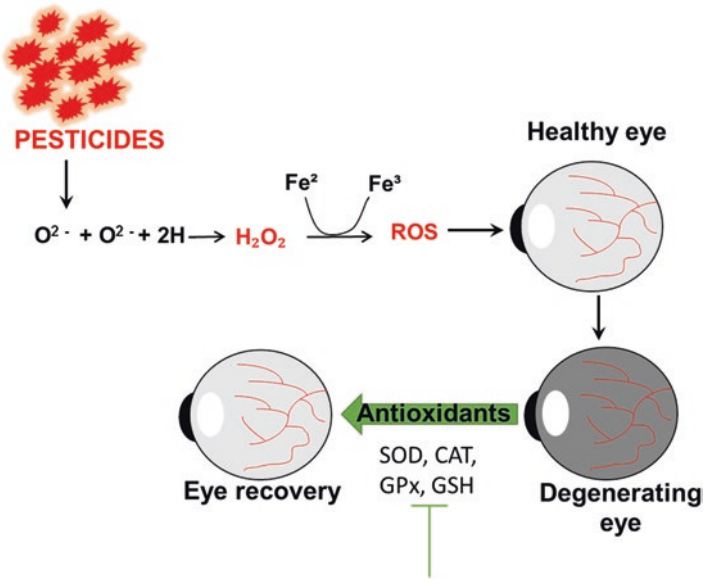

Fig. 9.2 Mechanism of pesticide-induced ocular toxicity and protective effect of antioxidant biomolecules. Pesticides enter into the human body through various routes and result in excess production of ROS. This ROS could lead to various disorders in the eye by impairing the inbuilt antioxidant enzymes. Supplementation with natural antioxidants could possibly maintain the levels of antioxidative enzymes and prevent/control pesticide-induced ocular toxicity. This image was made using the PowerPoint Drawing Toolkit from MOTIFOLIO

maintained the levels of GSH in the retina and kidney of these rats [57]. A very recent study has reported the efficiency of N-acetyl cysteine in promoting antioxidant defense over chlorpyrifos-induced oxidative stress in human retinal pigment epithelium (RPE) cell culture (ARPE19 cells) [47].

Though the various reported antioxidants have shown efficient preventive effect on pesticide-induced oxidative stress and further damage of various tissues in vitro and in vivo, they are still not approved therapeutics for the same. This may be due to one of the many reasons like hydrophobic nature, lack of target specificity, metabolism before reaching site (may be due to enzyme mediated degradation or modulation of activity by reacting with traces of pesticide in the body) of action, and lacking bioavailability [1, 58]. Hence discovering carrier-based drug delivery system which could overcome these problems and offer maximum therapeutic potential of these compounds has been a topic of great interest in the past few decades. Of late, nanotechnology product-based drug delivery has gained a great impact on improving the therapeutic potential of various drugs.

Biodegradable Polymeric Nanocarriers in Ocular Drug Delivery

Nanotechnology product-based drug delivery has been greatly appreciated for its ability to enhance the therapeutic potential of the encapsulated drugs [59]. In the recent past, encapsulation of natural antioxidants on biodegradable polymer-based nanocarriers has gained importance due to the scope of applications that natural antioxidants ensure. The nanoencapsulation of natural antioxidants is important to enhance the solubility, permeability, and stability and reduce the chance of metabolism before reaching the systemic circulation and providing a controlled release at the desired site of action and thus maximum use of therapeutic potential of these molecules [1, 60]. Figure 9.3 explains how nanoformulations of natural antioxidant biomolecules are better than their molecular forms.

Efficient ocular drug delivery has been a challenge for ophthalmologists due to the unique anatomy of the eye posing significant biological barriers for efficient drug delivery. Further the intraocular structures are protected from the external tight junctions of the corneal epithelium and the mucosal surface. It is therefore very important for any drug carrier to cross these external barriers to achieve therapeutically efficient drug concentration in the internal structures of the eye [60]. An ideal ocular drug delivery system should be target specific, should offer sustained and controlled drug release, and last but not the least should possess a patient-friendly delivery route to minimize the side effects arising from administrative methods [59]. In recent times, the use of biodegradable polymeric nanocarriers for ocular drug delivery has gained importance due to their relatively higher biocompatibility, ease of availability, biodegradability, mucoadhesive ability, and controllable size [61]. A wide range of biodegradable polymeric nanocarriers like polylactide (PLA),

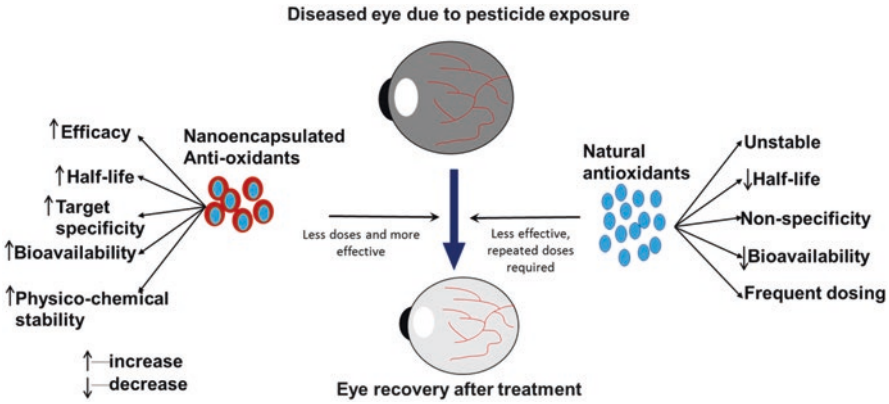

Fig. 9.3 Schematic representation explaining the fact that nanoformulations of antioxidants offer maximum therapeutic potential over molecular antioxidants. Nanoformulations of natural antioxidants are more efficient than their molecular forms for various reasons shown in the figure and thus offer more efficient protection over pesticide-induced ocular toxicity. This image was made using the PowerPoint Drawing Toolkit from MOTIFOLIO

chitosan, poly(lactic-glycolic acid) (PLGA), Eudragit, and alginate have been investigated for application in ocular systems [62] for delivering various ocular drugs through various available routes in the eye. The advantages of encapsulation of various ocular drugs in these biodegradable polymeric nanocarriers have been greatly reviewed in the past [63, 64]. Though the application of nanoencapsulated antioxidants in the ocular systems is vaguely reported, there are numerous studies that suggest that these polymeric nanoparticles are safe for drug delivery to the eye and have been used in enhancing the activity of various ocular drugs. Eudragit nanoformulation provided a sustained release both in vitro and in vivo at intraocular level for the ocular drug flurbiprofen and could achieve enhanced prevention of myosis due to the increased bioavailability of the drug [65]. Similarly Eudragit-encapsulated ibuprofen showed enhanced anti-inflammatory activity as a result of increased bioavailability of the drug in intraocular structures [66]. Alginate/HPMC was used to increase the retention time of gatifloxacin in ocular structures, suggesting the prospects of this polymeric system to be used as enhancer of ocular bioavailability of the drugs [67]. Polylactide (PLA) nanoparticles administered as intravitreous injection were reported to be efficiently localized in intraocular structures and further efficiently released the drug encapsulated in them [68]. Further systemically administered betamethasone phosphate poly(lactic acid) nanoparticles could efficiently inhibit experimental autoimmune uveoretinitis because of target specificity and controlled release of steroids [69]. Sparfloxacin-loaded poly(lactic-glycolic acid) (PLGA) nanoparticles showed enhanced precorneal residence time and ocular penetration [70]. In an ex vivo study, moxifloxacin-loaded PLGA nanoparticles showed an efficient antimicrobial activity compared to the present-day marketed drugs, which was due to sustained release of the drug [71]. Of late, research has been mainly focused on developing an efficient biodegradable polymeric nanocarrier which could be efficiently delivered as topical administration to decrease the mode

of administration-mediated side effects. Among the various biodegradable polymeric nanocarriers, the ones made of chitosan were largely studied in the recent past for the ocular drug delivery.

Chitosan has been extensively used as various formulations like solutions, gels, liposomes, nanostructured lipid carriers, and micro- and nanoparticles for delivery of various ocular drugs due to it being more biocompatible and biodegradable, having very few side effects, and being easily available from natural sources [72, 73]. The mucoadhesive property of chitosan makes it more ideal for drug delivery to the eye as this property enables enhanced residence time in the precorneal area [74]. Further the positive surface charge of the chitosan nanoparticles helps in easy and strong binding to the negatively charged eye surface (due to sialic acid of mucin on eye surface) [62]. Furthermore, the mucoadhesive property of chitosan makes it more compatible for topical drug delivery which could possibly increase the ease of drug administration for the patients.

Chitosan nanoparticles were reported to be less toxic to ocular system in various studies. In an in vivo study to evaluate the toxicity of chitosan nanoparticles to conjunctival cells, it was showed that even at high concentrations of 2 mg/ml, no toxicity was observed, and the cells were completely viable [75]. An in vitro and in vivo study evaluated the liposome-chitosan nanoparticle complexes (LCS-NP) for their tolerance by ocular surface. The study suggested LCS-NP posed a very minimal toxicity to cell line IOBA-NHC to LCS-NP. The in vivo results of the same study revealed that there were no changes in the ocular surface exposed to LCS-NP [76]. These conclusions on safety of chitosan nanoparticles for ocular drug delivery have attracted interest in encapsulation of many bioactive compounds for delivery to the eye. Some of the recent reported studies on efficacy of biodegradable polymeric nanocarriers for ocular drug delivery are summarized in Table 9.3.

Future Prospects

Pesticide-induced oxidative stress and further effects in vitro and in vivo have been greatly studied in various tissues, but their effect on ocular systems is scarcely reported. Increasing use of pesticides in day-to-day life warrants some detailed studies on the mechanistic aspects of pesticide-induced ocular toxicity and developing efficient therapeutics for the same. Natural antioxidant biomolecules have been efficient in controlling the pesticide-induced oxidative stress in tissues like the testis, liver, brain, etc., and these molecules were also found to prevent/control various ocular disorders like eye inflammation, cataract, diabetic retinopathy, etc., but the use of antioxidant biomolecules in prevention of pesticide-induced oxidative stress in ocular systems is scarcely reported. Hence we suggest that these bioactive molecules could be explored to control pesticide-induced oxidative stress in the ocular systems. The major hurdles in taking these antioxidant-based therapeutics to clinical level are that most of these molecules are hydrophobic in nature and their in vivo fate is undefined and uncertain. Furthermore, these molecules when administered orally in various studies lacked bioavailability at the target site. Though

Table 9.3 Summary of various ocular drugs encapsulated in natural biodegradable polymeric nanocarriers and observations of these studies

Biodegradable polymer	Drug encapsulated	Observation	Ref
PLA	Betamethasone	The nanoformulation offered efficient inhibition of experimental autoimmune uveoretinitis due to specific targeting and sustained release	[69]
PLGA	Sparfloxacin	The nanoformulation offered enhanced precorneal residence time and efficient ocular penetration	[70]
	Moxifloxacin	Efficient antimicrobial activity than the present-day marketed drugs as the nanoformulation provided sustained release of the drug	[71]
Eudragit	Flurbiprofen	The nanoformulation could achieve enhanced prevention of myosis	[65]
Alginate/HPMC	Gatifloxacin	The nanoformulation increased the retention time of the drug in ocular structures	[67]
Chitosan	Cyclosporine A	The nanoformulation increased the ocular bioavailability	[77]
	Indomethacin	The nanoformulation enhanced the bioavailability of the drug both in internal and external ocular structures	[78]
	Dorzolamide	The nanoformulation offered controlled release of the drug at physiological pH	[79]
	Gatifloxacinses-quihydrate	The nanoformulation offered controlled release of the drug, thus enhancing its antimicrobial activities	[80]
	Plasmid (pEGFP)	The nanoformulation showed enhance transfection capacity	[81]

PLA polylactide, *PLGA* poly(lactic-glycolic acid), *HPMC* hydroxypropyl methylcellulose

nanotechnology product-based delivery of these compounds has greatly improved their pharmacokinetic properties due to the increase of solubility, bioavailability, and longer residence time, the applications of antioxidant-based nanoformulations have not been studied in ocular systems. Chitosan nanoparticles are more favorable for developing antioxidant-based formulations which could be administered topically because of mucoadhesive nature of chitosan. Though chitosan nanoparticles offer various advantages for easy and efficient delivery of antioxidant-based nanoformulations, there are some important aspects that need to be addressed in this regard, like the issue of size, as the chitosan nanoparticles have a natural tendency to aggregate which could limit their applications in terms of ocular drug delivery due to the various barriers that ocular systems offer for foreign bodies of larger size. Hence, it is very crucial to develop user-friendly methods to synthesize chitosan nanoparticles with smaller size and possible ways to control their natural tendency to aggregate. Furthermore, despite large body of literature available proving the safety aspects and efficiency of antioxidants and chitosan nanoparticles, the use of chitosan to encapsulate the antioxidant biomolecules for ocular drug delivery especially to prevent/control pesticide-induced oxidative stress is scarcely reported. Hence there is a great scope for substantial research in this direction.

Concluding Remarks

Oxidative stress is one of the major pathways of pesticide-induced toxicity. Thus increased pesticide exposure could possibly lead to various oxidative stress-induced diseases in ocular systems. Various in vitro and in vivo studies suggest that natural antioxidant biomolecules like vitamins and polyphenols could effectively prevent/control pesticide-induced oxidative stress. Though these molecules showed potential inhibitory activities in in vitro and animal models, they are still not marketed as therapeutics for pesticide-induced ocular toxicities in human. This may be due to lack of target specificity and thus poor bioavailability of these molecules in human systems. Biodegradable polymeric chitosan nanocarriers could be efficiently used to deliver these natural antioxidant biomolecules so as to improve the ocular bioavailability and thus therapeutic potential when administered as topical applications. To conclude, an extensive scientific research both in vitro and in vivo has to be developed to make a detailed dose-dependent and time-dependent toxicity assessment of these novel nanocarriers before clinical application to prevent/control pesticide-induced ocular toxicity.

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Chapter 10
Advances in the Field of Microbial Infection in the Cornea and the Role of Nanotechnology in Treating Keratitis

Aseel Al-Mashahedah, Rupinder Kaur Kanwar, and Jagat Rakesh Kanwar

Abstract Microbial keratitis has long been associated with the activity of pathogenic microorganisms such as bacteria, fungi, parasites, and viruses, causing corneal epithelium disorder, decreased corneal material, and potential loss of vision. In fact, the ocular barriers have two contradictory roles during the infection pathway: the first involves protection of the eye from pathogens, while the second is involved in the obstruction of drug bioavailability. Here, we introduce a comprehensive overview of microbial keratitis as a world-wide concern and study some aspects of the mechanisms of microbial infection. We also review the role of the eye's natural defenses toward pathogens. More importantly, we highlight the potential of nanoparticles as therapy against increased multi-drug resistant microbes and the ability of these treatments to achieve drug bioavailability. Hence, nano-therapy provides a promising treatment for microbial keratitis in the future.

Keywords Microbial keratitis · Eye defenses · Immune response · Organic nanoparticles · Metal nanoparticles · Nanomedicine · Cornea · Drug delivery · Infection

Introduction

Ocular illnesses are common all over the world, and sometimes they can reach an epidemic degree. Eye infections such as keratitis, ulceration, conjunctivitis, blepharitis, and dacryocystitis can cause damage to eye structures, decreased vision, reduced corneal transparency, scarring, and even blindness if not treated promptly [1].

Corneal ulceration is a pathological condition indicative of a disorder of the epithelium and lack of material in the cornea [2, 3]. It is a common ocular disease and

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the second most common cause of blindness (after cataracts) in non-industrialized poor countries [4]. This disease has several distinctive characteristics, such as purulent infiltration; formation of stromal pus; inflammation of the anterior segment; and eventually corneal tissue damage, beginning with the epithelial layer and leading to the stroma [5, 6]. Corneal ulceration can occur as a result of exogenous injuries such as chemical wounds, physical trauma, contact lens (CL) use, and the 'dry eye phenomenon,' or it can be because of endogenous infections such as microbial infections (bacteria, fungi, parasites, and viruses) [3, 6, 7].

In general, keratitis can be categorized according to location, severity, and reason for ulceration, including superficial punctate keratitis that leads to cell death, which is located on the surface of the cornea; interstitial keratitis (stromal keratitis) that affects the stroma of the cornea (deeper layers) and results from infections and immune responses; herpes simplex viral keratitis where the herpes virus is responsible for the occurrence of infection through sexual transmission; traumatic keratitis, which occurs after scarring caused by a corneal wound; and keratoconjunctivitis resulting from an infection on the cornea and the conjunctiva at the same time [8, 9].

The ability of microorganisms to invade and damage corneal tissues or cells depends on virulence factors and resistance of the host defense mechanisms [10]. The eye has strong defense barriers, including the tear film, corneal tissue feature, and immune responses, but these barriers may fail to address the factors causing the disease [11]. Therefore, immediate diagnosis, detection of the causative agent of the disease, and good management are important keys for the control of keratitis and corneal fibrosis [9, 12]. In attempting to find a target therapy, we have seen the evolution of different treatments for different types of keratitis, especially microbial keratitis. A therapeutic approach has recently emerged based on drug delivery using nano carriers. These nano carrier formulations not only increase the drug residence time and reduce the frequency of administration, they can also improve drug stability and bioavailability by surpassing the eye barriers and improving the therapeutic outcome of the drug [13]. One of the most interesting aspects of this treatment is the use of a nanoformulation either alone or combined with other drugs as an antimicrobial agent to fight corneal infections [14, 15]. In this respect, nanomedicine is a promising future solution to diagnose, manage, and treat eye diseases.

Microbial Keratitis

Microbial keratitis is one of the five most frequent causes of loss of eyesight in the world [16]. This inflammation occurs in the cornea as a result of various types of pathogenic microorganisms, including bacteria, fungus, viruses, and parasites. Notably, it is associated with a series of distinctive symptoms such as aches, blurred vision, redness, photophobia, frank opacity within the cornea, and rupture or discharge in serious cases [4, 9]. However, it is often difficult to differentiate between the various types of microbial keratitis because of the similarity of the disease symptoms [17].

In fact, microbial keratitis can cause changes in and damage to the corneal shape and structure, reduced vision, and transparency, and corneal scarring may also accompany healing even after pathogens are eliminated by antibiotics [1, 16]. Injuries, CLs, and chronic disease or compromised immune systems are considered the main risk factors for microbial keratitis [18–20].

Undoubtedly, the cornea is the most exposed part of the eye to the external environment. This can contribute to increased cases of corneal contamination with a microbial source [7]. Farming activities, handwork, and domestic services can give rise to increased contact or contamination with pathogens. Increased cases of microbial keratitis have been observed in agricultural communities, especially in rural areas afflicted with poverty and a lower standard of living. For instance, the percentage of keratitis cases in Nepal and India are 10 times higher than in the USA. Also, climate conditions, including temperature and humidity, can contribute to an increase in corneal pathogen infections [16]. Furthermore, differing ratios and severity of corneal infection cases in various areas in the world may be as a result of the epidemiology of the microbe. Hence, diagnosis of microbial keratitis depends on the pathogen type, and this is considered the essential key to control of microbial keratitis [9, 12].

Bacterial Keratitis

A bacterium is one of the most common pathogenic microorganisms to be implicated in several ocular diseases such as bacterial keratitis, corneal ulcer and abscess formation, and conjunctival congestion, and eventually may lead to corneal scarring, haze, or blindness [6]. It should be noted that Gram-negative bacteria (e.g., *Pseudomonas aeruginosa* and *Chlamydia trachomatis*) and Gram-positive bacteria (e.g., *Staphylococcus aureus* and *Streptococcus pneumoniae*) are the most virulent causes of keratitis [21, 22].

External sources (injuries, contact lenses, burns, etc.) and internal agents (diabetes, age, etc.) are the main causes of bacterial keratitis [3, 6, 7].This has been confirmed by several studies, including a study conducted in Indonesia that found the main reason for the occurrence of bacterial keratitis was trauma, with an incidence of 74.7%, followed by chronic eyesight disorder, contact lens, and corneal surgery, with 12.1%, 4%, and 2%, respectively [6]. Also, results of an Indian study showed agreement with these findings with most cases of bacterial infection resulting from mechanical trauma at 72.4%, whereas ocular disorder cases were implicated in 9.2% [23]. Furthermore, 38% were from corneal trauma, 29% from blepharitis, and 20% from contaminated pharmaceutical procedures [19].

Contact lenses can also cause microbial keratitis through bacterial contamination and increased chronic hypoxic stress (lack of oxygen) on the corneal epithelial layer, especially when lenses are used while sleeping [24]. This leads to reduced corneal sensitivity, decreased epithelial mitosis and adhesion, early epithelial desquamation, and weakness and thinning of the epithelial cells, which negatively

affects the integrity of the cornea [8]. A study at the Jules Gonin Eye Hospital at the University of Lausanne in Switzerland showed that wearing contact lenses increased the incidence of bacterial keratitis by 36% and blepharitis by 21%, while wearing contact lenses increased corneal trauma by 20% [25].

The corneal epithelium is a formidable barrier against the spread of bacteria in the deep layers of the stroma because of the narrow space between superficial cells, various defense mechanisms such as innate and acquired immunity, and washing the eye with tear fluid [20, 26]. Bacterial keratitis occurs as a result of the discontinuity of the corneal epithelial layer or a disorder of the tear fluid system. Consequently, different types of pathogenic bacteria may enter the stroma, which consists of collagen types that have weak resistance against leukocyte enzymes and bacteria. After that, the epithelium and corneal stroma swell and are exposed to the necrosis process causing increased bacterial reproduction, inflammation, and partial or total ulceration at the wound site. This increases the possibility of corneal scarring [9].

Interestingly, the collapse of the corneal barrier helps the adhesion of bacteria to the corneal cell surface, which is the first step in creating the infection, and this adhesion often occurs between the host cell receptors and the pathogenic microorganism's receptors. For instance, during infection, the adhesion process with *Pseudomonas aeruginosa* occurs by binding of the flagellum of the bacteria and the mucin on the host corneal epithelial cells [27]. Also, components of the extracellular matrix (ECM) such as laminin, fibronectin, and collagen can assist pathogens in adhesion, stimulation of inflammatory responses, colonization, and occupation of host tissue [28].

During the infection stages, the inflammatory response plays a controversial role. It has been observed that the T helper-1 (Th-1) leads to acute corneal necrosis, while Th-2 plays a positive role in reducing infection without corrosion. Both are responsible for inducing antibody production during the infection phase [29]. Notably, the severity of keratitis (mild, moderate, and acute) depends on the corneal situation and pathogen type [9].

In Gram-negative bacteria, the cell wall of the bacterium contains lipopolysaccharides (LPS) as a virulence factor, which stimulate secretion of different cytokines produced by inflammatory cells. Tear fluid also helps LPS to penetrate into the corneal tissues during an injury [30]. One study revealed that the LPS of *Pseudomonas aeruginosa* can induce production of tumor necrosis factor-alpha (TNF-α), macrophage inflammatory peptide 2 (MIP-2), and interleukin-1beta (IL-1β), and reduce IL-10 in a corneal mouse model. Thus, dysregulation of cytokines provides nutrients for bacterial growth, enhancing corneal damage and aggravating the disease [31]. Moreover, several studies have reported that the role of LPS in *Pseudomonas aeruginosa* was to stimulate the expression of toll-like receptors-4/5 (TLR-4/5). These receptors are present on corneal macrophages and have the ability to induce transcription of chemokines such as KC/CXCL1, and cytokines such as IL-1α and IL-1β. It has been observed in cases of corneal ulcers that there is an increased expression of TLR2, TLR4, TLR5, and TLR9, as well as Nod-like receptor type NLRP3 and NLRC4 inflammasomes [29, 32].

Gram-positive bacteria are no less dangerous than Gram-negative bacteria. For instance, *Staphylococcus aureus* can stimulate the expression of different pro-

inflammatory cytokines like TNF-α, interleukin (IL-1β, IL-6, IL-8, and IL-12), MIP-2, and interferon-γ (IFN-γ), causing corneal inflammation in mouse models [33]. In brief, over 24–48 h after exposure to bacteria with strong virulence factors, the infection may develop rapidly and impact one or both eyes, leading to complete corneal damage if left untreated [9] (Fig. 10.1).

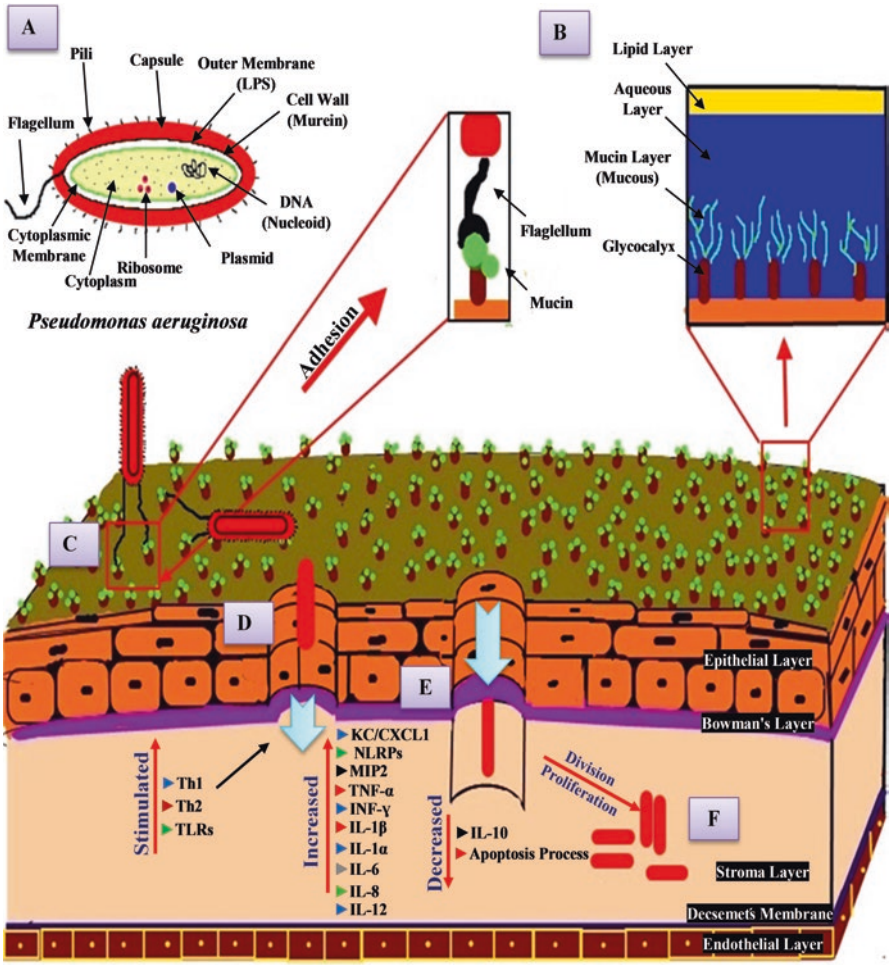

Fig. 10.1 The mechanism of bacterial keratitis. (**A**) The basic structure of *Pseudomonas aeruginosa*. (**B**) Three layers of the tear film that protect the eye surface. (**C**) The adhesion between bacterial flagella and corneal mucin layer. (**D**) The role of lipopolysaccharide, which is located in the outer membrane of Gram-positive bacteria to induce an inflammatory response by T-helper cells (Th-1, Th-2) and Toll-like receptors (TLRs), in order to increase necrosis and damage the corneal epithelium. (**E**) The inflammatory response causes stimulation of chemokines, pro-inflammatory cytokines and the necrosis process, while reducing apoptosis, and eventually the invading bacteria reach the stroma layer. (**F**) The occurrence of bacterial keratitis cases because of increased division and proliferation of bacteria in the corneal stroma

Fungal Keratitis

Fungal (mycotic) keratitis is one of the most common types of keratitis in the world, especially in tropical regions. On a global level, many studies have shown that fungal keratitis prevalence in South India, Ghana, Bangladesh, South Florida, and Nepal is 44%, 37.6%, 36%, 35%, and 17%, respectively [34]. Several reports in India have shown that the proportion of mycotic keratitis cases was 36.7% in South, 36.3% in Western, 25.6% in North-Eastern, and 7.3% in Northern India [35]. In China, it has also been observed that there has been an increased rate of mycotic keratitis infection in the past decade. In contrast, fungal keratitis infection rates remain very low in Britain and Northern USA [34]. This was confirmed by a study at the University of Texas Southwestern (UTSW), which showed that the infection rate of fungal keratitis was low at 15% compared with the infection rate of 85% for bacterial keratitis [36].

Trauma, compromised immune systems, climate conditions, overpopulation, and frequent long-term use of antibiotics such as steroids, corticosteroids, etc. are the major factors exposing the eye to infection by different types of fungi [35]. There are more than 70 types of fungi that can cause fungal keratitis, but the most common of these are *Candida spp.*, *Aspergillus spp.,* and *Fusarium spp.* [37]. Some studies in India showed that different types of fungi cause corneal infection at various rates. It was found that the major fungal isolates were 27.9% *Aspergillus spp.* and 23.2% *Fusarium spp.* in Eastern India [35]. In the Kumaon region, Uttarakhand (India), pathogenic fungi led to keratitis cases by 57.6% *Aspergillus spp.*, 33.3% *Fusarium spp*., 6.1% *Penicillium spp.,* and 3.03% *Candida spp.* [23]. *Fusarium spp.*, *Aspergillus spp*., *Penicillium spp.,* and *Candida spp.* were the most common in South Kerala, with incidences of 37.1%, 26.3%, 20.1%, and 1.8%, respectively [38]. Additionally, the proportion of fungal keratitis caused by *Fusarium spp.* was 48.3% and by *Aspergillus spp.* was 27.6% in the western part of Uttar Pradesh [4]. Although fungal keratitis cases are low in advanced countries, it was found that *Candida albicans* (48%), *Fusarium solani* (10%), and *Aspergillus fumigatus* (7%) were the causal fungi in fungal keratitis in New York [39]. Also, a study in the UK found that fungal keratitis was caused by yeast in 57.5% of cases and filamentary fungi in 42.5% [40]. Generally, there are two important groups in medicine that associated with corneal infection are filamentous fungi and yeast. Fungal keratitis such as *Candida* and *Aspergillus* keratitis are occurring in individuals who are suffering from immunocompromised. Aspergillus species such as *Aspergillus fumigatus*, *Aspergillus flavus*, and *Aspergillus niger*, and *Fusarium* species are responsible for one-third cases of traumatic infectious keratitis [4].

Fungal keratitis occurs when the fungus invades the surface of the cornea. Innate immune cells begin to identify the etiology by pattern-recognition receptors (PRRs), especially type C lectin receptors (CLRs). It has been recently revealed that Dectin-1 in the cornea determines the identity of the attacking fungi [41]. Generally, a PRR-mediated inflammatory response promotes elimination of fungi and repair of corneal tissue, and determines Toll-like receptor types TLR2 and TLR4 [29].

TLRs are the major elements of the innate immune response that are indispensable in selecting fungal zymosan and mannan, and release host defense responses [42]. Also, TLRs play a crucial role in keeping the cornea healthy and transparent by controlling the neutrophil infiltration and expression of IL-1β, IL-6, IL-12, and TNF-α, as well as monocyte chemoattractant protein type MCP1/CCL2 and MIP-2/CXCL2 [29, 32]. Therefore, decreased TLRs can lead to exacerbation of infection by pathogenic fungi, while over-activation of TLRs gives rise to increased inflammation, tissue injury, and even corneal necrosis. In this situation, the clinical prognosis relies on pathogen virulence and host immune response in order to protect the cornea and prevent scarring [4, 32] (Fig. 10.2).

Parasitic Keratitis

The most common parasites causing keratitis are *Acanthamoeba spp.*, *Mirosporidia spp.*, and *Onchocerca volvulus*. Less common parasites that impact the cornea are *Mansonella ozzardi*, *Leishmania spp.*, *Gnathostoma spp.,* and *Thelazia spp.* [8]. Another causal parasite is *Acanthamoeba* species, which includes *Acanthamoeba castellanii*, *A. polyphaga*, *A. culbertsoni*, *A. hatchetti*, *A. lugdunensis*, *A. palestinensis*, *A. rhysodes*, *A. quina,* and *A. griffin*, all free-living protozoa in habitats such as water and moist soil [8, 43, 44]. The life cycle of *Acanthamoeba* species has several characteristics including effective movement, nutrition, and partition into two phases: trophozoite and cyst with a double cyst wall. The cyst phase provides *Acanthamoeba spp*. with high resistance to extreme conditions, drying, antibiotics, and sterilization materials such as chlorine [44]. In *Acanthamoeba* keratitis cases, there were increased numbers of *Acanthamoeba* trophozoites in the anterior stroma, while the cysts were more common in the deeper layers of stroma with a lower inflammatory response. The main symptoms that can appear in patients with parasitic keratitis include eye ache, redness, reduced vision, light sensitivity, explicit opacity within the cornea, stromal infiltrate, and repeated disintegration of the epithelial layer [8, 45].

It has been observed that the incidence of parasite ocular infection is increasing globally [17]. Generally, the infection occurs because of polluted water and minor trauma involving soil, especially in regions with poor economic and social conditions and in conjunction with cases of human immunodeficiency virus (HIV) [46]. One of the more prominent *Acanthamoeba* characteristics is the ability to adhere to contact lenses. This adhesion depends on several factors, including contact lens manufacture materials and sterilization solutions, the concentration of *Acanthamoeba*, exposure period, and life phase of protozoon [24]. It was noted in developed countries such as the USA that during 2003–2008, levels of *Acanthamoeba* keratitis increased fourfold, and this was related to the high use of contact lenses [47]. Hence, the use of contact lenses in conjunction with poor hygiene is a risk factor leading to increased rates of ocular inflammation with *Acanthamoeba* parasites, increasing from 62.5% to 95% [8]. Although the corneal epithelium is an important

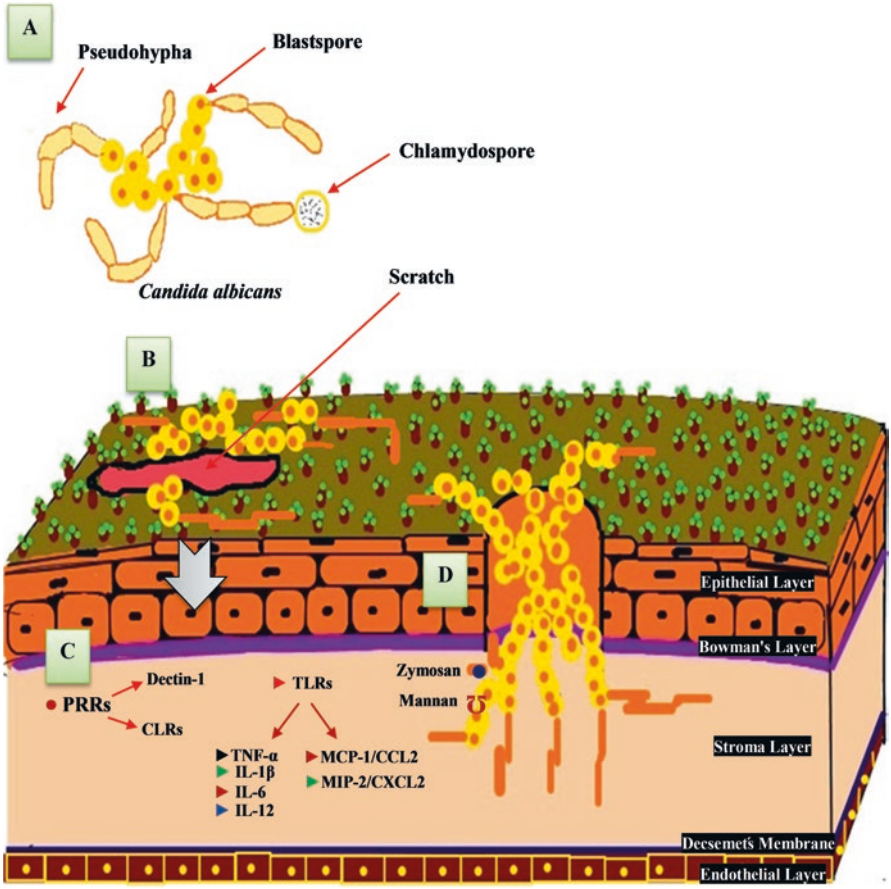

Fig. 10.2 Corneal injury with fungal keratitis. (**A**) The shape of *Candida albicans*. (**B**) Attack of the fungus on the infected corneal surface with scratch. (**C**) Innate immune cells of the cornea begin to identify fungi and attack mechanisms by pattern-recognition receptors (PRRs) include C lectin (CLRs) and Dectin-1 receptors. (**D**) TLRs are responsible for determining fungal zymosan and mannan, maintain control of neutrophil infiltration and expression of cytokines (IL-1β, IL-6, IL-12, and TNF-α) and monocyte chemoattractant proteins (MCP-1/CCL2 and MIP-2/CXCL2). The immune response is crucial for the eradication or prevalence of keratitis

barrier against the invasion of the parasite, the use of contact lenses can cause small scratches on the cornea, which are essential for entry of *Acanthamoeba*.

Corneal scratches lead to increased expression of mannose glycoproteins in the epithelial layer and consequent development of *Acanthamoeba* keratitis [8]. *Acanthamoeba* has the ability to selectively associate with mannose saccharides instead of non-mannosylated neoglycoproteins such as galactose, lactose, fucose, and galactosamine. The binding between mannose-binding protein (MBP) as a transmembrane protein on the typical *Acanthamoebae* surface cell and mannose receptors can assist *Acanthamoeba* to invade corneal stroma and induce

Acanthamoeba to produce the pathogenic protease enzymes [8, 47]. This happens via a complex series of events that involves the production of various enzymes such as collagenases and proteases [24]. These enzymes can cause basement membrane damage, stimulating cytolysis, enhancing the apoptosis process of corneal cellular elements, and increasing novel *Acanthamoeba* plasminogen activator (aPA) and inflammatory cytokines such as macrophage inflammatory proteins (MIPs), especially MIP133. In turn, MIP133 can give rise to different types of matrix metalloproteinases (MMPs) such as MMP1, MMP3, and MIP9 that have an important role in corneal fibrosis and corneal neovascularization. Consequently, penetration of proteases and collagenases into the stroma of the cornea and down to the deeper layers can lead to lysis of the collagen in the corneal stroma [8, 47, 48].

Generally, *Acanthamoeba* keratitis causes many histological changes such as ulceration of the corneal epithelium, lack of keratocytes in the stroma, and infection in two-thirds of the stroma with necrosis [8], which requires rapid therapeutic intervention to avoid losing sight [43] (Fig. 10.3).

Viral Keratitis

Viral keratitis is a disease that results in reduced vision or blindness in developed countries such as the USA [49–51]. *Rubeola virus* and *Vaccinia virus* have been implicated in viral keratitis cases [52], but the *Herpes simplex* virus (HSV) has been considered the most common cause of this optical disease [49, 53]. Globally, HSV leads to 1.5 million cases of *Herpes simplex* keratitis, including 40,000 severe cases of weak or lost vision every year [49]. A study in the USA has confirmed that 90% of adults have a positive serum to the herpes virus and about 500,000 cases of ocular herpes simplex infection are active annually [54].

There are two important kinds of *Herpes simplex virus* (type 1 and 2), which have oversized and double-stranded DNA [55]. In particular, HSV-1 is a pathogen implicated in dangerous cases of eye inflammation. However, HSV-2 can also negatively affect the eye, especially in newborns [51]. The main feature of viral keratitis is chronic and frequent ocular inflammation [56]. This may be due to the ability of the virus to disappear from the host's defenses causing frequent infection and irreversible corneal tissue damage [29]. Generally, *Herpes simplex* keratitis can cause harm in the anterior and posterior eye segments, and is most widespread in the cornea (dendritic ulcer). Thereby, HSV may impact on any or all corneal layers with a recurrence of disease in 20–48% of cases, which can lead to corneal neovascularization (CNV) and corneal scarring [54].

Several factors can contribute to the development of herpetic stromal keratitis; some of these agents are well-known such as diabetes and auto-immune deficiency syndrome (AIDS), and others are still unclear or under study. In this sense, this disease has opportunistic properties [55]. Corneal infection starts after the inflammation of mucous membranes. This inflammation is caused by HSV-1 through the initial adhesion between the glycoprotein receptor type B and/or C of virus and

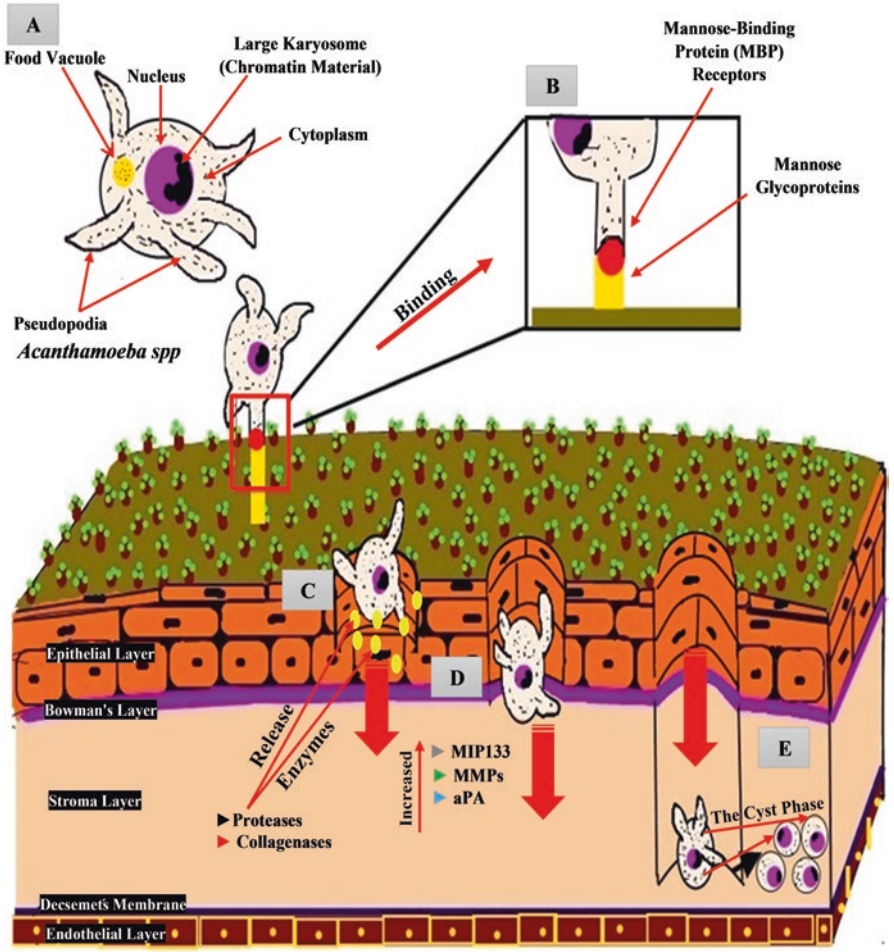

Fig. 10.3 The process of *Acanthamoeba spp.* keratitis (AK). (**A**) The structure of *Acanthamoeba spp*. (**B**) The binding between mannose glycoproteins on the corneal epithelium and mannose receptors (MBP) on the surface of *Acanthamoeba spp.* (**C**) *Acanthamoeba spp.* releases protease and collagenase enzymes (virulence factor) to enhance destruction of the host (corneal layers). (**D**) Protease and collagenase enzymes lead to stimulating inflammatory cytokines, damaging the basement membrane, increasing apoptosis and cytolysis, and penetrate to the deep corneal stroma layer by lysis of the collagen. (**E**) Transformation of *Acanthamoeba spp*. from the trophozoite phase to the cyst phase and stability in the deep layer of the stroma causing AK

heparan sulfate proteoglycan (HSPG) receptors of the corneal surface (host cell). This is followed by the secondary adhesion, which includes a series of interactions between virus receptors type GD, GH, GL, and GB, and the GD receptor on the cornea. This leads to the virus entering the host cell by fusion of the virion envelope with the membrane of an intracellular vesicle or the plasma membrane. After that, many processes occur, including releasing viral DNA and replicating in the nucleus, virus prevalence, inflammation, and apoptosis [57].

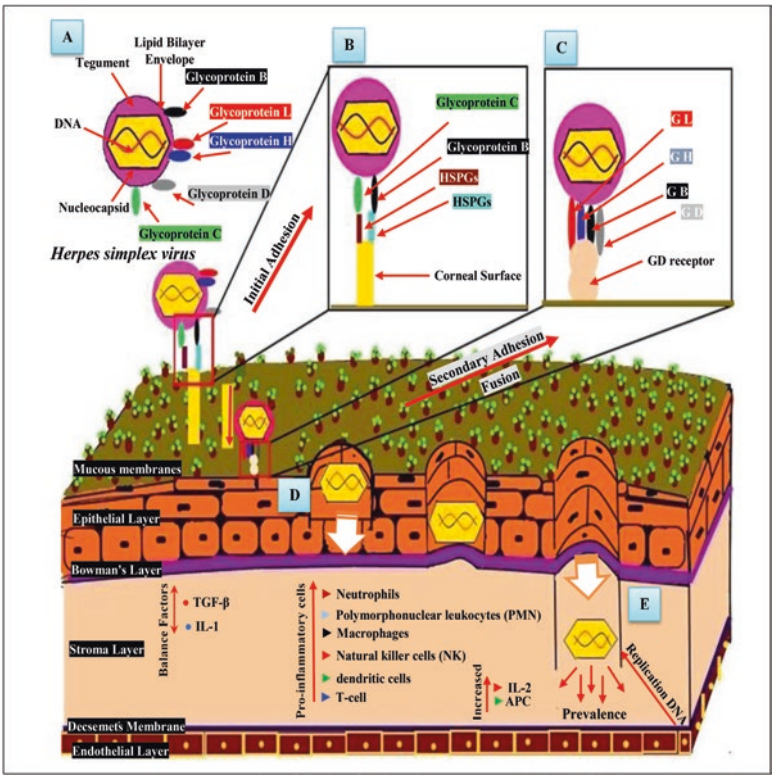

Fig. 10.4 The interactions of keratitis with *Herpes simplex virus* (HSV). (**A**) The most important components of the *Herpes simplex* virus. (**B**) The initial adhesion between virus glycoprotein receptors type B (GB) or/and C (GC) and heparan sulfate proteoglycan receptors (HSPGs) on corneal epithelium. (**C**) In order to release virus from nucleocapsid and tegument to the host cytoplasm through membrane fusion (Lipid Bilayer Envelope), interaction between virus receptors (GD, GH, GL, and GB) and corneal receptors (GD) is required. (**D**) During the membrane fusion process, destruction of the corneal surface occurs with the increase in pro-inflammatory cells and cytokines, and the virus invasion continues into the deep stromal layers. Immunosuppressive factors can play a role in the immunology response. (**E**) Replication DNA and the prevalence of a virus allow for an increase in herpetic stromal keratitis cases

During infection, the infected and non-infected corneal epithelial cells enlist several leukocytes into the corneal stroma such as neutrophils, polymorphonuclear leukocytes (PMN), macrophages, dendritic cells, natural killer cells (NKs), and T-cells in order to induce an immune response. In extreme immune responses, release of some antagonist receptors such as IL-1 and TGF-B occurs, which are considered to be factors involved in immunosuppression, thus decreasing corneal neovascularization, and reducing inflammation. In contrast, some cytokines can be released, such as IL-2 and an antigen-presenting cell (APC), causing a negative impact on the corneal stroma by developing HSK cases. Consequently, the immune response has two contradictory roles: one that improves the corneal health and one causing damage [29] (Fig. 10.4).

The Eye Defense Mechanisms

Tear Fluid

Tear fluid is a thin aquatic layer that covers the ocular surface. It is produced by the lacrimal gland located in the orbit over the lateral end of each eye and is also secreted from various tissues situated around the eye surface [58]. The tear fluid has many functions that are vital in the optical system. These functions include lubrication of the ocular surface, nutrition, providing growth factors to the corneal epithelium, a protective barrier against pathogens and the outside environment, and a refractive surface maintaining the ideal vision [59].

It is worth mentioning that the tear film is not a homogeneous entity and is divided into three distinct layers. The first layer is mucin, which is directly adjacent to the corneal surface with a thickness of 2.5–5 μm. It consists mostly of sugar-rich glycosylated proteins that are produced by epithelial cells and anchor to the epithelium. The gelatinous structure of the mucin maintains the wet surface easily and thereby contributes to the distribution of water after blinking [60, 61].

The second layer is an aqueous layer with a thickness of 4 μm. Lacrimal glands are responsible for secreting this layer. The main functions of the aqueous layer are preventing dehydration of the ocular surface, eliminating pathogens, protectection from pollution, as well as supplying nutrition to the cornea [60]. This layer is not a pure aqueous solution, but contains soluble and insoluble components, such as peptides, proteins, and electrolytes. The majority of proteins in tear fluid are lactoferrin, serum albumin, lipophilin, secretory immunoglobin A (IgG and IgA), beta-lysin, and the most abundant lysozyme and lipocalin [59]. Interestingly, the high availability of lysozyme in tear fluid is justified because lysozyme can inhibit microbial activity (antimicrobial) as a defense barrier by decomposing bacterial cell walls. Also, IgG and IgA in tear fluid neutralize some types of viruses and binding bacteria. This can reduce the microbe numbers in the tear film [11, 59, 62]. The change in the concentration of different proteins in the tear fluid reflects a normal or diseased case. The analysis of tear fluid with electrophoresis and chromatography has shown that diabetic patients have different patterns of tear protein compared to non-diabetic individuals [59].

The third layer of tear fluid is the lipid layer, which is relatively thin (0.015–0.160 μm) [63]. It is the outer layer of the tear fluid that has direct contact with the external environment. The lipid layer is secreted by small glands known as meibomian glands. These glands are located within the tarsal plates with many openings at the edge of the eyelids. This layer has several functions, such as decreasing surface tension of the tear film, reducing water evaporation, preventing the tear fluid from spilling over to the lid margins, and an antimicrobial function. Recent studies have shown that a deficiency of the tear fluid lipid layer is associated with the ‘dry eye syndrome’ [60]. Dry eye syndrome is defined as a multifactorial disease and is due to a disorder or unstable production of tear fluid, causing several symptoms such as increased discomfort and potential microbial infection, ocular surface harm,

and vision disruption [64]. Hence, tear fluid promotes the protection of the cornea as the first line of defense against the external environment.

Corneal Tissue

The cornea is an effective shield against external factors. The corneal epithelium is the first layer of the cornea, and consists of five to seven layers of cells. It is strongly associated with the stroma. In brief, corneal epithelium includes the basal layer, which is the deepest layer and consists of columnar cells. This is followed by the wing layer that contains two or three layers and involves polyhedral cells (mostly prickle cells). Finally, the squamous layer (superficial layer) contains three or four layers (stratified squamous cells) with flattened nuclei. In the superficial layer, the tight junctions are considered an important feature that obstructs the entry or progress of microbes to the stromal tissue [65].

Epithelial cells can secrete various cytokines to stimulate ocular immune defenses to eliminate pathogens. It has been observed that interleukin-1α (IL-1α) is released from corneal epithelial cells after exposure to infectious factors or trauma [11]. In contrast, the excessive secretion of IL-1α may promote microbial invasion, corneal neovascularization, and corneal tissue damage. However, corneal epithelial cells are capable of modifying the excessive secretion of IL-1α by producing IL-1RII (gene therapy), which is the natural antagonist of IL-1α [66, 67]. Hence, corneal epithelial cells contribute to a reduction of leukocyte infiltration, changing the cytokine profile selectively, inhibiting corneal neovascularization cases, and maintaining optimal vision [11].

Corneal stroma also has a defensive capacity against germ invasion. Keratocytes exist within the stromal layer and can limit microbial activity (antimicrobial) and enhance corneal wound healing. This occurs through synthesizing interleukin type IL-6 under the effect of IL-1α and tumor necrosis factor-alpha (TNF-α)) [11, 68]. The interaction between IL-6 with IL-1α and TNF-α acts as a therapy against microbial keratitis. Furthermore, keratocytes play a role in attracting neutrophil cells located in the conjunctiva. It has been shown that the expression of IL-8 resulting from keratocytes, is considered to be a chemoattractant agent for neutrophils in HSV keratitis cases. Conversely, IL-8 is completely absent in the corneal epithelial layer in cases of herpes infection [11, 68, 69].

In addition, corneal nerves play a fundamental role in innate defense by delivering sensory information, which in turn supports the ocular protection process as reflexive movements such as tear production, secretions, and wound healing [70]. The release of neuropeptides is essential after sensations such as pain and discomfort in order to stimulate the activity of cytokines [11]. Under the pain response, two types of neuropeptides are produced from sensory corneal neurons: calcitonin gene-related peptide and substance peptide. Both neuropeptides induce an immune response to repel microbial invasion [71, 72]. All of the above demonstrates the vital role of the corneal tissue as a potent barrier against microbial activity.

Immune Response

The immune response plays a key role in corneal protection during exposure to various injuries. The immune response includes innate and acquired immunity, and each has a defensive stage. The innate immune response is related to the early defense stage, while the acquired immune response is associated with the late stage of defense [11].

The early defense stage begins after a few minutes to several hours to eliminate pathogens [73]. During this stage, the innate immune response dominates through releasing various defenses. Interferons (IFNs) are a group of defense proteins also known as cytokines that have a role against microbial attacks. These cytokines consist of several types, including IFN-α, IFN-β, and IFN-ɤ. Notably, IFN-α and IFN-β are secreted by leukocytes and fibroblasts, respectively, while IFN-ɤ is released by T-cells and natural killer (NK) cells [74]. The main interferon functions in the innate immune response are promoting the capacity of infected cells to produce required proteins for T-cells in order to limit microbial activity. This process occurs by stimulating the expression of major histocompatibility complex class-I molecules (MHC-1) and proteins. Additionally, the activated NK cells use IFN-α and IFN-β to target and eliminate microbes [11].

Immune cells have a critical role in the innate immune response. Neutrophils are one type of these cells and exist in the cornea normally. Neutrophil cells are responsible for protecting the ocular surface by phagocytosis and killing pathogenic microorganisms during the invasion. Neutrophil extravasation, also called diapedesis, refers to neutrophil movement out of the circulatory system toward damaged tissue or the infection site. In the cornea, this process occurs by moving neutrophils in the limbal vasculature endothelial cells through the adhesion with receptors located on vascular endothelial cells [73, 75]. Also, eosinophil cells have immunoglobulin-E (IgE) receptors on the surface and the complementary components, which in turn provide protection against parasites. Therefore, it is assumed that eosinophil activation is necessary for parasitic toxicity as part of the innate immune response [11]. Furthermore, macrophage cells play a fundamental role against microbial infection during the innate immune response, including phagocytosis, antigen-presenting capability, release of inflammatory cytokines, and modulating effects on T-cell [65]. It has been confirmed recently that macrophage cells are located in the conjunctiva and corneal stroma in mouse models [11]. In addition, NK cells are large granular lymphocytes that no antigen receptors on the surface. NK cells are capable of recognizing MHC class-I molecules and can lysis the target cells, which have small amounts of these molecules, such as tumor cells, virally infected cells, and undifferentiated cells [76, 77].

In contrast, the microbial infection may overwhelm the first defense and thus the efforts of the innate immune system may fail. For instance, a bacterium has the ability to replicate in 1 h, and it reaches 2 million organisms after 24 h, thus overwhelming the initial immune defenses. This leads to the second defense stage, known as acquired immunity, being stimulated, which can occur from 24 h to 48 h after initial infection, and the full response may take days [11].

When the body recognizes the non-self-antigen during acquired immunity, the Langerhans cells are already working on processing the foreign antigen and transferring it by MHC (class-l or class-ll) to the corneal surface [78]. Then, T-cells are stimulated through displaying the non-self-antigen on the MHC molecule by linking to the T-cell receptor, eventually leading to maturation of the T-cell to CD4+ or CD8+ depending on the class of MHC molecules, i.e., I or II, respectively. Thus, T-cells have two main functions: killing the external antigens (CD8 + −cytotoxic cells) or releasing cytokines (CD4+ T-helper cells) to bring effector cells. The most common effector cell is the macrophage that destroys microbes and stimulates inflammatory cells. Hence, in ocular diseases, the interrelationship between the innate and acquired immune systems is very important in cases of acute and chronic inflammatory responses [79, 80].

Nanoparticles for Use Against Ophthalmic Pathogens

In spite of the enormous development that the world has witnessed in the antimicrobial field since 1960, multiple infectious diseases remain a big challenge with regard to therapy [81].

In ophthalmology, topical application of medicine is considered the most popular approach [82]. However, this method has several limitations. The eye's protection mechanisms (including the blink reflex, secretion from the lacrimal gland, and the structure of the corneal tissues) are highly efficient in preventing drug permeability and achievement of the desired treatment concentration at the target site [83]. Also, several antimicrobial compounds have difficulty passing through cell membranes. As these compounds have limited effectiveness within the cells, they have a weak impact on microbial activity. Thus, antibiotics may fail to eliminate pathogens, resulting in severe consequences and progression along the infection path from the fusion stage with early phagosomes, late endosomes, or with lysosomes (the pathogen elimination stage) to disease proliferation stage and chronic infection [81].

Furthermore, the evolution of microbial resistance to various antibiotics has made this an important global health issue. In addition, eye barriers and microbial resistance have led to the use of high doses of antibiotics, which can give rise to the generation of intolerable toxicity in healthy tissues [10].

Within this scenario, there are many pharmaceutical strategies such as gels, ointments, and viscous solutions that have been used with a view to enhancing and increasing bioavailability of traditional treatments on the ocular surface, but these strategies are not the best solution to overcome traditional therapy limitations [84]. Consequently, there is a need to design a new therapeutic approach that adopts the following principles: The first principle aims to achieve the ability to penetrate the corneal barriers, prolong the time on the eye surface with high bioavailability, and target the parts that harbor microbes. The second principle aims to overcome pathogen defenses [81, 82].

There is enough experimental evidence to show that nanoparticles have several unique physico-chemical and biological properties, including a small size on the nanometer scale, high permeability, increased surface area-to-volume ratio, a large capacity to deliver the drug, and lower toxicity in the cellular environment. Importantly, multiple ligands such as deoxyribonucleic acid (DNA), peptides, antibodies, molecular sensors, and therapeutic molecules can be transported to target sites by being loaded onto nanoparticles [85]. Recent studies show that the use of nanotechnology in ophthalmology applications can play a key role in passing through the ocular physical barriers, including the cornea, conjunctiva, sclera, and blood-retinal barriers [86, 87]. It has been recently shown that nanoparticles are a promising approach to treatment of a broad range of pathogenic microorganisms [81].

In brief, the small size of nanoparticles allows for easy penetration of the microbial cell membrane and exertion of antimicrobial activity, which includes limiting replication of DNA, obstructing cellular proteins, as well as the interaction between nanoparticles and the microbial membrane causing change and damage to the membrane structure, and, eventually, death of the pathogen [88, 89] (Fig. 10.5). However, these mechanisms are still under study and need further investigation.

Generally, nanoparticles are widely classified into organic and inorganic nanoparticles [90]. Some of the more well-known examples of nanomaterials as antimicrobials are discussed below.

Organic Nanoparticles

Chitosan

Chitosan is a linear polycationic polymer (N-deacetylated derivative) that is derived from chitin through treating crab or shrimp shells or other crustaceans with alkaline substances like sodium hydroxide (40–50% NaOH). Chitin is soluble in organic acids such as acetic acid, lactic acid, etc. as opposed to other solvents like water [91].

On the experimental level, chitosan has been used in nanoparticle formulations as a treatment against various diseases such as microbial keratitis [92]. This is due to the unique characteristics of chitosan such as being biocompatible, non-toxic, having a positive charge, and having a high absorption capacity, which make it an active agent against pathogens [93].

Chitosan's efficiency as an antimicrobial agent is based on the interaction of chitosan (positive charge) with the microbial cell membrane (negative charge), leading to destabilization of the microbial membrane and leaking of proteinaceous and other intracellular constituents, followed by lysis and cell death [90]. It has been shown that the polysaccharide (N-carboxybutyl /cationic) in chitosan has the ability to interact and form complex compounds with acidic polymers that are implicated

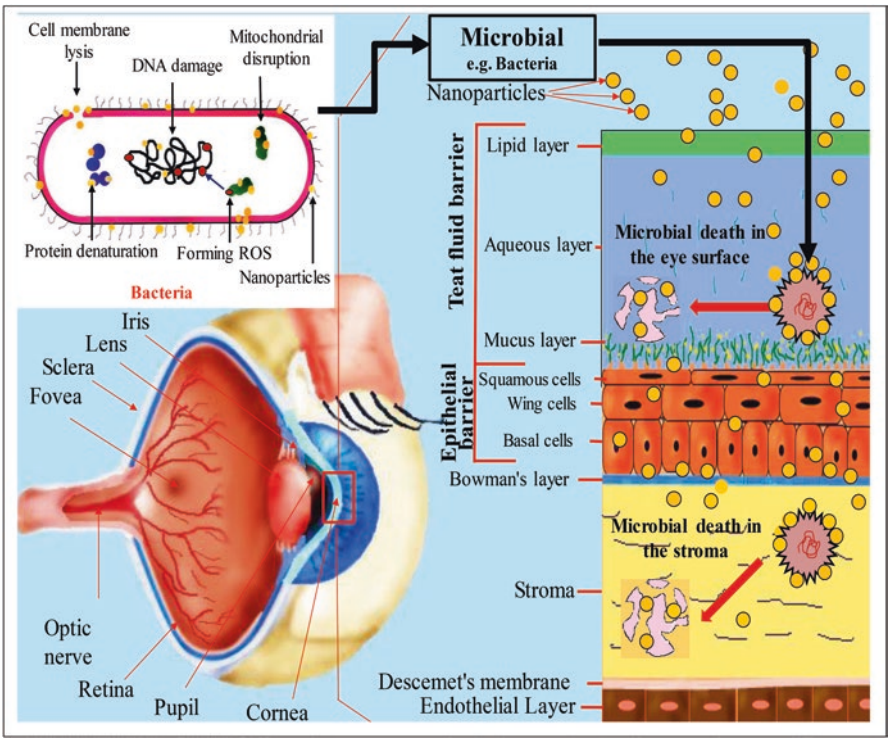

Fig. 10.5 Permeability of nanoparticles via the ocular barriers and their role to eliminate pathogens. Nanoparticles have the ability to overcome the corneal barriers (tear fluid and corneal tissue), reaching the microbe. Nanoparticles can easily penetrate microbial cell membranes, causing damage in the membrane structure, obstructing DNA replication and proteins, mitochondrial disruption, and reactive oxygen species (ROS) formation, stimulating apoptosis, and, eventually, lysis and death of the pathogenic microorganisms

in the pathogenesis of bacteria. For instance, N-carboxybutyl interacts with lipopolysaccharides, which are present on the outer surface of Gram-negative bacteria, or with the peptidoglycan (teichoic acid) of Gram-positive bacteria, leading to limiting pathogen virulence factors [94]. Also, chitosan has a chelating ability through selectively binding with trace metals, and can thus inhibit toxin production and limit microbial growth. Furthermore, chitosan activates the host tissue defenses and acts as a water-binding agent as well as inhibiting several enzymes. It has been observed that chitosan penetration via microbial cell membranes and movement to the nuclei may allow binding with microbial DNA and interference with synthesis of the microbial mRNA and proteins, and, eventually, the disorder of the microbial replication process [91]. However, the details of this mechanism are still unclear and under investigation.

Poly-ε-lysine

Poly-ε-lysine is a cationic homo-polypeptide of L-lysine (high positive charge density). The positive charge allows for the formation of soluble complexes with macromolecules that are negatively charged. Notably, poly-ε-lysine nanoparticles can affect a wide range of bacteria, especially Gram-positive bacterial strains such as *Bacillus coagulans* and *Bacillus subtilis* [90]. This effect is attributed to the disruption of microbial membranes through electrostatic adsorption of a bacterial cell surface, then outer membrane stripping, followed by abnormal cytoplasm distribution and damage to the bacterial cell [95].

Quaternary Ammonium Compounds

Quaternary ammonium compounds are cationic polymers that consist of non-ionic polyethylene glycol chains with different molecular weights. Numerous studies have referred to the role of these compounds as antimicrobial agents against a broad spectrum of Gram-negative and -positive bacteria, fungi, parasites, and viruses depending on the chain length (N-alkyl chain). Also, the features of quaternary ammonium compounds such as polymer morphology, molecular charge density, binding affinity to quaternary compounds, and the solubility of poly-cations in water, can affect the microbial activity [96, 97].

Briefly, the quaternary ammonium compound mechanism as an antimicrobial occurs through weak electrostatic interactions between the positive charge of these polymers and the negative charge of bacterial membranes, followed by the hydrophobic compound tail being inserted into the hydrophobic core of the bacterial membrane leading to the denaturing of the structural protein, and eventually bacterial cell death [98].

N-halamine Compounds

N-halamine complexes consist of one or more nitrogen−halogen covalent bonds that can be made by the halogenation of amide, imide, or amine groups. In general, an N-halamine compound can have two groups: the first refers to inorganic groups such as phosphate, sulfate, etc., and the second group is organic such as an alkyl group, a carbonyl group, etc. Thus, the presence of these groups indicates if it is an inorganic and organic N-halamine structure [99].

The biocidal properties of N-halamine compounds may be due to the return of halide atoms in the chloramine group (>N − Cl) or the bromamine group (>N – Br) to the oxidation state. These oxidizing halogens act by using active element transfer directly to biological receptors or by free halogen dissociation and release into the aqueous media, causing the microbial cell inhibition [90, 99].

Inorganic Nanoparticles

Silver

Silver is one of the metallic elements that has intrinsic therapeutic properties. Previously, it had been widely used in different forms such as metallic silver, silver nitrate, and silver sulfadiazine to treat microbial infections, wounds, and burns. Nevertheless, the use of silver compounds decreased markedly as a result of antibiotic discovery. Due to the emergence and development of microbial resistance to antibiotics, there was a push to find a more integrated approach. Accordingly, the comeback of silver with a nano-metal formulation may provide a potential antimicrobial agent [89].

There are differences in the effects of pure ionic silver (Ag+) in various compounds such as silver nitrate/$AgNO_3$ and silver nanoparticles (AgNPs) on bacterial activity. The use of the silver ion alone can create a low molecular weight area in the bacterial center as a defense mechanism against bacteria. This mechanism supports the bacterial DNA by providing protection from toxic materials when the bacterium senses membrane disturbance. In contrast, the region of low molecular weight did not form when treated with silver nanoparticles, thus proving the efficiency of AgNP as an antimicrobial agent [100].

The experimental evidence shows that the silver nanoparticle (AgNP) is effective against bacteria (Gram-negative and -positive), fungi, parasites, and viruses [90]. It has been shown that the effectiveness of AgNP as an antimicrobial agent depends on size, shape, ionic density and strength, pH condition, and capping agent [101].

A summary of AgNp's working mechanism against microbes is presented here. The large surface area of AgNP provides better contact with pathogens and AgNP can easily attach to the microbial cell membrane. This is followed by penetration of AgNP into the microbial cell [89, 101]. It has been shown that the small size of AgNP allows it to break through microbial cell walls easily, including microbial biofilm layers [102]. Also, the presence of the positive charge on the Ag ions (Ag+) is considered to be the vital component for antimicrobial activity through the protein structure disruption by binding to thiol (carbon-bonded sulfhydryl/ R–SH) and amino groups [90]. The bacterial cell membrane consists of sulfur-containing proteins, which can interact with AgNP, which has Ag + ions [100]. This encourages increased permeability of the bacterial cell membrane, leaving the bacterium unable to regulate material transport via the plasma membrane. Furthermore, lipopolysaccharide molecules in the outer membrane of Gram-negative bacteria allow for permeability of the bacterial barrier. Recent studies have discussed the role of metals like Ag against *E. coli* as a Gram-negative model, and the results showed that the metal can cause outer membrane distortion, formation of irregular-shaped pits, and changes in the permeability of the membrane, leading to release of lipopolysaccharide molecules and other proteins in the membrane. Hence, the membrane structure of *E. coli* degrades during treatment with AgNP [103].

When the AgNP enters bacterial cells during treatment, AgNP molecules tend to react with sulfur-containing proteins and phosphorus-containing compounds like DNA. Thus, several complex processes occur, causing protein denaturation, loss of DNA replication, inhibiting cell division, and, eventually, cell disruption and death [100, 103].

Notably, AgNP can also affect bacteria by interacting with the mitochondrial membrane, causing the activation of apoptosis-related genes that induce apoptosis. Also, the mitochondrial damage generates reactive oxygen species (ROS), which play an important role in DNA degradation [81]. In this context, it has been observed that the biosynthesis of AgNPs produced by *Aspergillus niger* has the ability to significantly inhibit *E. coli* by perforating the cell membrane, causing leakage of cell components, forming ROS, and, finally, bacterial cell death [104].

In ophthalmology research, the results have shown silver nanoparticles to have antimicrobial activity through inhibiting different microbes such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Acanthamoeba castellanii*, which cause keratitis. In addition, the use of AgNPs in a solution form or incorporated into contact lenses can reduce bacterial availability, limit bacterial adhesion, and prevent bacterial colonies from forming (the lens surface colonization). This may prevent the correlation between microbial keratitis and contaminated contact lenses [105].

Gold

Recently, gold nanoparticles (AuNPs) have been described as promising agents for various biological applications. Several studies have investigated the role of AuNPs in order to find ideal ways to utilize their features in an antimicrobial manner. Notably, the main goal of using AuNPs is to overcome microbial resistance to antibiotics such as methicillin-resistant *Staphylococcus aureus* (MRSA), which has serious virulence features [106]. Some researchers have confirmed that AuNPs are less effective against microorganisms when used alone compared to when used in combination with antibiotics such as vancomycin and ampicillin [90].

In brief, AuNPs can attack and eliminate microbes via two steps: The first step is inhibition of the metabolism process through increasing potential changes in the membrane surface morphology and reducing the synthesis of adenosine triphosphate (ATP). The second step is the collapse of the biological mechanism by declining the ribosome subunit for tRNA binding. At the same time, it has been shown that AuNPs have low toxicity to mammal cells. Thus, gold nanoparticles are considered suitable candidates to use against pathogens [107].

Nitric Oxide

Nitric oxide (NO) has been shown to have antimicrobial properties [108]. It has been recommended to use NO in a NO-nano formulation as a potential strategy against microbial activity. Nitric oxide is a diatomic free radical that plays an important role in the innate immune response by limiting infection [109]. It is worth mentioning that NONPs have high efficiency against a broad spectrum of Gram-negative and Gram-positive bacteria, as well as the ability to reduce the formation of bacterial biofilms. The antimicrobial activity of NO occurs by producing reactive nitrogen species (RNS) rather than ROS [90]. It has been confirmed that the NONPs can inhibit the activity of methicillin-resistant *Staphylococcus aureus* (MRSA) in *in vitro* and *in vivo* abscesses in a mouse model [109]. In this regard, NONPs may be useful therapeutics for microbial skin abscesses and microbial keratitis.

Conclusion

Microbial adhesion on the corneal surface is considered a critical step that results in infection after the destruction of corneal barriers by external or internal injury. The immune response (innate and acquired immunity) involves releasing cytokines, interferons, and NK cells. The immune response plays a key role in eliminating pathogens, but if it fails, microbes may enter the eye and cause corneal damage. It has been shown that microbial resistance to antibiotics and low drug bioavailability with the presence of side effects are the main factors aggravating microbial infections. Therefore, scientific research is directed towards finding successful medical management of microbial infections using a new technique known as nano-formulation such as use of nanoparticles. The evidence clearly indicates that the unique properties of organic and inorganic nanoparticles have the ability to disrupt the microbial cell membrane, cause nucleus (DNA) damage, overcome microbial resistance, and achieve ideal therapeutic properties.

Highlights

- Microbial keratitis is a widespread disease at the global level and the main cause of blindness.
- Corneal damage, pathogen adhesion, and the failure of eye immune defenses are considered the major causes of microbial keratitis.
- In ophthalmology, traditional treatment limitations and increased microbial resistance have necessitated the discovery of a more integrated therapeutic approach.
- The experimental evidence from several studies has shown the nanoparticle approach to be a promising therapy due to the ability of nanoparticles to provide a high level of bioavailability and eliminate pathogens.

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Chapter 11
Nanomedicine-Based Delivery to the Posterior Segment of the Eye: Brighter Tomorrow

Afrah Jalil Abd, Rupinder Kaur Kanwar, Yashwant V. Pathak, Maysaa Al Mohammedawi, and Jagat Rakesh Kanwar

Abstract Therapeutic strategies for the posterior ocular segment face tremendous challenges due to the presence of anatomical and physiological ocular barriers. Although several efforts have been conducted to manage the retinal dysfunction via various modes of administration, current therapeutic options have their disadvantages since these routes are invasive and followed by postinjection complications. Due to the possibility of encapsulating medications and to maintain their bioavailability in abundance, nanotechnology has been widely employed in the ophthalmology field particularly to manage disorders regarding the distal point of the eye. In this chapter, we elaborated the concept of using nanoparticles to treat the posterior part of the eye.

Keywords Nanomedicine · Posterior segment of eye · Ocular barriers · Drug delivery · Retinal diseases

Introduction

Nanomedicine is an emerging field, which involves development and employment of materials and devices at the size of intracellular structures and biomacromolecules [1]. This field is aimed to repair and defend biological systems at the molecular level [2]. The human eye is a complicated organ that is constantly exposed to a wide variety of insults, in addition to the presence of anatomical and physiological barriers [2, 3]; therefore, the eye is a unique target for nanotechnology.

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The incidence of neurodegenerative diseases is widely developed during the last decade's especially choroidal neovascularisation and diabetic retinopathy [4, 5]. The anatomically placed ocular barriers prevent the delivery of drugs from the anterior part to the posterior one, e.g. the systemic administration is notably limited by the presence of blood-retinal barrier. Hence, several drug administration routes were introduced to deliver medicine to the posterior segment of the eye represented by transscleral, intravitreal, subretinal and subconjunctival injections [6, 7]. However, these approaches are invasive [5] and followed by serious complications such as endophthalmitis, retinal detachment and ocular toxicity [7]. Thus, nanotechnology has emerged to reduce the frequency of injection and thereby reduce the incidence of adverse effects [6, 7]. In this chapter, we highlighted obstacles facing therapeutic strategies regarding the posterior segment of the eye and in nanotechnology-based techniques for ocular drug delivery.

Eye Barriers: Obstacles to Ocular Drug Delivery

The concept of eye barriers was investigated vastly in several studies [8–10]. Human eye barriers act to regulate ocular microenvironment and maintain the transparency of the anterior segment in addition to separate this portion from blood entering the eye. There are two main ocular barriers: blood-aqueous barrier and blood-retinal barrier [9].

The blood-aqueous barrier involves the ciliary body and iris; it contributes to the nourishment of the cornea and lens. A non-pigmented ciliary epithelium is connected tightly to each other; the tight junction of these cells constitutes an epithelial barrier against the free access of molecules [8, 9]. Further endothelial barrier formed by the tight junction of the vascular endothelial cells of the iris, similar to the ciliary epithelial barrier. Both ciliary and iris barriers prevent free diffusion of molecules to the posterior segment of the eye [8].

Blood-retinal barrier (BRB) is composed of two main portions: the outer and the inner blood-retinal barrier. The outer barrier, retinal pigmented epithelium (RPE) and Bruch's membrane, acts to regulate the access of nutrients from the choroid to the subretinal space, while the inner barrier lines the inner retinal microvasculature [9–11]. Both of inner and outer blood-retinal barriers possess tight junction proteins (Occludins and Claudins) which constitute supremely selective barrier that limits the access of macromolecules to the photoreceptors and permits the access of only 1–2% of the administrated medications to reach the posterior segment of the eye [9, 11]. Such barriers would make drug delivery to the retina and vitreous body really challenging.

Additionally, when medication drug/therapeutic is applied topically, it gets removed rapidly from the ocular surface via the lacrimal fluid. Occasionally, drug is absorbed to the systemic circulation [12]. The human tear fluid is constantly restored within 2–3 min [13]. Tear fluid involves three layers: the external one is composed of the lipid layer followed by the intermediate one, an aqueous layer, containing mucin, salts, protein and metabolic enzymes. The inner layer is known as glycoca-

lyx, constitutes of lysosomes and mucin [14]. The tear fluid is thought to be the first line of defence to protect the eye against external environment. However, it is capable of diluting drug molecules and reduces their availability; moreover, it prevents the entrance of hydrophilic medications from the plasma to the aqueous humour [6]. This could be considered additional obstacle against mobility of the drugs from the anterior segment of the eye to the posterior one.

Currently, there are frequent efforts towards delivering of treatments to the posterior segment of the eye since this process is problematic and considered as a major challenge. Additionally, the available techniques are invasive and have their own drawbacks [6]. Hence, the development of alternative options is critically needed to overcome the obstacles regarding maintaining a constant drug release of medication to the posterior segment of the eye. In this regard, nanotechnology-based approach is promising to enable a prolonged drug release, reduce the number of injections and, therefore, decrease the expected negative complications.

Nanomedicine Tools for Retinal Diseases

Nanomedicine definition according to the National Institutes of Health Roadmap for Medical Research in Nanomedicine (NIH 2006) is "an offshoot of nanotechnology, which refers to highly specific medical interventions at the molecular scale for curing disease or repairing damaged tissues, such as bone, muscle, or nerve" [15]. Nanoparticles from natural or synthetic lipids, polypeptide, polysaccharide-based systems, polymeric and metallic nanoparticles can be used for eye delivery [16]. Lipid-based nanoparticles were employed to synthesise the liposomes which are composed of phospholipid bilayers encapsulating drug molecules [17]. Liposomes (Fig. 11.1) are generally non-immunogenic, biocompatible and nontoxic. These liposomes can be used for encapsulation of hydrophilic or hydrophobic molecules. A current approach focused on improving liposomes' intravitreal half-life and retina targeting, in case of posterior delivery [18].

Dendrimers are another nano-therapeutic delivery tool/molecules possessing highly branched structures connected to each other by a central core (Fig. 11.1)

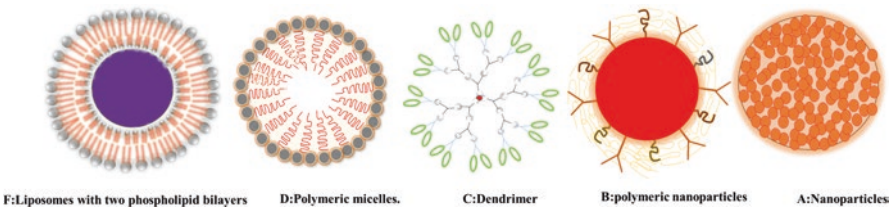

Fig. 11.1 Nanotechnological tools used for treatment of posterior ocular disorder. A: nanoparticles; B: polymeric nanoparticles; C: dendrimer; D: polymeric micelles; E: liposomes with two phospholipid bilayers. This image was made using the PowerPoint Drawing Toolkit from MOTIFOLIO

[19]. Dendrimers are clearly distinct in their size and composition in comparison to other nanoparticles. Dendrimers could be extended using stepwise synthesis; this process permits controlling the molecular weight, shape, size, stability and composition of dendrimers [20].

Polyglycolide and polylactic polymers are different sorts of nanoparticles used as drug delivery system; they are often mixed to establish a copolymer used in various ratios to synthesise PLGA. PLGA has been tested and shown to be nontoxic, non-immunogenic, biocompatible and biodegradable [21]; PLGA encapsulated several therapeutic molecules that had been approved by the Food and Drug Administration [21–23]. PLGA also was extensively investigated for delivering gene therapy [24]. Polypeptide nanoparticles are frequently synthesised using poly-L-lysine or albumin, while polysaccharide nanoparticles are composed of either heparin, cyclodextrin, hyaluronic acid or chitosan formulated as a nanocarriers [5, 25–27].

Metal/inorganic nanoparticles such as gold and silver are roughly used among metal nanoparticles due to their desirable features since they are nontoxic, inert and non-immunogenic [28–30]. Gold nanoparticles are used successfully to cross blood-retinal barrier with a minimum size 20 nm [30, 31] indicating the possibility of delivering gold nanoparticles to the posterior segment of the eye safely. Moreover, chitosan, gold nanoparticles and poly-L-lysine dendrimers possess anti-angiogenic property [32–34]. This feature could be used to deliver cargo to manage disorders characterised by angiogenesis such as aged macular degeneration and diabetic retinopathy.

Biodistribution of the Nanoparticles Through the Posterior Segment of the Eye

Investigation of the biodistribution of nanoparticles to the target tissue is very important since it gives us an indication about the duration of the therapeutic action, cellular uptake, bioavailability and safety profile. Several factors affect the biodistribution of nanotherapy and their drainage from the posterior part of the eye such as the size of particles, mode of the administration, surface charge and composition [35]. Fluorescently labelled polystyrene nanoparticles have been studied in various sizes (50 nm–2 μm) when administrated intravitreally in pigmented rabbit model. Particles with larger size (>2 μm) have shown to aggregate close to the trabecular meshwork in the vitreous cavity, while nanoparticle sizes 200 nm were distributed evenly in the vitreous cavity. However, small-sized nanoparticles have been found to cross the retinal barrier and identified in the retina [36]. Gold nanoparticles were administrated intravenously in two different sizes (20 nm and 100 nm); their biodistribution has been investigated in a C57BL/6 mice model, after 24 h using transmission electron microscopy. The data revealed a higher rate of biodistribution; 75% of small-sized nanoparticles (20 nm) were presented in retina, while large

nanoparticles did not appear in retina suggesting the preferential impact of nanoparticles size on overcoming eye barriers [36]. Another study investigated the role of route administration and nanoparticle size on the retinal biodistribution in a rat model using both subconjunctival and transscleral modes of delivery. Small-sized nanoparticles 20 nm were cleared rapidly from the site of injection while 200 nm were aggregated at the site of injection [37, 38]. Mode of administration also played a critical role in biodistribution of the therapy as described later in this chapter.

Zeta potential, the key indicator of the nanoparticle stability as colloidal dispersions, is also known to influence the biodistribution of nanoparticles. Zeta potential is a measure of the magnitude of electrical charge of particles (electrostatic or charge repulsion/attraction between particles); cationic nanoparticles would adhere to the vitreous network, while anionic ones are distributed equally through the vitreous and penetrate retinal layers, since vitreous has anionic charge [39]. Hence, the presence of polyethylene glycol enables nanoparticles to cross the anionic vitreous and reach to the retina [40].

Surface chemistry and its influence on nanoparticles' biodistribution in the retina have been investigated thoroughly. Self-assembled amphiphilic nanoparticles possessing different surface charge have been administrated intravitreally in the healthy rat model. Cationic polyethylenimine (PEI) nanoparticles were unable to cross the vitreous barrier and accumulate in the vitreous body. While glycol chitosan (GC) nanoparticles which are also cationic nanoparticles but since they possess glycol group prevent adhesive interaction for these nanoparticles with the vitreous elements and were capable of delivering therapeutic agent brimonidine to the retina. GC nanoparticles showed a promising potential for the direct delivery of drugs to the retinal ganglion cells for glaucoma treatment, while anionic nanoparticles were capable of crossing the entire retina and reaching retinal pigmented epithelium [39, 41]. Therefore, in addition to the surface chemistry, both the size and nanoparticle engineering can profoundly influence nanoparticle distribution to the posterior segment of the eye.

Mode of Nanoparticles Administration to the Posterior Segment of the Eye

As mentioned earlier in this chapter, ocular drug delivery has faced a major challenge due to anatomical and physiological ocular barriers. Therefore, therapeutic formulations are required to be considered during the synthesis phase of ophthalmic formulations and in choosing the suitable routes of administration to the posterior part of the eye. The mode of delivering therapies is known to be either through subconjunctival, intravitreal, subretinal or systemic. Subconjunctival administration has enabled a constant drug release to the posterior segment of the eye; however, the variability in the clearance rate was dependent on the particle size and their degradation rate [7, 42] (Fig. 11.2).

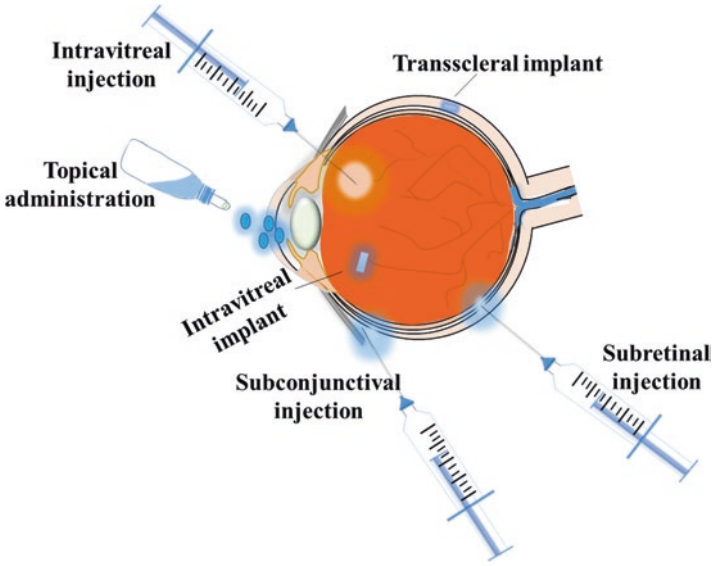

Fig. 11.2 Routes of administration of nanotherapy for the posterior segment of the eye. This image was made using the PowerPoint Drawing Toolkit from MOTIFOLIO

Subconjunctival administration was also used to deliver biodegradable hydrogels. These hydrogels showed a higher safety profile for in vitro R28 retinal neuron cells and exhibited biodegradability for a constant insulin release to the posterior segment after implantation using subconjunctival route. This approach was successful and offered a constant drug release to the retina [43]. Triamcinolone acetonide polylactic acid (PLA) nanoparticles were accumulated in choroid-PRE; when administrated via transscleral, this could be due to the melanin binding property of TA; and both of choroid and PRE are rich in melanin [5, 42].

Intravitreal administration offers the potency for mobilising medication directly to the vitreous enabling their diffusion to the retina and RPE [44]. PLA nanoparticles loaded with either Rhodamine-6G (Rh) or Nile red (Nr) were accumulated at the RPE following intravitreal administration; furthermore, their sustained release lasted for several months following single injection [45]. TG-0054, a potent small molecule, inhibited the binding of CXCR4 to stromal cell-derived factor (SDF); this process is implicated in angiogenesis pathogenesis [46]. TG-0054 encapsulated with PLA nanoparticle retention rates was investigated in New Zealand rabbit eyes that showed a sustain release for 3 months.

Intravitreal therapy was used to deliver free medications like plain fluconazole; however, injected therapy was cleared rapidly from the vitreous cavity via circulation and vitreous turnover [5] in comparison to liposome-entrapped fluconazole. A short medication half-life requires follow-up injections and raises the risk for postinjection complications. Moreover, rapid elimination of free medication via transretinal route could lead to accumulation of a higher dose of fluconazole that

could be retinotoxic. Therefore, nanoparticle technique can avoid the issue of quick clearance and elevated retention time through the constant release of the therapy [47]. Sustained delivery of the epidermal growth factor receptor tyrosine kinase inhibitors (EGFR TKI) was shown to promote neuron regeneration and survival. This phenomenon was exploited by introducing poly(lactic-co-glycolic acid) (PLGA) nanospheres to encapsulate EGFR TKI4-(3-chloroaniline)-6,7-dimethoxyquinazoline (AG1478) and showed the promise to regenerate optic nerve at 4 weeks after intravitreal delivery through a single administration [48].

Anti-inflammatory steroid and nonsteroid medications have been used to treat retinal disorders [49]. In this study, fluocinolone acetonide was delivered using polyamidoamine (PAMAM) dendrimers to manage retinitis pigmentosa. These dendrimers exhibited a selective localisation to the activated microglial cells in the retina within retinal degeneration rat model, but not with the healthy ones. The dendrimers also showed a maintained drug release for 3 months after a single injection and exhibited the efficacy in preserving photoreceptor outer nuclear cells, arresting retinal degeneration and attenuating activated microglia. This technique showed to maintain the biodistribution for a long period with more potency as compared to the injected free drug besides the reduction in the number of injections [49].

Additional strategy was developed to avoid vitreous barrier by using subretinal injection [50]. Compact DNA nanoparticles have been investigated when CK30PEG-DNA nanoparticles were directly shifted to the PRE after subretinal injection; CK30PEG-DNA is a single molecule of plasmid DNA compacted with 30-mers of polylysine conjugated to 10 kDa polyethylene glycol; these nanoparticles internalised with photoreceptors rapidly and restored cone and rod function as compared to the free DNA therapy when injected in retinitis pigmentosa mice model [51]. The study authors reported that CK30PEG-DNA nanoparticles have an acceptable safety profile and presented no adverse effects when injected in subretinal space.

Systemic mode is a further strategy used to treat ocular disorders. This strategy faces a major challenge due to the presence of inner blood-retinal barrier [52]. However, a novel approach was developed to create reversible opening of the inner blood-retinal barrier when targeting Claudin-5 in the retina using siRNA; the resulted selective permeability allows crossing of micromolecules without creating retinal oedema. However, the revisable opening permits delivery of less than 1 kDa molecules by using this approach. Photoreceptor function was improved, while the adverse effects of retinitis pigmentosa were inhibited [53]. The surface-functionalised PLGA nanoparticles loaded with anti-vascular endothelial growth factor (VEGF) intraceptor Flt23K, a recombinant construct of VEGF-binding domains 2 and 3 of VEGFR-1/Flt-1 receptor coupled with the endoplasmic reticulum (ER) retention signalling sequence Lys-Asp-Glu-Leu (KDEL) targeting CNV have been delivered via systemic route in a rat model [54]. These nanoparticles were shown to be aggregated at the site of angiogenesis when the treated Brown Norway rats were exposed to a laser-induced choroidal neovascularisation in contrast to the normal animals. This technique confirmed the selective targeting by the injected nanoparticles at the site of angiogenesis. These surface-functionalised nanoparticles loaded with VEGF

intraceptor Flt23K showed the promise to reduce the size of angiogenesis area and presented acceptable safety profile since no inflammatory reaction, cellular infiltration or atrophy was observed via haematoxylin and eosin staining, indicating that these NP are non-immunogenic and nontoxic.

Retinal Diseases: The Target for Nanotechnology

As mentioned earlier in this chapter, neurodegenerative and neovascular retinal diseases are mainly responsible for vision impairment worldwide and boosted the concern in the last few decades worldwide including the Middle East countries [5, 55]. Further, we reviewed the recent strategies focus on reducing the frequency of injection to diminish the risk regarding post administrative complications and to improve patient compliance. Nanotechnology could assist to develop a new approach for crossing eye barriers, augmented controlled drug biodistribution and targeted delivery as well [7, 56, 57]. In the next section, we discussed about targeting posterior segment of eye disorders.

Targeting Aged Macular Degeneration with Nanotechnology

Over the next few decades, ageing population is growing dramatically among societies. This is further accompanied by significant rise of the number of individuals suffering from aged macular degeneration (AMD) [55]. AMD can be classified into two types: dry AMD and wet AMD (also called choroidal neovascularisation (CNV)) [58, 59]. CNV is responsible for 90% of central vision loss due to initiation of incomplete blood vessels from choroid towards the subretinal space; these blood vessels are constantly leaking and contributing to the macular oedema, subretinal haemorrhage, retinal detachment and scar formation [7, 60]. Recent approaches with nanotechnology are mainly targeting angiogenesis and focus on the sustained release of medication [2, 7, 57]. Previous study confirmed the efficacy of using dexamethasone acetate (DA)-encapsulated PLGA nanoparticles in vitro and maintaining a controlled linear release for 40 days. Moreover, DA nanoparticles present in vitreous of CNV mice model within an effective range for more than 50 days that is capable to supress the inflammatory response [16]. Polylactic acid/polylactic acid-polyethylene oxide encapsulating integrin-antagonist peptide (C16Y) was shown the promise to suppress the angiogenesis in CNV that form after 5 days of injection and also to reduce the size of neovascularisation after 12 days of injection; these nanoparticles also showed a high localisation to the RPE and maintained a continuous drug release [61]. Additional Macugen aptamer [62] was FDA approved to treat AMD patients and demonstrated the efficacy to suppress angiogenesis and improve vision [63].

Gene therapy has been introduced to target CNV through using PLGA-chitosan nanoparticles encapsulated proteolytic plasminogen kringle 5 (K5) and supress VEGF via HIF 1-α pathway. K5 exhibited a strong inhibitory effect on retinal neovascularisation and retinal vessel leakage in oxygen-induced retinopathy model and presents successful results to supress angiogenesis [64]. Additional ones like anti-VEGF sense oligonucleotide [65], hypoxia-inducible factor1α short hairpin RNA plasmid DNA-loaded poly(D,L-lactide-co-glycolide) nanoparticles (shRNA pDNA) [66], PEGylated liposome-protamine-hyaluronic acid nanoparticles (PEG-LPH-NP) encapsulated with siRNA (PEG-LPH-NP-S) targeted VEGF receptor 1 in vitro and in vivo [67], polyion complex (PIC) micelle encapsulating plasmid DNA (pDNA) [68] and integrin αvβ3 targeted NP containing a dominant negative Raf mutant gene (NP-ATPμ-Raf) [69] are further described in Table 11.1.

Drug molecules are delivered mainly via intravitreally although this technique is accompanied by several adverse effects [82]. However, nanoparticles could maintain a constant drug release and reduce the number of injections thereby minimise the risk of post-operative injections. As reviewed here, therapeutic strategies and the management for disorders regarding posterior segment of the eye are still a challenging task due to the anatomical ocular barrier that interferes with the access of therapy to the macular area; therefore, we still need to develop more suitable mode which is less invasive and enhance the therapeutic bioavailability.

Diabetic Retinopathy

Diabetic retinopathy (DR) is originated from sustained hyperglycaemia, characterised by formation of abnormal retinal blood vessels due to ischaemia to innervate the hypoxic retina; DR is accompanied by diabetic macular oedema (DME) following breakdown of BRB as a result of leakage originated from hyperpermeable diluted blood vessels [83]. Nanotechnology-based approaches were established to treat diabetic retinopathy. Triamcinolone acetonide encapsulated nanostructured lipid carriers (NLC) showed anti-angiogenic property and exerted a prolonged drug release when diffused across rabbit sclera [84–86].

Additional effort was achieved using dexamethasone implant to sustain the release of medication [74]. Dexamethasone implant exhibited a new hope to ameliorate macular oedema and retinal vein occlusion; such implantation was approved in 2009 [85]. Fluocinolone acetonide is a further anti-inflammatory delivered in a non-biodegradable implant. It is applied to manage post segmented ocular complications; fluocinolone acetonide showed efficient results during treatment of patients suffering from DME as a major complication of diabetic retinopathy [85, 87].

Suppressing angiogenesis with diabetic retinopathy was investigated deeply by using anti-angiogenic agents. Anti-VEGF has been used widely to manage this disorder, which is similar to that used with CNV [85]; ranibizumab, pegaptanib and aflibercept were used to target angiogenesis; and these agents were encapsulated and delivered to the back of the eye using several injection approaches [59, 72, 85].

Table 11.1 Different drug delivery approaches for the treatment of posterior ocular segment dysfunctions

S.n	Incorporated drug	Dysfunction used for	Nanoparticle type	References
1	Hypoxia- inducible factor 1α short hairpin RNA plasmid DNA	CNV	PLGA NPs	[66]
2	Bevacizumab	CNV	Poly(ethylene glycol)-b-poly(D,L-lactic acid) microspheres and poly(DL-lactide-co-glycolide) nanospheres	[70]
3	Ranibizumab biosimilar	CNV	(Mab)/polyethyleneglycol (PEG)-conjugated small gold nanoparticles	[71]
4	Anti-vascular endothelial growth factor (VEGF) oligonucleotide (ODN-1)	CNV	Loaded with lipophilic amino-acid dendrimer	[65]
5	Pegaptanib	CNV	Aptamer binds to and blocks $VEGF_{165}$	[59, 72, 73]
6	VEGFR1 siRNA	CNV	PEGylated liposome-protamine-hyaluronic acid NPs	[67]
7	Dexamethasone	Macular oedema	Intravitreal implant	[74]
8	Triamcinolone acetonide	DR, AMD	Implantable polymeric devices	[75]
9	Doxorubicin	CNV	Polyethylene glycol and poly(sebacic acid) (DXR-PSA-PEG3)	[76]
10	Water-soluble integrin-antagonist peptide (C16Y)	CNV	Encapsulated with polylactic acid/polylactic acid-polyethylene oxide nanoparticles (PLA/PLA-PEO)	[61]
11	Plasmid DNA (pDNA) of soluble Fms-like tyrosine kinase-1 (psFlt-1)	CNV	Polyion complex (PIC) micelle	[68]
12	Anti-vascular endothelial growth factor (VEGF) intraceptor plasmid (Flt23K)	CNV	Dual-functionalised poly-(lactide-co-glycolide) nanoparticles	[54]
13	Fluocinolone acetonide	Diabetic macular oedema (DME)	Intravitreal implant	[77]
14	Dominant negative Raf mutant gene (NP-ATPμ-Raf)		Cationic nanoparticle coupled to an integrin αvβ3-targeting ligand (NP)	[69]
15	Combretastatin A4	CNV	Liposomes	[78]
16	Paclitaxel, succinyl-paclitaxel	CNV	Loaded with cationic liposome	[79]

(continued)

Table 11.1 (continued)

S.n	Incorporated drug	Dysfunction used for	Nanoparticle type	References
17	Plasminogen kringle 5 (K5)	CNV, DR	Loaded with PLGA-chitosan nanoparticles	[64, 80]
18	A polymeric formulation of TNP-470 (anti-angiogenic drug) (Lodamin)	CNV	Conjugated to monometoxy poly(etylen)glycol-poly(lactic acid) (mPEG-PLA) micelles	[81]

VEGF is augmented by hypoxia and needs to be neutralised to protect vision of the affected patients; therefore, anti-VEGF agents are widely used and consider as the gold strategy to control angiogenesis with the affected patients [16, 85]. The protein kinase inhibitor, growth hormone inhibitor, antioxidants, anti-inflammatory and gene therapy are some other additional therapeutic approaches targeting angiogenesis [85], administrated to improve patient compliance and obtain better results using nanomedicine.

Retinoblastoma

Retinoblastoma is a kind of paediatric ocular tumour presented widely in the live births [88]. The disease therapy has faced advancement after administrating nanotherapeutic approaches. Dendrimeric nanoparticles loaded with carboplatin to suppress retinoblastoma tumour size in vivo were investigated earlier [89] and proved significantly effective in maintaining drug release as compared to the free drug. While poly(D,L-lactide-co-glycolide)-poly(ethylene glycol)-folate (PLGA-PEG-FOL) micelles (DOXM) have been used to sustain doxorubicin (DOX) release targeting folate receptors in vitro, these micelles were effective to maintain constant release of DOX for 2 weeks [90]. Since retinoblastoma cells overexpressed folate receptors, targeting of these receptors using micelles exhibited higher efficacy as compared to the free drug. Additional strategy was developed using mesoporous silica nanoparticles loaded with photosensitiser accompanied with chemotherapy [91]. These nanoparticles are encapsulating both of photosensitiser and camptothecin and were functionalised with galactose or mannose to target receptors on Y-79 calls. The outcomes were more satisfactory through using biotherapy as compared to the monophototherapy.

RB was investigated recently using anticancer therapy with Shepherdin which acts as a peptido-mimetic to destabilise heat shock protein (HSP90) and suppress HSP90-Survivin interaction; results referred to the potency of antitumour effect of Shepherdin to manage RB while showing no effect on the normal MIOM-1 cells [92]. Bovine lactoferrin an innate immunity protein with anticancer activity was studied to treat retinoblastoma cells and showed interesting outcomes. A recent study from our laboratory referred to the efficacy of using bovine lactoferrin in

upregulating sodium iodide symporter (NIS) gene which is an intrinsic membrane glycoprotein and attracts the attention in cancer radiotherapy [93]. Similarly, carboplatin-loaded bovine lactoferrin nanoparticles demonstrated a strong anti-proliferative activity, sustained release and greater intercellular intake [94]. Lactoferrin required further investigation with retinoblastoma since it is natural and has a potent antitumour effect.

Glaucoma

Glaucoma is mainly induced by chronic inflammation and microfibril defect through its effect on signalling via transforming growth factor β (TGFβ) or by modifying the biochemical property for the adjacent tissue [95]. Glaucoma is accompanied by increase intraocular pressure (IOP) by creating resistance to outflow through meshwork; such increase is thought to induce retinal ganglion cell damage and visual impairment. Therefore, glaucoma targets various tissues in the anterior segment and posterior one as well [95, 96]. Nanotechnology-based targeting was focused on lowering IOP through augmenting the outflow or reducing the production of aqueous humour [57, 95, 96] and developed to prolong the retention time and the bioavailability of glaucoma medication.

Hyaluronic acid-modified chitosan nanoparticles were used to enhance the synergistic effect of mucoadhesion. These nanoparticles are loaded with timolol maleate (TM) to overcome the issue regarding poor bioavailability due to the solution drainage; the outcome showed a significant reduction in the IOP in comparison to the plain solution [97]. The field of nanomedicine has further offered an effective platform to deal with a variety of diseases like glaucoma, a novel unique type of unimolecular micelle nanoparticles (unimNP) has been designed to exhibit a neuroprotective effect through their inhibitory effects on oxidative/inflammatory stresses and apoptosis in the retinal ganglionic cell layer, and the unimNP were conjugated with the cholera toxin B domain (CTB) for retinal ganglion cell (RGC) targeting, tracing with Cy5.5, and loaded with dehydroepiandrosterone (DHEA) as a model drug to activate sigma-1 receptor (S1R) [98].

Brimonidine tartrate-Eudragit nanoparticles have been synthesised with improved drug loading efficacy. These nanoparticles were well tolerated and smaller particle size with better drug loading capacity. Prolonged drug release was notified in addition to the significant reduction in intraocular pressure with in vivo glaucoma rabbit's model [99]. Similarly, additional nanotechnology tools were employed to manage glaucoma by using contact lens-based release of timolol to maintain drug bioavailability and to increase patient compliance. Contact lenses loaded with timolol could maintain the release of therapy for 1 month at room temperature and subsequently reduced IOP [100]. While glaucoma drainage implants are sorts of drainage devices used to increase the outflow of the aqueous humour to supress IOP, these devices were effective and safe in reducing IOP, especially with devices that have larger end plates [101].

Multiple efforts have been made to protect the posterior segment of the eye through introducing PLGA microparticles loaded with glial cell line-derived neurotrophic factor GDNF, designed for neurodegenerative diseases, which maintained a constant release for 11 weeks when used to treat glaucoma. Moreover, GDNF microspheres stimulated significantly the viability of retinal ganglion cells compared to blank microspheres [102]. Also, heat shock protein (HSP) induction has been noticed to induce ocular neuroprotection and has attracted a considerable attention in the glaucoma therapy; therefore, induction of HSPs was done using local magnetic hyperthermia via engineered superparamagnetic nanoparticles (EMZF-SPNPAs) [103]. The biological safety and biocompatibility were confirmed through in vitro and in vivo studies. These nanoparticles hold a promising hope as being an excellent therapeutic agent for ocular neuroprotection and to reduce the secondary complications for glaucoma.

Conclusion

Nanoparticle system has proven to be a potent tool to manage ocular disorders, especially the posterior ocular segment. Loading medications via nanoparticles could avoid ocular barriers and enhance bioavailability and biodistribution of the delivered therapy in addition to minimising the frequency of injection and thereby reducing the risk of postinjection complications. Further investigations are required to promote our understanding regarding the fundamentals of nanosystem and facilitate the development of more suitable mode of applications.

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Part III
Transscleral Iontophoresis for Retina and Posterior Segment Disease

Chapter 12
Transscleral Drug Delivery to Retina and Posterior Segment Disease

Ann-Marie Ako-Adounvo and Pradeep K. Karla

Abstract The complexities of the anatomical and physiological barriers pose a challenge for the treatment of posterior segment eye disease. Ineffective delivery of the drug to the posterior site is regarded a major contributing factor for treatment failure. Transscleral drug delivery aims the large surface area of the sclera for increased drug absorption when compared to the intravitreal injections. This chapter addresses posterior segment diseases, treatment approaches and routes of drug administration, with emphasis on transscleral drug delivery. Application of novel strategies such as iontophoresis have been useful for improved permeability and drug delivery of ionized drugs. Further, newer techniques such as, polymeric colloids, implants, and thermoresponsive gels are currently investigated for efficiency.

Introduction

Human ocular anatomy is complex posing a significant challenge for therapeutic drug delivery. The eye is anatomically divided into two segments: anterior and posterior [1, 2]. The anterior segment consists of the cornea, pupil, iris, ciliary body, conjunctiva, posterior chamber, and lens, with aqueous humor occupying both the anterior and posterior chambers. Posterior segment of the eye comprises of the vitreous humor, macula, retina, choroid, sclera, and optic nerve [1, 3, 4]. Ocular anatomy is protected by the blood-aqueous and blood-retinal barriers (BAB and BRB). These barriers restrict the movement of substances between the aqueous humor, retina, and blood to maintain a homeostatic environment [5–7]. Both barriers play a vital role in limiting the intraocular bioavailability of therapeutic agents administered for posterior segment disease states. Unlike the anterior segment, poor accessibility to posterior segment makes the treatment of disease states quite challenging (Fig. 12.1).

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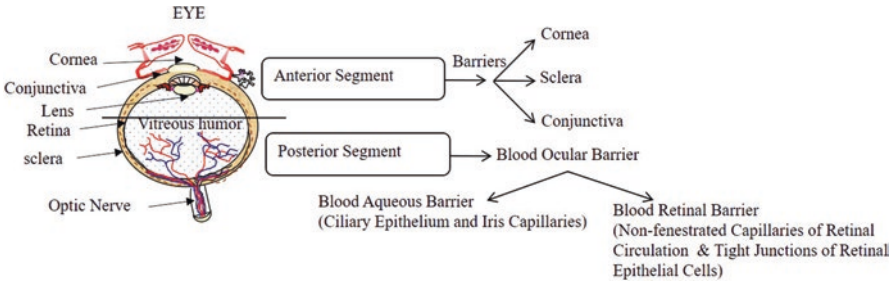

Fig. 12.1 Schematic presentation of the anatomy of the human eye and primary barriers for drug delivery

Visual impairment affects millions of people globally. The World Health Organization (WHO) statistics report that 253 million people across the globe suffer from moderate to severe visual impairment. An estimated 14.2% (36 million) are reported to be blind, and 85.8% (217 million) are reported to exhibit moderate to severe vision impairment [8, 9]. WHO reports that posterior segment diseases are a major cause of blindness [10]. Diseases affecting the posterior segment account for 55% of all ocular diseases leading to irreversible blindness [11]. Major disease states contributing to vision impairment include age-related macular degeneration (AMD), glaucoma, diabetic retinopathy, cytomegalovirus (CMV) retinitis, proliferative vitreoretinopathy (PVR), posterior uveitis, retinitis pigmentosa, retinal vascular occlusions, and choroid neovascularization (CNV) [12–14].

Complex anatomical and physiological barriers render the treatment of posterior segment disease state challenging. Ineffective delivery of the drug to the posterior site is regarded a major contributing factor for treatment failure. The current chapter discusses posterior segment disease states, treatment approaches, and routes of drug administration, with emphasis on transscleral drug delivery.

Pathology of Common Diseases Affecting the Posterior Eye Segment

Age-Related Macular Degeneration (AMD)

According to the National Eye Institute (NEI) report, age-related macular degeneration is a leading cause of vision loss among people aged 50 years and over worldwide [15–17]. In the United States, over 1.75 million people are currently affected by AMD, and 12% of the US population that are 80 years and above are reported to be suffering from AMD [17]. There are two forms of the disease: dry AMD and wet AMD [18, 19]. The macula is a small area in the middle of the retina that is responsible for focused vision. The degeneration of the macula is characterized by central vision loss. The early stage of dry AMD is marked by the accumulation of

extracellular aggregates in the retina, gradually leading to degeneration of the retinal pigment epithelial (RPE) cells and photoreceptors at later stages of the disease [18, 20, 21]. Wet AMD is marked by choroidal neovascularization (CNV) and is often observed at late stages of dry AMD [22]. Unlike dry AMD, wet AMD progresses rapidly and is known to be more vision-threatening [4, 18, 23].

Glaucoma

Glaucoma is a leading cause of irreversible blindness in the world. The disease is asymptomatic in its early stages and is characterized by an increase in intraocular pressure (IOP). Excessive aqueous humor production and reduced drainage due to clogging are regarded as the primary reasons for elevated IOP. If not adequately treated, the elevated IOP can cause damage to the optic nerve leading to irreversible blindness. There is no cure for glaucoma, and the disease is primarily managed by compounds that either decrease the fluid production or increase the fluid drainage. There are two types of glaucoma: primary open-angle glaucoma (POAG) and angle-closure glaucoma, with POAG being the most prevalent. Primary treatment regimen includes topical eyedrop therapy, and surgery is preferred in severe cases.

Cytomegalovirus (CMV) Retinitis

Cytomegalovirus is a double-stranded DNA virus that affects immunocompromised individuals. The virus tends to affect vital organs in the host body. Cytomegalovirus retinitis is the most common type of CMV infection and is a leading cause of visual impairment in individuals with acquired immunodeficiency syndrome (AIDS) [24, 25]. An estimated 25–42% of AIDS patients, with CD4+ T-lymphocyte counts of less than 50 cells, develop CMV retinitis [26–28].

Diabetic Retinopathy (DR)

According to the NEI, diabetic retinopathy commonly affects people with diabetes and is a leading cause of blindness in working-age population [29]. Diabetic retinopathy affects blood vessels in the retina causing them to leak resulting in contorting vision that can lead to blindness, if left untreated. The disease is asymptomatic during initial stages, and in the advanced stages, patients experience floaters, blurred vision, distortion, and progressive loss of visual acuity. The disease can be classified as nonproliferative (NPDR) and proliferative (PDR) [30]. PDR is characterized by retinal neovascularization, which involves the proliferation of new retinal blood vessels [30]. The disease starts as NPDR and progresses through mild, moderate,

and severe stages of NPDR where the retinal capillary walls are damaged. Chronic hyperglycemia in diabetic patients eventually leads to the hemorrhage of retinal capillary walls [30]. It then progresses to a more severe stage, PDR, where abnormal retinal neovascularization occurs [31, 32].

Retinitis Pigmentosa

Retinitis pigmentosa is a hereditary eye disorder that affects the retina, leading to a gradual degeneration of photoreceptor cells in the retina. The degeneration changes the morphology of retinal pigment epithelium, glial cells and retinal blood vessels [33]. The disease is characterized by a progressive loss of peripheral vision, photopsia (seeing flashes of light), and nyctalopia (difficulty seeing at night), with a subsequent loss of central vision in some individuals [34].

Routes of Drug Administration to the Posterior Segment

The vitreous, retina, and choroid are the primary sites of interest for drug delivery to posterior segment. Drug administration to the posterior segment aims to achieve a high drug concentration in the vitreous [35]. Prominent routes of posterior segment drug delivery are discussed below (Fig. 12.2).

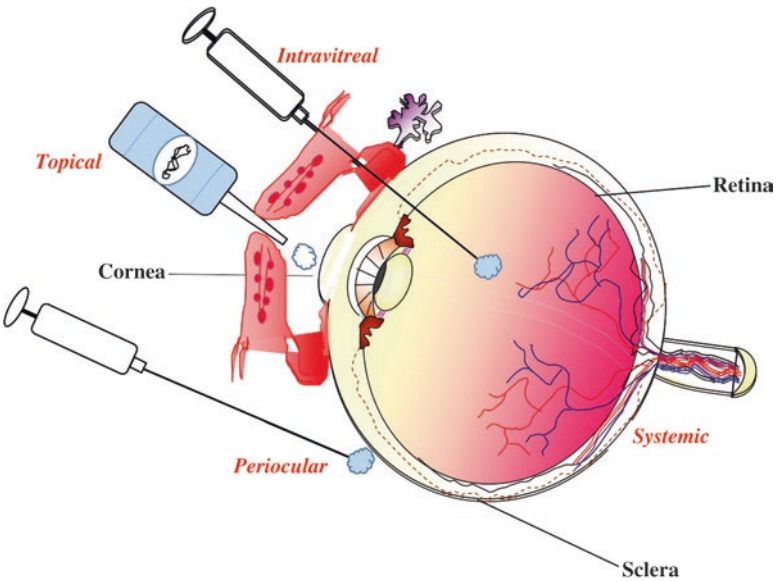

Fig. 12.2 Schematic representation of the primary routes of posterior segment ophthalmic drug delivery

Topical

Topical drug administration via eyedrops is primarily employed for anterior segment drug delivery [11, 36]. However, the route has been explored for delivery of therapeutic agents to the posterior segment. Factors impacting topical drug delivery include nasolacrimal drainage, tear dilution, tear turnover, poor permeability of corneal epithelium, and systemic absorption [36]. These factors significantly reduce the bioavailability of drugs to both anterior and posterior segments. It is estimated that approximately 80% of topically administered drugs are systemically absorbed through the nasopharyngeal mucosa and other non-corneal routes [37]. Only 1–10% of the administered dose is estimated to be absorbed via the cornea [38–40]. Despite the challenges, few therapeutic agents administered via the topical route have been shown to successfully reach the posterior segment at therapeutic concentrations [41–45]. Ease of application, noninvasiveness, and patient compliance make this a preferable route.

Systemic

The systemic route involves either intravenous (parenteral) or oral (enteral) drug administration. Post-systemic bioavailability of drugs is limited by the poor permeation of the drugs across BRB. The BRB comprises of two cell types. Retinal capillary endothelial forms the inner cell barrier, and retinal pigment epithelia forms the outer cell barrier [46]. The retinal pigment epithelium (RPE), characterized by tight junctions, forms the rate-limiting barrier for intraocular absorption of molecules from the blood [47]. Small molecular weight lipophilic molecules permeate RPE comparatively better than hydrophilic or large molecular weight compounds [11, 13, 47]. With systemic route, a strategy for improving bioavailability and permeability across the BRB is to maintain a higher blood-posterior drug concentrations by administering larger doses. This strategy, however, is associated with systemic adverse effects. Classes of drugs such as antibiotics, steroids, diuretics, and nonsteroidal anti-inflammatory drugs (NSAIDs) are current in clinical use for systemic treatment of ocular diseases. Systemic administration, similar to the topical route, has high patient compliance. However, systemic drug exposure and poor bioavailability limit the use of systemic routes [12, 48].

Intraocular Injection

Intraocular injection delivers formulations through the globe of the eye into the vitreous. It is also referred to as intravitreal injection. Unlike the topical and systemic, high bioavailability in the posterior segment is achieved with this route [49]. Examples of drugs that are currently in clinical use and administered intravitreally

include anti-vascular endothelial growth factor (anti-VEGF) agents (Macugen®, Lucentis®, Eylea®, Aflibercept®, and Visudyne®) for the treatment of AMD [50–53]. Because of the high intraocular drug bioavailability, therapeutic agents intended for the posterior segment are commonly administered via intravitreal route. However, the process is invasive, painful, and expensive, rendering the route less patient compliant. Due to the relatively viscose environment, distribution of the injected drug is uneven within the vitreous particularly for large molecules, whose diffusion is impeded by the viscose vitreous humor [54]. Further, smaller drug molecules can be cleared out faster, thereby reducing the intraocular half-life. In addition, repeated injections, often required with this delivery route, resulted in side effects such as retinal detachment, vitreous hemorrhage, increased intraocular pressure, endophthalmitis, and cataract [12, 13, 55].

Periocular

Periocular administration involves the deposition of therapeutic agents into areas adjacent to or surrounding the eye. Various periocular drug delivery strategies include subconjunctival, subtenon, peribulbar, retrobulbar, and posterior juxtascleral injections [55–58]. These routes are commonly used for delivery of local anesthetics and corticosteroids [59]. Following periocular delivery, drugs are known to reach the posterior segment via the following pathways: transscleral, systemic circulation and the anterior segment [60]. Compared to the intravitreal injection, periocular administration is less invasive and has low side effect profile. The bioavailability of certain drugs administered via the periocular route is comparable to topical and systemic routes [61, 62].

Transscleral Pathway for Delivery of Drug to the Retina and Posterior Segment

Research studies demonstrated the transscleral pathway to be a preferred route of drug absorption into the posterior segment following periocular route [63]. The sclera covers a large part (~ 95%) of the surface of the eyeball [35] and is separated from the cornea by the corneal limbus. Hence, it has a larger surface area (~ 16.3–17 cm^2) available for drug absorption [64]. In addition, the sclera is composed of dense collagen fibers (predominantly type I) making it less resistant and more permeable for the drugs [65, 66]. The sclera is made up of three layers, the episclera, sclera proper, and lamina fusca. The episclera forms the exterior part of the sclera and is a thin densely vascularized layer of connective tissues intermingled with fibroblast, macrophages, and lymphocytes. The sclera proper is avascular but consists of dense bundles of collagen fibers. The lamina fusca forms the inner surface of the sclera and is composed of collagen bundles dispersed with melanocytes [63]. The lamina fusca connects with the outer surface of the choroid (Fig. 12.3).

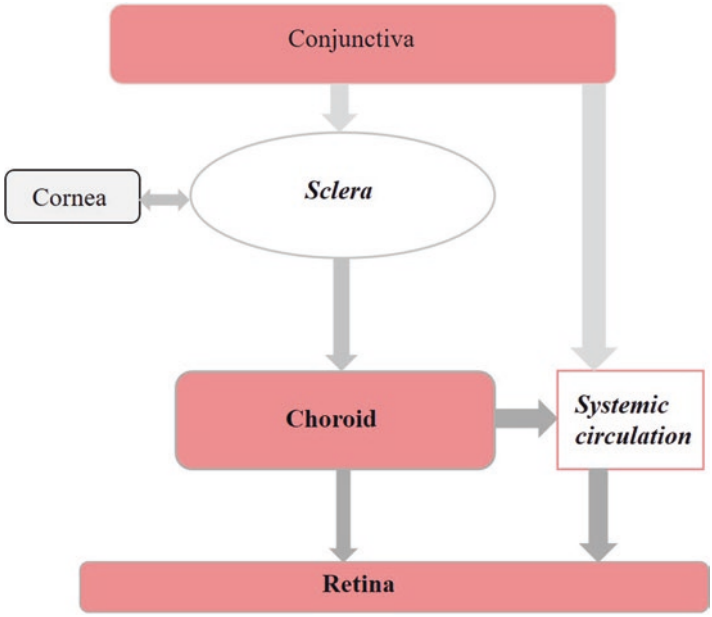

Fig. 12.3 Schematic representation of transscleral drug delivery to the retina

Scleral Permeability and Transscleral Pharmacokinetics

Factors affecting the scleral permeability of small and large molecular weight drugs have been established [67–69]. Shuler et al. demonstrated the permeability of fluorescein-labelled single-stranded oligonucleotide (MW = 7998.3) across the human scleral tissue from an in vitro diffusion study, employing a perfusion chamber [70]. Permeability constant of the oligonucleotide was analyzed from collecting choroidal perfusate fractions over a 24-hour period. The following permeability rate constant was employed, $K_{trans} = (R_{total}/(A \times t)) \times (1/D)$, where K_{trans} is the permeability constant, R_{total} is the total moles that permeated the sclera in time t, A is the total surface area (cm^2) of the sclera from the study, and D is the initial concentration of the fluorescein-labelled oligonucleotide solution (mol/mL) in the donor chamber. The study reported permeability constant values of $7.67 \pm 1.8 \times 10^{-7}$ cm/s and $1.32 \pm 0.42 \times 10^{-7}$ cm/s for transscleral diffusion and intrascleral injection, respectively [70].

Ambati et al. studied the in vitro permeability of high molecular weight compound across the sclera [71]. Rabbit sclera, mounted on a two-chamber diffusion apparatus, was employed to evaluate the diffusion of sodium fluorescein, FITC-conjugated bovine serum albumin (FITC-BSA), FITC-rabbit IgG, and FITC-conjugated dextran with molecular weights ranging from 4 to 150 kDa. The study demonstrated decreased scleral permeability with increased molecular weight and molecular radius. The permeability coefficient values were $84.5 \pm 16.1 \times 10^{-6}$ cm/sec (for the smaller compound) and $1.34 \pm 0.88 \times 10^{-6}$ cm/sec (for 150 kDa

FITC-dextran, with the largest molecular radius). Further, results indicated a better scleral permeability of globular proteins compared to linear dextran of similar molecular weight [71].

Anderson et al. studied the permeability of fluorescein isothiocyanate (FITC)-labelled bovine serum albumin across human scleral tissues from 15 donors. Further, the study investigated the effect of geographical location and donor age on scleral permeability [72]. The study demonstrated that geographical location had no significant impact on transscleral permeability. However, there was a significant decrease in the permeability of albumin across scleral tissue specimens with increasing donor age [72]. The conclusion that donor age influences scleral permeability contradicts an earlier work by Olson et al. [73] who tested 97 scleral tissue specimens and concluded that there was no significant decline in scleral permeability with age [73]. These recent research studies demonstrated that sclera is permeable to a wide range of low molecular weight, high molecular weight, hydrophilic, and lipophilic molecules.

Barriers for Transscleral Drug Delivery

The transscleral pathway is restricted by barriers that significantly reduce the bioavailability of therapeutic agents in the retina. The anatomical structures of the sclera, choroid, and RPE constitute static barriers through which drug molecules penetrate [74], creating a drug concentration gradient across the sclera, choroid, Bruch's membrane, and RPE [11]. The RPE is known to be a major barrier due to its tight intracellular junctions, hindering the permeation of drug molecules from the choroid to the retina [47, 75]. The studies indicate the presence of melanin in the choroid-RPE. The melanin-rich layer has high affinity for basic and neutral molecules, impeding their partition into the retina [58, 76, 77]. Blood and lymphatic flow constitute the dynamic barrier impacting drug clearance from the sclera, conjunctiva, and choroid [48, 68, 78, 79]. The impact of dynamic barrier on drug clearance is verified in an in vivo study, where the animal was euthanized post transscleral drug administration. The euthanization effectively halted the lymphatic and blood flow to the conjunctiva and choroid, reducing the drug clearance [80]. RPE is known to express high cytochrome P450 levels. This drug-metabolizing enzyme in addition to lysosomes affects the fraction of drug available for retinal uptake [80].

Current Efforts to Improve Transscleral Drug Delivery to Posterior Segment

Transscleral route resulted in a significant increase in posterior segment drug concentrations compared to other routes such as systemic and topical. Further, transscleral route has better patient compliance and side effect profile than intravitreal route. However, the transscleral barriers limit the permeability of moderate to high

molecular weight molecules reducing the posterior segment drug concentration. Current research focuses on developing novel technologies to improve transscleral permeability of moderate to high molecular weight drug molecules.

One such novel strategy is iontophoresis. Ocular iontophoresis is noninvasive and employs a low electric current to increase the penetration of ionized drug molecules. The technology is employed both transcorneally and transsclerally [74, 81, 82]. This technology resulted in a higher drug concentration in the posterior segment. Various therapeutic agents including antibiotics, antifungals, antivirals, steroids, and nonsteroidal anti-inflammatory drugs (NSAIDs) have been delivered employing iontophoresis [82]. Other strategies to improve transscleral drug delivery include the use of biodegradable polymeric colloidal drug carriers [43, 83, 84], implants [85], and thermoresponsive gels [84, 85].

Conclusion

Posterior segment eye diseases are a leading cause of visual impairment and blindness in the world. The chapter describes the common posterior segment disease states and drug delivery routes. Extensive research and clinical data demonstrated that the transscleral pathway confers a significant advantage when compared to the other routes of posterior segment drug delivery. Hence, the chapter emphasizes on the structure, barriers, and transscleral permeability pharmacokinetics of the sclera. In addition, the chapter provides an insight into the novel technologies such as iontophoresis, polymeric colloids, implants, and thermoresponsive gels for improved transscleral drug delivery.

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Chapter 13
Colloidal Carrier Systems for Transscleral Drug Delivery

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Abstract This chapter aims to describe colloidal carrier systems for transscleral drug delivery as a possible treatment approach for posterior segment ocular diseases. Complexities of eye anatomy pose several difficulties in the treatment of posterior segment ocular diseases. The transscleral route of drug delivery has demonstrated an alternative method to the already widely accepted topical, oral, and intraocular routes for drug administration into retinal segment of the eye. By combining this route of administration with a colloidal carrier system, the active pharmaceutical ingredient shows a higher potential of reaching its target location at a higher concentration while simultaneously mounting patient compliance.

Introduction

There are numerous efforts currently being made to find the most effective, efficient, safe, and patient- preferred method to treat ocular diseases of the posterior segment of the eye including but not limited to age-related macular degeneration, choroidal neovascularization, and diabetic retinopathy [14, 35, 45]. Various efficient routes of drug administration to the posterior segment of the eye include intravitreal, subconjunctival, and transscleral routes. Intravitreal injections though provide the direct drug exposure to the posterior segment of the eye; multiple injections place the risk of development of cataract, retinal detachment, vitreous hemorrhage, and endophthalmitis. Conjunctival retinal drug administration though provides enough space for the drug delivery and is less efficient due to blood retinal barrier and retinal pigment epithelial cells hindering the absorption of drug via conjunctival sac. A new route of pharmaceutical drug administration is transscleral drug delivery which has evidence to prove that bioavailability can be improved with targeted drug release. The bioavailability is improved by direct

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penetration of the drug through the sclera to the posterior segment of the eye [30]. This helps to decrease the amount of drug loss by lacrimal drainage or systemic circulation, decrease the amount of drug that is removed by the body's efflux transporters, or increase the amount of drug that can permeate through the intrinsic barriers to its intended target. The controlled and targeted release of the drug through the transscleral route is accomplished via the formulation of the drug delivery system. Till date colloidal carriers are widely exploited in the science field with various applications. Colloidal carrier systems present as one of the formulation methods in which the release of the active pharmaceutical ingredient can be manipulated [2, 38].

Anatomy of the Sclera

Human Sclera

It is important to understand the anatomy of the eye, specifically the sclera and its surrounding barriers, before considering how to deliver a pharmaceutical ingredient to the posterior part of the eye through the transscleral route. The sclera in combination with the cornea forms the exterior of the eye and is sometimes called "the white of the eye" [46]. The sclera extends from the anterior portion of the eye where it is continuous with the cornea to the posterior of the eye where it is punctured by the optic nerve. This anatomical relationship can be seen in Fig. 13.1 which identifies the different structures

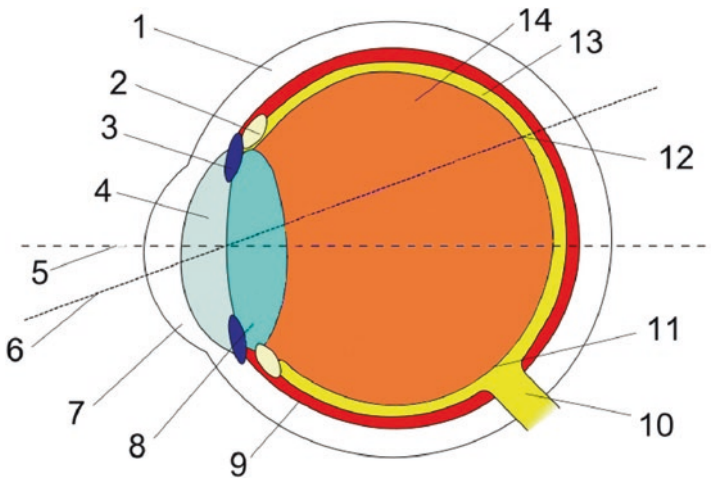

Fig. 13.1 Anatomy of the human eye. This image depicts the most important anatomical structures of the human eye. The sclera is continuous with the cornea in the anterior segment and is penetrated by the optic nerve in the posterior segment. (1, sclera; 2, ciliary body; 3, iris; 4, pupil and anterior chamber filled with aqueous humor; 5, optical axis; 6, line of sight; 7, cornea; 8, crystalline lens; 9, choroidea; 10, nervus opticus; 11, optic disc; 12, fovea; 13, retina; 14, corpus vitreum)

of the ocular region, including the sclera. The sclera contains Tenon's capsule, the episclera, the stroma, the spur, and the lamina fusca, and it is mainly made up of type I, III, V, and VI collagen fibers as well as proteoglycans which typically display a negative charge. Due to the presence of the proteoglycans, hydrophilic molecules diffuse more efficiently through the sclera than lipophilic drugs. Another important note about the sclera is its avascular property. It receives its nutrients mostly from the choroid [46]. The sclera is thickest at its most posterior region near the optic nerve, and it is thinner near the equator of the eye. According to the publication by Jigar N shah et al., the thickness of the sclera has an inverse relationship with the permeability of the sclera; therefore the ideal location for transscleral drug delivery is near the equator [36].

Ocular Barriers

Although the transscleral route of drug administration is proving to be the most effective while simultaneously minimizing invasiveness, the intrinsic barriers of the eye present difficulties to this new method. These barriers are categorized as static, dynamic, and metabolic. The static barriers are tissues that the drug must diffuse across, and they include the sclera, the choroid, Bruch's membrane, and the retinal pigmented epithelium (RPE). The dynamic barriers act to eliminate the drug from its local site of action, and they include blood circulation, lymphatic circulation, and proteins that act as efflux transporters. The metabolic barriers are the enzymes present in the body that acts to break down foreign substances, most prominently the CYP450 enzyme system [9, 36]. Each of these barriers presents its own challenge for successful drug delivery to the posterior segment of the eye, some of which will be discussed later in this chapter.

Transscleral Route

There are several already approved methods commonly employed to treat diseases related to the posterior segment of the ocular region. The need for alternative methods lies in the fact that each of the already commonplace techniques presents significant draw backs in their uses. The main types of drug delivery are topical administration, systemic administration, intraocular injections, and periocular injections via transscleral delivery. Topical administration can be effective and proves to be preferred by patients due to its ease of use; however, high drug concentrations are necessary because it is estimated that less than 5% of the pharmaceutical drug is able to successfully access its intended site due to nasolacrimal drainage and other intrinsic barriers [3, 38]. Systemic administration demonstrates very similar benefits and drawbacks as topical administration. Intravitreal injections have also proven to be effective for treatment of back of the eye diseases; however, there are possible side effects including hemorrhage, increase in intraocular pressure, glaucoma, and cataracts due to its

Table 13.1 Routes of transscleral drug delivery

Regions of administration via periocular route	Injection volume (mL)	Advantages	Disadvantages	Common uses
Subconjunctival	0.5 mL	High C_{max} in vitreous humor	Possible subconjunctival hemorrhage	Rhegmatogenous retinal detachment
Subtenon	1–5 mL	Large retention space	Injection complications	Anesthesia, chronic uveitis, CME*
Retrobulbar	2–3 mL	Targeted near macular region	Systemic complications and possible hemorrhage	Anesthesia, CME*, protein-polymer implants
Peribulbar	8–10 mL	Targeted near macular region	System complications, possible hemorrhage, less effective than retrobulbar	CME*, anesthesia
Posterior juxtascleral	N/A	One injection every 6 months	Requires surgery	Age-related macular degeneration, CNV*

This table summarizes the key points of the five main routes of transscleral drug delivery [38]. The possible complications arising from subtenon injection include accidental injection into choroidal or retinal circulation [24]. The posterior juxtascleral route does not have a standard injection volume due to its newness; however, a study done by Jokovich and co-researchers, 2005, [21] demonstrated this technique with 0.5–1 mL. This table is not all-inclusive
**CME* cystoid macular edema, *CNV* choroidal neovascularization

invasive nature [18, 34, 42]. In this chapter the focus will remain on periocular injections which are the administration technique for transscleral drug delivery systems. There are five main ways that a drug can be delivered through this periocular transscleral route. These routes include subconjunctival, subtenon, retrobulbar, peribulbar, and posterior juxtascleral. As evident in Table 13.1, each of these routes has its own advantages and disadvantages; also due to the anatomy of the eye, each route also has a maximum injection volume. For example, the subtenon route has the advantage of a large retention space due to the large filling capacity of the subtenon region, and therefore it is the most commonly used route for anesthetics. All of these routes have the common advantageous factor that the eyeball is not being punctured in the injection process. The posterior juxtascleral route is unique from the others in that it classifies as a depot suspension in which administration is required only once every 6 months due to the sustained release of the active pharmaceutical ingredient from the deposited suspension [7]. These periocular injections for transscleral delivery release the drug beneath the conjunctiva, and then the drug diffuses through the ocular region into the sclera and then to its site of action. Figure 13.2 demonstrates this pathology of how the administered drug reaches its target location and can demonstrate why drug delivery to the eye can be difficult due to these many barriers [31].

Another important aspect of transscleral drug delivery is the properties of the actual drug molecule. The permeability of the active pharmaceutical ingredient

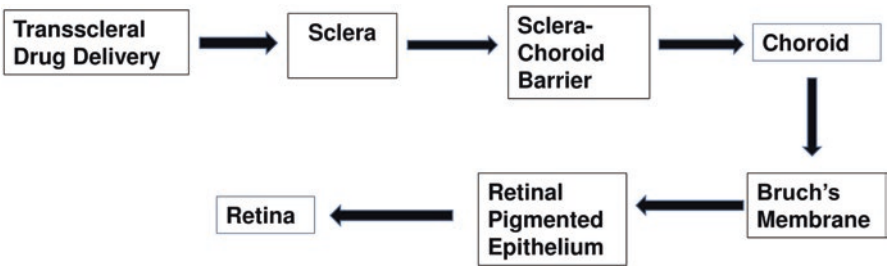

Fig. 13.2 Pathology of transscleral drug delivery. This image displays the general path that the drug molecule must cross before it reaches the retina or the vitreous humor [31]

through the different barriers discussed above is dependent on several pharmacokinetic factors. Some of these factors affecting the absorption and distribution of the drug molecule are molecular weight, molecular radius, particle size, charge, hydrophilicity, lipophilicity, degree of ionization, and protein binding. According to a study done by Ambati and co-researchers, 2000, the permeability of the sclera decreases exponentially with increasing molecular weight and molecular radius. This study found that the sclera is permeable to 70 kDa dextran when in globular form and 40 kDa when in linear form. It has also been shown that the ideal molecular radius for scleral permeation is 20–200 nm [36]. In terms of the molecule's charge, positively charged molecules demonstrate poor permeability due to their attraction to the negative charge present on the proteoglycans within the sclera [16]. This charge on the sclera partially explains the hydrated quality of the sclera. The sclera demonstrates a higher permeability to hydrophilic molecules; therefore, as lipophilicity increases, the permeability decreases [44]. Hydrophilic drug candidates may permeate more easily through the aqueous medium of proteoglycans in the fiber matrix [12, 26]. Additionally, thickness of the sclera also differs at various regions ranging from 0.39 mm near equator to 0.9–1.0 mm near the optic nerve. Thickness of human sclera near the limbus is found to be 0.53 mm [29], and the posterior portion of sclera comprises loose collagen fibers which are firm in the anterior portion [13]. Due to the varying physiological characteristics of the sclera and the physicochemical properties that are required for the absorption of the drug, colloidal carrier systems display a beneficial drug delivery system in order for the drug to reach steady state at the specific target site.

Colloidal Carrier Systems

Colloidal carrier systems are a novel form of drug delivery that will aid in treating diseases that are hard to access like those in the posterior segment of the eye. Only recently did the researchers begin studying colloidal systems in transscleral drug delivery where in the past its focus has been on transcorneal and intravitreal delivery [4, 23]. Colloidal carrier systems include but are not limited to the particulate system

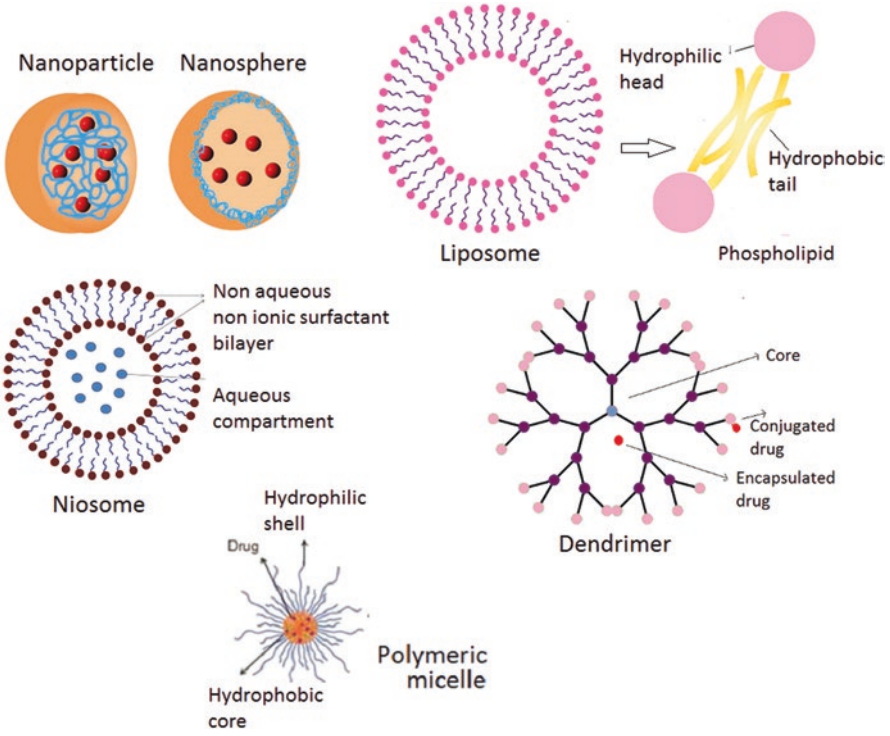

Fig. 13.3 This image depicts various colloidal carrier systems that can encapsulate drug and can be further utilized for targeted drug delivery system via transscleral route for the treatment of posterior segment of the eye

of nano- and micro particles, liposomes, dendrimers, niosomes, and micelles as shown in Fig. 13.3 [37]. Each of these systems has its own preparation method as well as its own advantages and disadvantages. Further in the chapter, various colloidal carrier systems will be discussed along with its scope for transscleral drug delivery.

Nano-Particulate System

Application of nanotechnology for the drug delivery in the posterior segment of the retina can be a promising tool in treatment of ambit of diseases affecting the mentioned area of eye. Nano-particulate technology meets the finest objectives of drug delivery including enhanced drug permeation and controlled and targeted release of drug. The first step of the preparation of drug- loaded micro- or nano particles includes the choice of polymer used to create the colloidal carrier system. These polymers can be described as biodegradable or non-biodegradable, and some examples include poly lactic acid (PLA), poly lactic glycolic acid (PLGA),

thermoreversible gels, polyethylene glycol (PEG), poly vinyl alcohol, and chitosan [38]. When considering which polymer to choose, one must focus on a few key points: the intended rate of drug release, the amount of drug required per dose, and if this required dose exceeds the maximum amount that can be loaded into the polymer. For example, PLA releases drugs in the span of months, where as PLGA releases drugs in a 2–4- week time span [47]**.** The polymers PLA, PLGA, and PEG are beneficial for delivery of hydrophobic drugs to the posterior of the eye. The hydrophobic characteristic of these drugs leads to poor absorption, and therefore the bioavailability is increased by the protection that the polymer provides. In order to encapsulate these hydrophobic drugs in one of the above mentioned polymers, one common method is emulsification. This process involves dissolving the polymer and the compound in an organic solvent, adding aqueous solvent in order to induce emulsification, and then applying some type of external force on this mixture to finish encapsulating the drug molecule.

Depending on the physicochemical properties of the drug compound, there are different types of emulsification techniques for hydrophobic drugs including oil in water, oil in oil, solvent in oil in water, and others. An example of how to choose which method can be demonstrated with the hydrocortisone drug molecule. This compound shows significant aqueous solubility; therefore, if the o/w method was chosen, the encapsulation efficiency would be low due to the tendency of the drug molecule toward the aqueous phase. Therefore, for the most efficient encapsulation of hydrocortisone, the oil in oil method would be preferred [47]. The end goal of the emulsification technique is to obtain spherical particles of equal size and shape with a high encapsulation efficiency of the drug particles.

Recent studies have highlighted the pertinency of transscleral drug delivery of macromolecules efficiently [5, 6]. Colloidal carrier systems for such globular proteins was developed and evaluated for transscleral drug delivery of the macromolecule by Carrasquillo et al. The investigators had demonstrated microspheres of anti-vascular endothelial growth factor (VEGF) RNA aptamer using biodegradable polymer, poly lactic glycolic acid (PLGA). Microspheres were prepared using oil in oil solvent evaporation method. PLGA microspheres of aptamer were able to sustain the drug release by the release rate of 2 μg/day for 20 days [8].

Liposomes

In recent years many researchers have significantly explored the use of liposomes in ophthalmic therapy. Liposomes are reported to protect the drug molecules from the metabolic enzymes present at the tear and corneal epithelium interface and help to come into immediate contact of ocular surfaces, thereby providing target drug delivery [22]. Liposome formulations are mainly composed of phosphatidylcholine-, cholesterol-, and lipid- conjugated polymers. Inherent properties of liposomes make it biodegradable and biocompatible in nature [27]. Hironaka et al. investigated liposomal drug delivery system for targeting the retinal segment. Submicron- sized

multilamellar vesicles were prepared of egg phosphatidylcholine and L-α-distearoyl phosphatidylcholine. Results depicted ability of liposomes for delivery of hydrophobic molecules into the retina [20]. Tan and Ling reported liposomal transscleral drug delivery system made up of unsaturated lipids like 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine and saturated lipids like 1,2-dimyristoyl-sn-glycero-3-phosphocholine. Results demonstrated high potential of unsaturated lipids to transport across the sclera [39].

Niosomes

Niosomes are surfactant- based vesicular systems exploited for targeted drug delivery. These are more or less similar to liposomes in structure and constituents. Lipid phosphatidylcholine is replaced by surfactant and hence imparts more chemical stability and low toxicity owing to their nonionic nature than other vesicular systems. Niosomes are biodegradable, biocompatible, and non-immunogenic that make it more viable drug delivery system [1, 33, 40].

Recently many investigators have demonstrated niosomal formulations for ocular drug delivery. Abdelkader and co-researchers, 2010, prepared niosomes of naltrexone hydrochloride for the treatment of diabetic keratopathy including impaired corneal sensation and delayed wound repair by improving its permeation into the eye. Niosomes were fabricated using nonionic surfactants like sorbitan esters and polyoxyethylene alkyl ethers. Results suggested potential thermoresponsive properties of niosomes desirable for ocular delivery of naltrexone hydrochloride.

Dendrimers

Dendrimers are branched- like nanostructured polymers with size ranging from 2 to 20 nm with narrow polydispersity. The controlled terminal groups present on the surface of dendrimers present a potential in biomedical applications and drug delivery [11, 19, 28]. Advantages of dendrimers in drug delivery include biocompatibility and water solubility, thereby improving bioavailability [15]. The very first family of dendrimer polymeric nanostructures includes poly(amidoamine) dendrimers (PAMAM). PAMAM dendrimers are exploited for ocular delivery of pilocarpine nitrate and tropicamide for controlled drug delivery in the eye. Results depicted increase in the residence time of the drug in ocular region [43].

Yavuz and co-researchers, 2015, evaluated dexamethasone-PAMAM dendrimers for drug delivery into retinal portion. Hydroxyl- terminated PAMAM dendrimers of dexamethasone were formulated to sustain the drug in the posterior segment of the retina [48].

Micelles

In the past few years, polymeric micelles are investigated extensively as promising drug delivery systems for the treatment of ocular disease affecting anterior as well as posterior segment of the eye [25]. Micelles comprise of amphiphilic molecules that accumulate in aqueous media to form organized molecular body. Self-accumulation takes place above certain concentration known as critical micelle concentration. Size and shape of micelles depend on the molecular weights of center and periphery forming entities [41]. Hydrophilic periphery helps in easy solubilization of the entire molecular body. Examples of core polymers investigated include poly(lactide), poly(propylene oxide) (PPO), poly(glycolide), poly(lactide-co-glycolide), and poly(ε-caprolactone) (PCL). Poly(ethylene glycol) (PEG) is mostly exploited for corona layer of the micelle. Other copolymers investigated for ocular drug delivery include polyhydroxyethyl aspartamide (PHEA), bearing in the side chains polyethylene glycol (PEG) and/or hexadecylamine (C16) [10], and poly(L-lactide)-b-poly(methacrylic acid-co-3-acrylamidophenylboronic acid) [32].

Elsaid and co-researchers, 2012, [17] reported positively charged amphiphilic chitosan derivative of rapamycin to facilitate transscleral drug delivery. O-octanoyl-chitosan-polyethylene glycol (OChiPEG) micelle was prepared using thin film method. Rapamycin micelles were prepared successfully with particle size of 40.6 nm and zeta potential +6.84 mV, and CMC was found to be 16.6 μM. Prepared micelles of rapamycin showed sclera retention of 14.8 ± 0.81 μg/g with efficiently transscleral permeation of $5.57 \pm 1.04 \times 10^{-8}$ cm^2 s^{-1}.

Future Directions

Succinctly it could be concluded that transscleral drug delivery is a better option for the therapy of disease pertaining to posterior segment of the eye. Furthermore, colloidal carrier systems are the novel approaches that are successfully utilized in one or the other way to efficiently utilize the transscleral route for drug delivery into the eye. Different colloidal carriers facilitate sustained and targeted delivery of drug in the posterior segment of the eye. Apart from sustained release, various colloidal systems will also provide stability to the drug by encapsulation.

Presently intravitreal route is widely utilized for the administration of drug into the posterior segment of the eye. But it is the highly invasive method of administration hence not following patient compliance. Future directions may include formulation and design of smart colloidal carrier systems with an objective of controlled and sustained drug delivery to the posterior segment of the eye via transscleral route.

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Chapter 14
Transscleral Iontophoretic Drug Delivery for Treating Retinal Diseases

Dherya Bahl, Rinda Devi Bachu, Mrudula Chitti, Pallabita Chowdhury, Jwala Renukuntla, and Sai H. S. Boddu

Abstract Drug delivery to the eye still is a challenge due to its intricate anatomical structure. Posterior segment delivery is much more challenging due to the acellular nature of the vitreous humor and the longer diffusion distance to the retina. Iontophoresis is a noninvasive technique that facilitates the movement of charged drug molecules into tissues by an electric field. Using iontophoresis, it is possible to achieve therapeutic concentrations faster by modulating the intensity and duration of the applied current. Transscleral iontophoretic delivery is gaining pace and is considered as an alternative for a safe and more effective treatment to retinal disorders. This chapter intends to highlight various aspects of iontophoresis, with a special emphasis on transscleral delivery of drugs and drug-loaded nanocarrier systems for treating disorders in the back of the eye. Finally, a section on toxic effects of iontophoresis to various ocular tissues is included.

Keywords Electrorepulsion · Ions · Current intensity · Tissue barriers · Ocular drug delivery · Transscleral iontophoresis · Posterior eye · Retina

Introduction

Drug delivery to the eye remains a challenge due to intricate anatomical and physiological barriers. The anatomical position of the eye allows local delivery of drugs, along with noninvasive clinical assessment of disease, while the physiological barriers preclude the entry of drug substances. For efficient treatment of diseases, drug molecules should circumvent the protective physiological barriers without causing

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permanent tissue damage. Both anterior and posterior segments provide unique barriers to the entry of drugs [1, 2]. These barriers vary based on the route of administration such as topical, systemic, and injectable. For instance, topical administration results in low ocular bioavailability (<5%) due to various barriers such as nonproductive absorption, tear production, tear turnover, solution drainage, transient residence time, and impermeable corneal epithelium. Ocular tissue barriers and clearance mechanisms that prevent topical drug absorption into the eye are presented in Fig. 14.1.

Drug delivery to the posterior segment eye diseases is even more challenging. The retina and choroid are the target sites for most posterior segment diseases. Topically applied conventional dosage forms such as eye drops, ointments, and suspensions deliver less than 1/100,000th of the dose to the choroid and retina [3, 4]. The common approaches to the treatment of posterior segment diseases generally include systemic delivery and intraocular injections/implants. Systemic delivery results in severe adverse effects, with less than 2% of the administered dose reaching the retina due to the presence of blood-retinal barrier. Intravitreal administration delivers a high drug concentration to the retina; however, the inherent side effects, like cataract [5], hemorrhage [6], increased intraocular pressure [7], and endophthalmitis [8], lead to complications limiting long-term therapy [9]. Furthermore, the chronic and progressive nature of retinal diseases demands multiple intraocular injections at regular time intervals, which are associated with the risk of retinal detachment and cataract progression. Though implants have overcome some disadvantages linked with intravitreal injections, the risks of drug precipitation and invasive surgical procedure are the key deterrents in such delivery [10, 11]. Moreover, the absolute number of adverse reactions will increase as more drugs using these delivery approaches receive FDA approval. To address the growing demand from

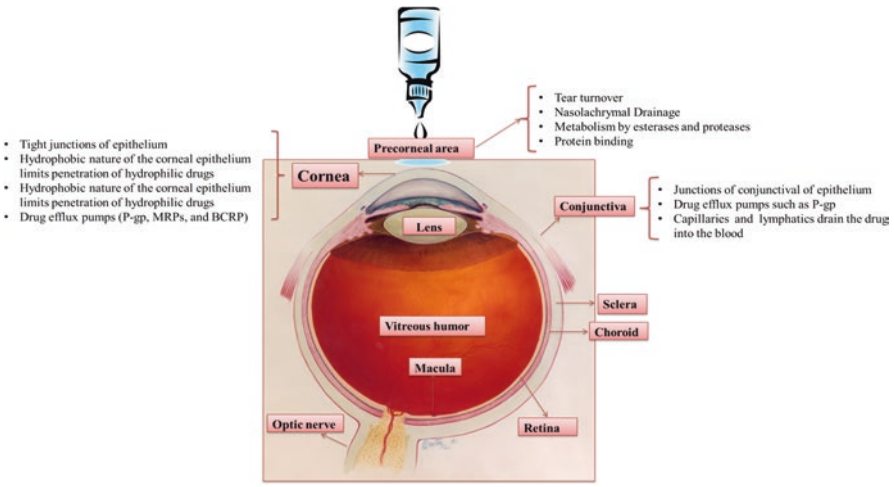

Fig. 14.1 Ocular tissue barriers and clearance mechanisms that prevent the penetration of drugs to the back of the eye. (Modified from the National Eye Institute, National Institutes of Health (Ref#: NEA04))

the aging population suffering from chronic posterior eye diseases, emphasis has been placed on the development of novel drug delivery strategies to minimize complications and to improve patient compliance. Clinical and pharmaceutical researchers working on this area are looking for newer approaches/technologies to facilitate the delivery of small molecules and biologics for treating both anterior and posterior segment eye diseases [12]. These include injectable implants [13], osmotic pumps [14], microneedles [15], nanocarriers [16–18], microcannulation [19], light-sensitive vectosomes [20], and iontophoresis [21]. Some of these approaches have been discussed in a review article by Thrimawithana et al. [22].

Ocular iontophoresis involves the placement of a donor electrode loaded with the drug in the eye with another electrode placed on a nearby body surface to complete the electrical circuit (Fig. 14.2). The application of electric field helps in the delivery of drugs into the eye. Iontophoresis has been used in the field of ophthalmology for many years, mainly via transcorneal and transscleral routes [23]. Transcorneal iontophoresis is preferred in treating anterior segment diseases affecting the cornea, aqueous humor, ciliary body, iris, and lens, whereas transscleral iontophoresis is the choice for treating posterior segment diseases affecting the choroid and retina. When applied topically, the lens-iris diaphragm forms a major barrier for drugs reaching the posterior region of the eye. With transscleral iontophoresis, it is possible to overcome this barrier and deliver drugs to the vitreous and retina through the choroid. Transscleral iontophoresis is efficient and safe, especially at low current densities, compared to traditional intravitreal/subconjunctival injections or systemic therapy. It has been used for treating posterior ocular disorders such as retinitis, retinoblastoma, endophthalmitis, optic nerve atrophy, uveitis, and age-related macular degeneration (AMD) [24]. This chapter aims to review the essential features of iontophoresis and the use of transscleral iontophoresis as an exciting platform for translating advances in the basic sciences from bench to bedside and the potential of such strategy to improve patient compliance and increase drug efficacy in treating retinal diseases.

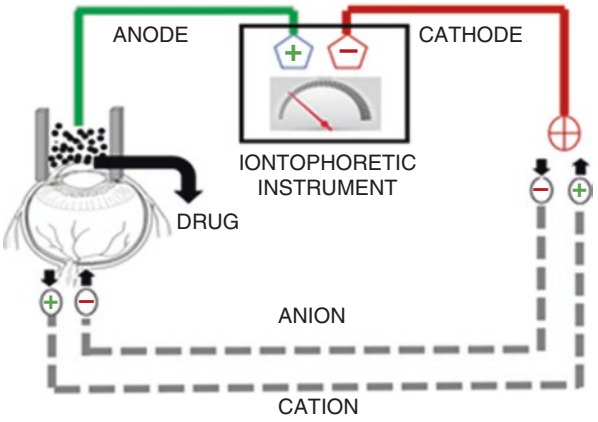

Fig. 14.2 Diagram of ocular iontophoresis. (Modified from Vivek et al. [129])

History of Ocular Iontophoresis

Iontophoresis is a localized, noninvasive rapid process in which absorption of water-soluble charged particles is facilitated across tissue barriers with the aid of physiologically acceptable levels of voltage drop/electric field through electrostatic effects [25–27]. The electrical potential, along with the concentration gradient, provides the necessary driving force for the transfer of drug molecules across the tissue layers [28]. Iontophoresis employs low voltages (<10 V) and low currents (~ few mA) over a short duration of time (a few minutes) to provide a sustained and regulated drug release.

Greek civilization marks the use of electricity in clinical settings [29]. Interestingly, the word iontophoresis was also described in Greek language [29]. A Greek physician, Etius, prescribed torpedo fish shocks for gout relief [30]. The entry of the iontophoresis technique in modern ophthalmic drug delivery was delayed compared to transdermal delivery. In 1908, Wirtz, a German analyst, demonstrated the treatment of corneal ulcers, keratitis, and episcleritis with the aid of electric current through electrolyte-saturated cotton sponges over the exposed cornea [31]. Later various European investigators [32] such as Birkhauser [33], Fietta [34], and Morisot [35] started indicating its effectiveness in corneal leukoma, recalcitrant posterior synechiae, scleritis, glaucoma, cataract, and optic atrophy. Erlanger [35] introduced this technique in the United States for the first time. This was followed by the delivery of antibiotics (e.g., tetracyclines, chloramphenicol, penicillin, streptomycin, neomycin, bacitracin) in a rabbit model, examined by Witzel and colleagues [36] in the 1940s. The efficacy of this technique was enhanced by the efforts of Ludwig von Sallmann in the 1950s [36–40]. The method suffered fading from mainstream ophthalmic delivery due to the ambiguity in toxicity associated with ocular iontophoresis and the lack of technological development in the devices/electrodes, which were considered to be inept and inconvenient. Transscleral iontophoresis gained momentum in 1980s. Maurice and Barza et al. conducted transscleral iontophoresis by placing the iontophoretic probe over the pars plana. The technique was able to deliver high drug concentrations to the vitreous humor by overcoming the lens-iris barrier [41–43]. These initial studies of transscleral iontophoresis were conducted with devices that were inconvenient, as the charged drug reservoir in the solution was directly placed over the iontophoretic site. In the last two decades, there has been a steady growth in the development of optimal protocols and devices for safe and effective use of ocular iontophoresis.

Mechanism of Transscleral Iontophoresis

For transdermal applications, pathways like inter- and intracellular, trans-epidermal, trans-follicular (sweat, sebaceous, and eccrine openings), disturbance of stratum corneum structure, flip-flop of protein and lipid structures and cation flow tendency of the skin have been described as major mechanisms that are involved in iontophoretic delivery of drugs [44]. However, it is difficult to extrapolate these mechanisms to ocular systems due to differences in physiological and anatomical conditions [45].

Transscleral iontophoresis enables noninvasive intraocular drug delivery via the sclera, a porous tissue with high surface area. The sclera is composed of collagens with an isoelectric point pI ~3–4. Under physiological conditions, the sclera carries a net negative charge, similar to the skin and nail plate. However, compared to the skin and nails, the sclera is porous in nature with an effective pore radius estimated to be around 10–40 nm. This high porosity of sclera allows the penetration of macromolecules and nanoparticles [46]. Due to the collagenous nature and high porosity of the sclera, its electrical resistance is relatively low, with no significant tissue alteration during iontophoresis. This indicates the sclera's higher tolerance of (20 mA/cm^2) electric currents as compared to the skin (0.5 mA/cm^2) [46]. Three mechanisms explaining the iontophoretic enhancement of drug permeation have been reported: electrophoresis, electromigration, and electroosmosis [47]. The enhanced movement of an ionic species in the presence of an applied electric field is called electrophoresis. Electroosmosis involves the passage of both charged and neutral species by an electric field-induced convective solvent flow [48]. Electromigration is stated as an orderly transport of ions across membranes [10]. The relative contribution of electrophoresis and electroosmosis toward the iontophoretic flux depends on the molecular size and charge of the permeant [46]. When same level of electric current (current density <0.5 mA/cm^2) is applied, the transscleral iontophoretic flux enhancement is relatively small in comparison to that of transdermal iontophoresis. In order to achieve high flux enhancement values in transscleral iontophoresis, higher electric current density should be employed. This is attributed to the low electrical resistance of the porous sclera relative to the skin [46].

Transscleral Iontophoretic Devices

The earliest transscleral devices were tubular in design, which caused small burns at the application site due to the high current density. In the 1990s, these were modified to have a larger surface area, thus lowering the potential for damage. The basic design of an iontophoretic device comprises two electrodes and a direct current power source. Typically, the ionized drug is placed in the electrode compartment with the same charge, while the ground electrode is placed at a distal site on the body. The drug is retained in the iontophoretic device in two ways.

By Filling the Eye Cup with Drug Solution

In this method, an eye cup is filled with the drug solution, and a metal electrode extended from the current supply is submerged. The eye cup has an internal diameter of 5–10 mm and is placed over the eye. During the iontophoretic treatment, the drug solution is continuously infused into the cup. There are two ports in the eye cup. One port delivers the drug solution, while the other port holds the metal electrode and removes air bubbles that can disturb the current supply. The other

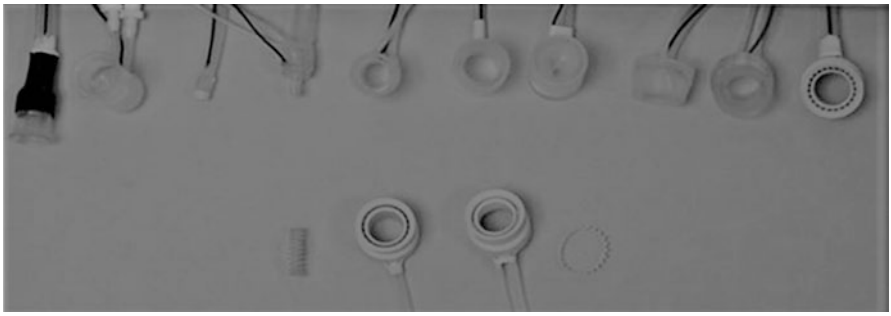

Fig. 14.3 Variety of transscleral iontophoretic device in different shapes and sizes. (Reproduced with permission from Behar-Cohen et al. [44])

electrode (ground electrode) is usually attached to the ear, closer to the former electrode, to obtain minimal resistance [21, 49, 50]. Different shapes of eye cups have been developed by the Bascom Palmer Eye Institute (J.M. Parel and F. Behar-Cohen and the team) (Fig. 14.3) [51–54].

The current applied to tissue results in the generation of heat, which in turn might affect the hydration level of the tissue. Also, the resistance might change in the damaged tissue with time, resulting in varied electrical fields. As a consequence, there could be a change in the iontophoretic character of the drug with time. To avoid this problem, the coulomb-controlled iontophoretic (CCI) system was developed by the Biophysics Center of the Bascom Palmer Eye Institute, Miami, in cooperation with OPTIS, France. The cup covers the whole circumference around the cornea, with a 14 mm inner diameter and 17 mm outer diameter (Fig. 14.4). This system can automatically adjust the fluctuations in resistance by altering the current so that continuous delivery of the drug is maintained [50, 51]. In addition, this device could indicate a circuit break and measure the total amount of current and drug flowing through the system.

The EyeGate apparatus is another iontophoretic device (OPTIS, France) made up of a flexible medical grade rubber and sized as per the conjunctival layer exposure area [51, 55]. It consists of an annular applicator with a front diameter of 2 mm and a slightly larger shaft [55]. Inside the applicator is a tungsten anode (0.5 cubic cm) surrounded by the drug solution. Between the wall of applicator and the anode are two silicone tubes, one with a vacuum for adherence enhancement and another for the suction of waste fluids, which helps maintain IOP [55]. This device does not cloud the vision and is therefore considered to be appropriate for patient compliance. Using the modified EyeGate delivery system (Fig. 14.5), it was recently shown that a single transscleral iontophoretic dose of a dexamethasone phosphate acidic solution (40 mg/mL at 10, 20, or 30 mA/min) in the rabbit eye effectively delivered dexamethasone phosphate and dexamethasone to both the anterior and posterior ocular regions (aqueous humor, choroid, retina, and vitreous humor) 15 min post-dose. The measured concentrations were directly proportional to the dexamethasone phosphate concentration utilized and the applied current [56].

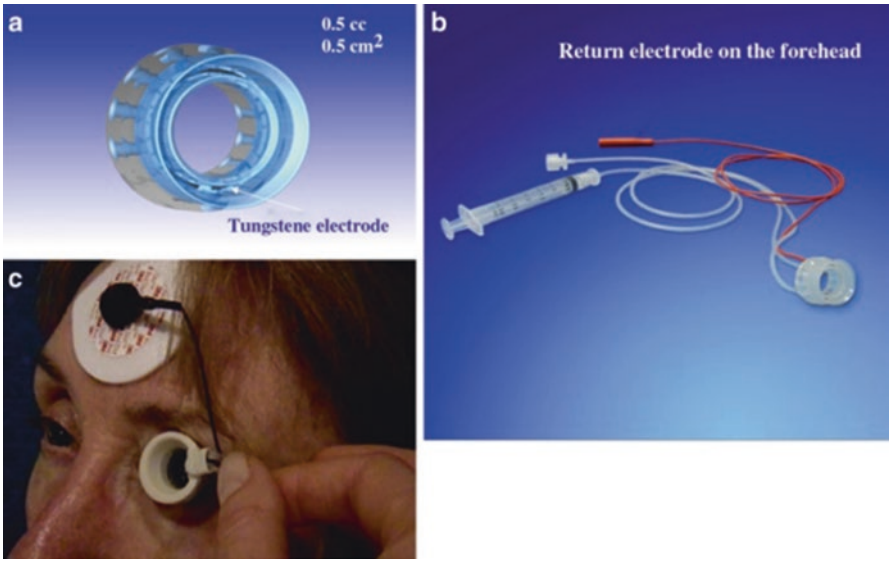

Fig. 14.4 Coulomb-controlled iontophoretic (CCI) system employed in a clinical trial. (**a**) Diagram of the probe with a drug reservoir of 0.5 cm^2 and tungsten electrode at the bottom. (**b**) The entire system attached to a syringe for introducing the drug into the reservoir and another tube to extract the fluid for maintaining a constant flux during the procedure. The forehead return electrode and probe are connected to a generator. (**c**) A patient undergoing the procedure after topical anesthesia. (Reproduced with permission from Behar-Cohen et al. [44])

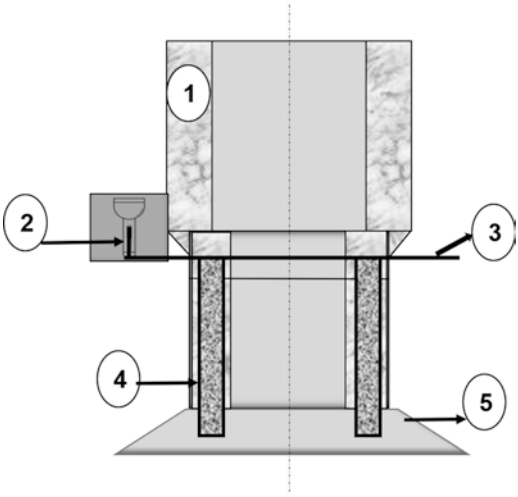

Fig. 14.5 Diagram of the EyeGate transscleral iontophoretic device with labeled parts. The annular device consist of (1) a strong back support, (2) wired connector for battery and electrode, (3) electrode, (4) reservoir (17 mm OD × 14 mm ID × 3 mm H), and (5) distal part, touching the cornea. (Redrawn from Gungor et al. [56])

By Using Drug-Saturated Gel as the Delivery Probe

Jones and Maurice adopted this method to deliver fluorescein into the anterior chamber of the eye with a fluorescein-saturated agar gel [57]. The use of agar was later discouraged due to its fragile nature. Moreover, its use in the eye is not approved by the FDA. Novel iontophoretic applicators using the drug-saturated gel approach were reported in the recent past. The hydrogels help in modulating drug release and thereby facilitate drug handling and minimize tissue hydration. Hydrogel polymers used are cross-linked with hydrophilic groups, such as –OH, –COOH, –$CONH_2$, –SO_3H, or –COOR, hydrophobic in nature, allowing the gel to hold large amounts of water (50–1000% water/dry polymer) [58]. These have been demonstrated to be safer for ocular usage as probes [59, 60]. Drug-loaded hydrogels prepared using hydroxyethyl methacrylate (HEMA) cross-linked with ethylene glycol dimethacrylate (EGDMA) (0.5–4%), at different water concentrations (53–80%), were studied by Frucht-Pery et al. The optimized hydrogel for the study was prepared from HEMA, 2% v/v EGDMA and 75% water [58, 59, 61].

Iomed Inc. and Aciont Inc. based in Salt Lake City (Utah, USA) have developed hydrogel-based applicators, OcuPhor™ and Visulex™, for transscleral iontophoresis with different drugs. OcuPhor™ hydrogel applicator consists of a patented silver-silver chloride ink conductive element contained in a small silicone shell, a hydrogel pad for absorbing the drug formulation, and a small, flexible wire that connects the conductive element to the dose controller. The dry hydrogel matrix is hydrated with the drug solution and placed against the sclera in the lower cul-de-sac at the time of administration. The electrical circuit is met by positioning the return electrode anywhere on the body [62, 63]. Visulex™ applicator (Aciont Inc., USA) was developed by Hastings and Li [64]. Visulex™ is made up of a unique selective membrane that increases drug transport through the scleral tissue by excluding the transport of non-drug ions [21].

In vitro experiments with freshly excised scleral tissues or intact eyeball have also been performed. In an in vitro experiment, the eye bulb was sealed in a polypropylene support surrounded by donor drug solution and held on a beaker with receptor fluid, with eye mimicking conditions (Fig. 14.6). A circular AgCl cathode (Iomed Inc., Vista, CA) was fixed facing the scleral layer, and the anode (Ag) was connected to donor solution. At the end of experiment, posterior tissues were extracted and analyzed for 40 kDa dextran. Drug accumulation was observed in the sclera with a sustained release effect [65].

Another device called “mini-ion iontophoretic unit” is a battery-operated system (Fig. 14.7). This device works when the applied current is in the range of 0.1–1.0 mA for 10–120 s. It uses a charged drug-loaded hydrogel for delivery into various tissues following transscleral iontophoresis [59, 61, 66]. This has been previously used for transscleral delivery of gentamicin at 1 mA for 4 min. Four hours after iontophoresis, the amount of gentamicin/g of tissue in the retina and sclera were found to be 110.8 μg ± 55.9 μg and 56.3 μg ± 23.9 μg, respectively [59]. Also, gentamicin concentrations were found to be well above the minimal inhibitory concentrations (MIC) for most infections (e.g., *Pseudomonas*) [67, 68].

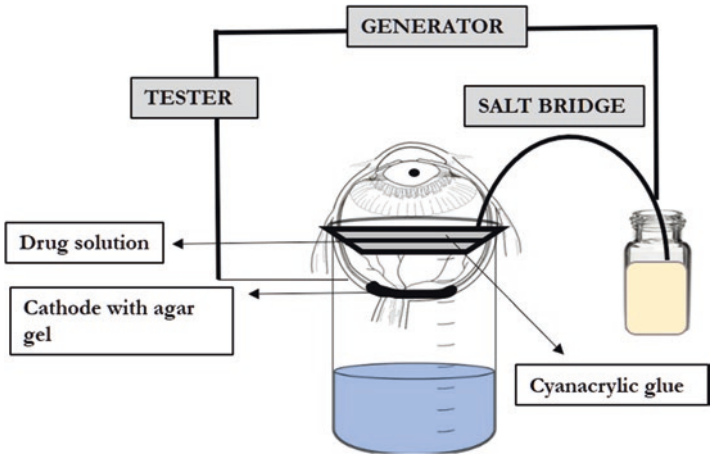

Fig. 14.6 Schematic of an in vitro experimental setup used for iontophoretic permeation experiments with intact eye bulb without a dynamic barrier. (Redrawn from Pescina et al. [65])

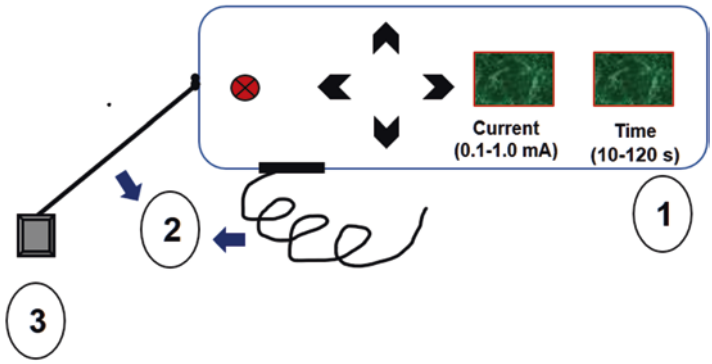

Fig. 14.7 The mini-ion device; composed of (1) a control panel for time and current control, (2) two electrodes, and (3) a cylindrical well for the insertion of disposable hydrogel. (Redrawn from Myles et al. [63])

Factors Influencing Iontophoretic Delivery of Drugs

Much of methodical studies and mechanistic aspects related to the factors influencing iontophoresis of drugs are derived from its application in transdermal drug delivery. The key factors influencing the iontophoretic drug delivery include (a) physicochemical properties of the compound such as molecular size, charge, polarity, and concentration; (b) drug formulation factors such as types of vehicle, buffer, pH, viscosity, presence of other ions and ionic strength; (c) equipment factors such as current intensity, constant vs. pulsed current, type of electrode, and the duration of application; and (d) biological variations such as membrane site, regional blood flow, age, sex, intersubject variation, and regional temperature.

Physiochemical Properties of the Drug

The molecular size of the drug has been seen to be a major deciding factor in the overall feasibility of the system. Generally iontophoretic systems allow smaller and more hydrophilic ions to penetrate rapidly [69]. The effect of transscleral iontophoresis on the in vitro permeation of high-molecular-weight neutral dextrans was studied across porcine and human sclera. This study concluded that the transscleral permeability of large molecules (MW between 4.4 and 120 kDa) through both pig and human sclera was enhanced by the application of anodal iontophoresis. This enhancement was attributed to the convective solvent flow in the direction of anode to cathode, caused by the presence of a net negative charge on the sclera. The average electroosmotic flow (4 μl cm^{-2} h^{-1}) was found to be same in both human and pig sclera [70].

Charge is also considered as a determining factor, which decides the pathway by which iontophoresis proceeds, i.e., either electrorepulsion or electroosmosis [71]. Cations exhibit better transport across tissues than other ions like amino acids, but an excess of positive charge could change the pH and accumulate on the surface, which could slow down the overall process [71, 72]. The effect of charge on the transscleral delivery of permeants such as bovine serum albumin (BSA) and polystyrene sulfonic acid (PSS) as well as a model drug bevacizumab (BEV) was studied. At a pH of 7.4, anodal delivery of these molecules was found to be higher than the cathodal delivery due to the apparent overshadowing by electroosmosis [73]. Whereas, positive molecules such as tetraethylammonium (TEA) and salicylic acid (SA) demonstrated increase in the permeation following cathodal iontophoresis to the posterior eye due to molecular shape, electrophoresis, and relaxation effects [74]. Other mechanisms such as changes in the scleral properties caused by the macromolecules and/or electric field during iontophoresis were also hypothesized to be involved [75]. In addition, hydrophilic compounds have been reported to be most favorable when it comes to attaining maximum flux across scleral tissues. One such investigation was performed with nalbuphine and its ester showing a lipophilicity-dependent decrease in flux values following retinal iontophoretic delivery [76].

Concentration is also a major factor in iontophoretic delivery. The flux values tend to increase with the drug concentration. In a transcorneoscleral iontophoretic study, a dose-dependent increase in effectivity (50% improvement in tumor mortality) after multiple CCI applications of carboplatin (7 mg/ml, 2.57 mA/cm^2, 5 min) was observed [52, 77]. In a different study, the effects of permeant [salicylate (SA) and tetraethylammonium (TEA)] concentration upon transscleral iontophoretic transport were studied using conductivity experiments in the rabbit sclera. A constant DC iontophoresis at permeant concentration ranging from 0.015 to 1.0 M in the donor chamber without background electrolyte at 0.4–4 mA (current density, 2–20 mA/cm^2) was used in the study. The fluxes of the ionic permeants increased linearly with the electric current but were relatively independent of their donor drug

concentrations. These contradictory findings suggest that further evaluation of the effect of dose and concentration of drug on transscleral iontophoresis is required [73]. Further understanding on how the physicochemical properties of the drug could influence the delivery is needed to optimize the transscleral iontophoretic drug delivery.

Drug Formulation Characteristics

pH is an important factor in iontophoretic delivery [78]. pH could have a dual effect on the overall performance of the system. First, the pH on the inside of the tissue influences the pH on the surface and makes it selective to certain moieties. This, in turn, positively affects the transfer of cationic species, owing to the ionization of acidic portions of the membrane and thus causing anodal transfer to happen. pH changes could also impact the transport of weakly basic/acidic drugs. They would be ionized to a lower magnitude pertaining to a lower dissociation constant than the pH and thus mainly transport via electroosmosis than by electrorepulsion [79]. This phenomenon has been validated by numerous studies. For optimum delivery by iontophoresis, a majority of the drug needs to remain in an ionized form and its salt form; and this could be achieved by adjusting the pH. In addition, pH should be balanced at a value that is nonirritant for the subject [80]. The effects of ionic strength and pH on the transscleral anodal iontophoretic (AI) delivery of immunoglobulin G (IgG) to conjunctiva and sclera of rabbits were studied in vitro and in vivo. A decrease in the formulation pH from 6.5 to 2.5 displayed a 40-fold decrease in the in vitro permeability coefficient (*P*) value, which was attributed to a compromise in electroosmosis due to a fall in the pH value [81].

Equipment Factors

The flux of the iontophoresis system is linearly related to voltage, based on the Nernst-Planck equation. Usually it is kept constant during one cycle [82]. Current strength has also been found to have a linear relationship with flux values. Current delivered per unit surface area or the current density has to be optimized for iontophoresis, as higher values could cause harm to the application site and lower values would have difficulty crossing a threshold to provide desired flux suitable for the delivery [27]. A major role was played by current density beyond 2.5 mA/cm^2 in transscleral delivery of neutral high molecular weight hydrophilic dextrans [65]. Also, a higher current density of 40 mA/cm^2 was seen to enhance the transscleral delivery of fluorescein to the vitreous body [83]. With increase in electric current, an enhancement in transscleral delivery of dexamethasone phosphate, methylprednisolone, amikacin, cefazolin, ticarcillin, and gentamicin was observed in the posterior

eye tissues [42, 51, 60, 62]. Magnetic resonance imaging (MRI) has been considered as a useful technique in studying the ocular penetration of delivery systems loaded with a contrast agent or a drug molecule tagged with a contrast agent. MRI studies also showed that a longer duration of current (20–60 min) enhances the delivery of the drug into the posterior regions of the eye [42, 51, 62, 83]. Pulsation of DC current compared to continuous has also shown to enhance the flux values of iontophoretic delivery [84] in the case of peptides and proteins [80]. The cutoff time between the pulsed flow allows the skin to become depolarized, aiding drug absorption.

The duration of the applied current also affects the delivery parameters in iontophoresis linearly [85]. A study involving anodal and cathodal constant current transscleral iontophoresis was conducted in vitro (excised sclera in side-by-side diffusion cells) and in vivo (rabbits) for the delivery of model permeants, including manganese ions (Mn^{+2}) and manganese ethylenediaminetetraacetic acid complex ($MnEDTA^{2-}$). The distribution of the permeants was determined with MRI, and the results were compared to those obtained following subconjunctival injection and passive delivery. The total current applied was 2 and 4 mA (current density 10 and 20 mA/cm^2) for a duration of 20–60 min. Both anodal and cathodal iontophoreses were found to be effective in ocular delivery when compared to passive and subconjunctival injections. The study concluded that the position and duration of application highly altered the effectiveness of the delivery in vivo. Also, the ciliary body (pars plana) was determined to be the least resistant pathway for iontophoretic transport [44, 83].

Biological Factors

Tissue absorption of drugs was found to be dependent on blood supply at the target site. However, in some studies, blood supply was found to be rather ineffective on the associated flux values. This was confirmed by Cross and Roberts (1995), who observed similar drug concentrations in the epidermal layers of anesthetized and euthanized rats [27]. Ocular clearance, choroidal vasculature clearance, and lymphatic clearance have been seen as the major barriers to transscleral delivery [86–89].

In a recent study, MRI technique was used to compare transscleral and transcorneal iontophoresis (30 min, 2 mA anodal constant current, and 10 mA/cm^2 current density) and further monitor the distribution of a Mn^{2+} probe ion in the anterior chamber and vitreous after iontophoretic delivery. This study concluded that the short application time did not deliver a significant amount via passive diffusion, while transscleral and transcorneal iontophoresis could deliver the ion into the vitreous and anterior chamber, respectively. Also, the probe ion penetrated a distance of approximately 0.1 cm into the vitreous over the 30-min transscleral iontophoresis transport [90]. A study was conducted to investigate the ocular barrier and barrier alterations in transscleral iontophoretic delivery (constant current of 2 mA with a current density 10 mA/cm^2) with MRI. In vitro experiments were performed in

excised sclera in side-by-side diffusion cells in vivo, and postmortem experiments were conducted in rabbits after subconjunctival injection. This study showed the electric field-induced barrier alterations as an important absorption-enhancing mechanism following ocular iontophoresis. In vivo and postmortem results suggest that electroporation or electro-permeabilization could be a flux-enhancing mechanism in transscleral iontophoresis [24].

In Vitro and In Vivo Preclinical Studies Involving Transscleral Iontophoresis

Preclinical Studies

Transscleral iontophoresis has been studied mainly with antibiotics, antiviral drugs, and corticosteroids. Rabbits are commonly used for conducting preclinical transscleral iontophoresis. Compared to other animal models, a rabbit eye is large enough to place iontophoretic devices [91]. Transscleral iontophoresis has been used widely in the delivery of antibiotics. Barza et al. studied transscleral iontophoresis of commonly used antibiotics including cefazolin, ticarcillin, and gentamicin in rabbits. Iontophoresis at 2 mA for 10 min showed a mean vitreous concentration of the drugs in the range of 94–207 μg/ml. Particularly, the levels of gentamicin were about twice as high, suggesting a greater benefit of the technique for gentamicin than for the beta-lactam drugs. Also, the drug penetration showed a correlation with current strength and duration of treatment [42]. The same group also reported a study wherein the bacterial count was lowered after two sessions of transscleral iontophoresis of gentamicin in the treatment of *Pseudomonas* endophthalmitis in rabbit retinas [92, 93].

Choi et al. examined the ability of the technique to deliver vancomycin into the vitreous humor of rabbit eyes. Rabbits were treated with iontophoresis at 3.5 mA for 10 min, whereas the control eyes were treated with subconjunctival injection. This study revealed that eyes receiving transscleral iontophoresis showed a significantly higher vitreal drug concentration when compared to the control [94]. The levels of ciprofloxacin following transscleral iontophoresis (5 mA for 15 min) were studied in the vitreous body of rabbits by Yoshizumi et al. The study tested both the positively and negatively charged forms of the drug molecule. The results indicate that the drug did not reach the vitreous body of rabbits in therapeutic concentrations with either of the forms. However, higher concentrations were attained when the drug was negatively charged [95].

Vollmer et al. evaluated the amount and distribution of amikacin in the posterior ocular tissues with respect to escalating current intensities (2, 3, and 4 mA). Significant enhancement in drug levels was observed in the sclera, choroid, and retina when compared to the control (no iontophoretic treatment). Moreover, the drug levels were 7- to 92-fold higher in the retina and choroid than those for in vitro MIC

for *S. aureus*, *S. epidermis*, and *P. aeruginosa*. Also, the drug transport was found to be directly proportional to the applied charge [62]. Another in vivo study evaluated the delivery of gentamicin solid hydrogel using the mini-ion device. The solid gels were prepared using hydroxyethyl methacrylate (HEMA) cross-linked with ethylene glycol dimethacrylate (EGDMA). The test rabbits were treated with solid hydrogel iontophoresis using an applied current ranging from 0 to 1.5 mA over a period of 30–120 s, while the control rabbits were treated using fortified gentamicin eye drops. This study reported high concentrations of gentamicin sulfate in the sclera and retina 4 h after iontophoresis with a current density of 1.5 mA for 60 s [96].

Corticosteroids such as dexamethasone and prednisolone derivatives have been administered by transscleral iontophoresis as an alternative to intravitreal injections. Drugs such as dexamethasone and methylprednisolone have shown improved penetration following transscleral iontophoresis and were found to be effective in controlling intraocular inflammation [50, 51, 97, 98]. An ocular sustained release system containing triamcinolone acetonide phosphate and a precipitating cationic counterion was tested in New Zealand white rabbits. Iontophoresis experiments were conducted using the Visulex™ iontophoretic system (Aciont, Salt Lake City, UT). The sustained release formulation resulted in a higher half-life of the drug compared to the control (nonsustained release system) and demonstrated the feasibility of using the Visulex™ system for noninvasive iontophoretic delivery [99]. The pharmacokinetics of methylprednisolone hemisuccinate (HMP) following its delivery using transscleral CCI or after i.v. injection was studied by Behar-Cohen et al. Two different concentrations of the drug, as well as various current intensities (0.4, 1, 2 mA/0.5cm^2) and durations of treatment (4, 10 min), were tested in rabbits. For every parameter tested, six rabbit eyes were used. The control rabbits were treated with HMP infused in the CCI device without any application of electric current. Sustained and high levels of HMP were achieved in the choroid and retina of rabbit eyes when treated with 2 mA/0.5cm^2 for 10 min, whereas negligible amounts of the drug were found in ocular tissues of the control. Moreover, the levels were higher than those attained after i.v. administration [51].

Another study by Eljarrat-Binstock et al. evaluated the transscleral iontophoresis technique across the rabbit eye using drug-loaded hydrogels for delivery of methylprednisolone hemisuccinate. The hydrogels tested were mounted in a portable iontophoretic device, and the evaluations were compared to that of 10mg/kg i.v. infusion. Cathodal iontophoresis (2.6 mA/cm^2 for 10 min) demonstrated a significantly higher concentration in the retina, aqueous humor, and vitreous chamber when compared to the passive control groups. Moreover, concentrations of approximately 178.59 ± 21.63 μg/g, 6.74 ± 2.38 μg/ml, and 2.71 ± 0.57μg/ml were reported to be found in the retina, aqueous humor, and vitreous chamber, respectively, while non-detectable concentrations were observed after 2 h of i.v. infusion [100]. A comparative pharmacokinetic study was conducted by Voigt and colleagues using acetylsalicylic acid for a duration of 10 min at 2.5 mA. Forty-one rabbits received either a single CCI, topical application, or i.v. injection. Following treatment, the distribution of the drug was evaluated in all the ocular tissues and fluids at 0.5, 1, 2, 4, 6, 8 h. The results of this study indicated higher drug concentration with CCI

when compared to the other study methods. With CCI, the highest drug levels in all the tissues were immediately observed in about 30 min, whereas with intravenous administration, there was a delay in the drug peak for about 2 h. However, drug concentrations were in the same range after 8 h with both CCI and intravenous administration [53]. The potential of repetitive transscleral CCI for the ocular delivery of acetylsalicylic acid was studied in rabbits. Fourteen rabbits were given serial CCI treatment with acetylsalicylic acid, while another 14 rabbits underwent CCI with balanced salt solution (BSS) for 6 days at either 24 h or 48 h time intervals. In another set of animals, a single CCI application was given to 18 rabbits, while another 18 rabbits were intravenously injected with 15 mg acetylsalicylic acid/kg body weight. The results indicate a significantly higher concentration of salicylic acid in ocular tissues following iontophoresis when compared to the controls. In addition, levels were sustained without any side effects [101].

The local and general toxicity following repeated dosing of dexamethasone phosphate by transscleral iontophoresis was studied in rabbits. Test rabbits were administered with 40 mg/ml of ophthalmic dexamethasone solution in their right eyes using an EyeGate® II iontophoretic device biweekly for 24 consecutive weeks (with varying current doses of 10, 14, 20 mA/min, and −4 mA/3.5–5 min). Two sets of controls were used in the study: (a) rabbits treated with citrate buffer using the device without any application of current, and (b) rabbits treated with iontophoretic citrate buffer at 20 mA/min and −4 mA/5 min. The left eye in all the rabbits was untreated and served as an internal control. The results of the study indicated that both the iontophoretic drug solution and citrate buffer were well tolerated for the entire test period. Also, no changes in intraocular pressure, electroretinography, or histopathology were reported. However, transient signs of conjunctival hyperemia, mild corneal opacity, and fluorescein staining of the cornea were observed and were attributed to the temporary placement of iontophoretic device [102]. Dexamethasone transport from iontophoretic sustained-release micellar systems was studied across excised human sclera. Simple and mixed micelles of the drug were prepared from either sodium taurocholate (TA) or with egg lecithin (LE) and were characterized for solubilization and encapsulation. Side-by-side Franz diffusion cells were used for the transport experiments (passive and 2 mA iontophoretic), and saturated dexamethasone solution was used as a control. It was found that solubilization of micelles increased with the total lipid concentration of the system. Also, the drug release was significantly prolonged following iontophoretic delivery when compared to the control. Less than 20% of the drug was released from the sclera in 2 h, whereas more than 50% was released from the control [103].

Transscleral iontophoresis has also been used in delivering chemotherapeutic agents. Platinum compounds such as cisplatin and carboplatin are used in treating retinoblastoma. However, systemic carboplatin therapy is known to increase the risk of second cancers in childhood survivors [104]. Hayden et al. compared carboplatin delivery to the posterior segment of rabbit eyes after a single intravenous infusion of carboplatin (18.7 mg/kg of body weight), a single subconjunctival carboplatin injection (5.0 mg/400 μL), or a single application of carboplatin delivered by CCI (14 mg/mL carboplatin, 5.0 mA/cm^2, 20 min). This study concluded

that focal administration of carboplatin using subconjunctival or CCI resulted in higher drug concentrations in the target tissues of the retina, choroid, vitreous, and optic nerve compared to intravenous infusion [52]. Another in vivo study evaluated the delivery of a methotrexate hydrogel using a portable iontophoretic device. The test rabbits were treated with iontophoretic hydrogel at 5 mA/cm^2 for 5 min, while the control rabbits were treated using intravitreal injection and mock iontophoresis. Therapeutic concentrations of the drug were obtained in both mock and iontophoretic groups. The drug concentrations observed in the retina and vitreous chamber following iontophoresis were two to four times higher than in those treated with mock iontophoresis. Also, the drug concentration in the sclera was significantly higher than the levels after mock iontophoresis. On the other hand, the drug levels were higher after intravitreal injection in the vitreous and retina when compared to the iontophoretic treatment. However, no significant difference was reported in the drug levels in the sclera for iontophoretic versus intravitreal-injected groups [105]. Transscleral iontophoresis allows local administration of anticancer drugs and appears to be a promising alternative compared to systemic and intravitreal injections. However, side effects associated with the long-term therapy remains to be evaluated.

Transscleral iontophoresis was also tested in the treatment of cytomegalovirus (CMV) retinitis in a rabbit eye model. Ganciclovir at a single dose of 20% (w/w) in aqueous solution was delivered with the help of transscleral iontophoresis (1.0 mA for 15 min). A high retinal concentration of the drug was detected, even after 24 h of study, compared to the traditional control [106]. Sarraf et al. conducted a study to investigate the effect of transscleral iontophoresis for the delivery of foscarnet into the vitreous chamber. Half a milliliter of 24 mg/ml drug solution was iontophoretically administered to 72 rabbits with 1 mA current for 10 min. Vitreous aspirations revealed that a peak concentration of 200 ± 31 μM was attained in 4 h, and therapeutic levels were maintained until 60 h, following iontophoresis. The peak concentration was well below the concentration known to cause retinal toxicity. In addition, biomicroscopy revealed no toxic effects for the anterior segment of the eye [107]. Another study for improving the treatment of cytomegalovirus was conducted by Yoshizumi and colleagues. Transscleral iontophoresis was performed at the para-limbal site using foscarnet at 3-day intervals for a period of 21 days. The mean vitreous humor concentration (189 ± 50.6 μM) obtained was within the therapeutic range for the treatment of CMV. Moreover, histological studies showed no signs of toxicity. Therefore, the study concluded that iontophoresis could be a good alternative for localized management of CMV in humans [108].

A few researchers have attempted to deliver macromolecules using transscleral iontophoresis. Transscleral iontophoresis was also used to deliver 6-carboxyfluorescein-labeled phosphorothioate oligonucleotides (S-ODNs) along with a 4.7 kb plasmid. The delivery in an albino rabbit model resulted in the expression of green fluorescent protein (GFP). Following iontophoretic treatment, S-ODNs

were observed in the vitreous chamber and the posterior retina after 10 and 20 min, respectively. In addition, there was no alteration in the length and fluorescence emitted by the transferred S-ODNs [109]. Voigt and co-workers used transcorneoscleral CCI to improve the intraocular penetration of anti-nitric oxide synthase II oligonucleotides (anti-NOSII ODNs). The evaluation was performed in a rat model of endotoxin-induced uveitis. The anti-NOSII ODNs were not only detected after 1 h in all the corneal layers, peripheral retinal layers, and sclera but were also observed in the retina/choroid even after 6 h. This resulted in the downregulation of NOSII mRNA when compared to the control groups, and these observations suggest that transscleral iontophoresis can be used for the ocular delivery of oligonucleotides and genes without affecting their physical integrity and biological function [110]. Bevacizumab, a recombinant humanized IgG1 monoclonal antibody, is used for the treatment of neovascularization in conditions such as diabetic retinopathy and wet macular degeneration. In vitro anodal iontophoresis using fluorescein isothiocyanate (FITC)-bevacizumab across human sclera for a duration of 2 h and a current density of 3.8 mA/cm^2 was investigated by Pescina et al. The results from the permeation study revealed that anodal iontophoresis significantly increased the transport of bevacizumab by a factor of 7.5, even without the drug being essentially charged [111].

In vitro permeability of cytochrome c, a positively charged molecular protein (MW 12.4 kDa), across ocular tissues following transscleral iontophoresis was investigated by Tratta et al. Anodal iontophoresis was studied as a function of current intensity (0.9–3.5 mA), current density (1.5–5.8 mA/cm^2), and donor concentration (5–70 mg/ml). In addition, the possibility of tuning the rate of cytochrome c permeation was studied using 70 mg/ml of protein solution, by alternating passive permeation and iontophoresis at various intensities. Iontophoresis at 2.9 mA/cm^2 enhanced the permeation across the sclera at all the concentrations tested. Moreover, the permeated amount was about ten times higher after 2 h, and this effect was proportional to the current density. In addition, precise modulation of the release was observed (i.e., desired transscleral flux), suggesting the possibility of tuning the release as per specific therapeutic needs [112]. These studies could form the basis of further research in the delivery of macromolecules for treating posterior segment diseases by iontophoresis.

Clinical Studies

Very few clinical trials of transscleral iontophoresis have been reported in the literature. A phase II clinical trial was conducted using CCI for the delivery of methylprednisolone sodium succinate (Solu-Medrol Solution, Reconstituted). This study reported that iontophoresis was well tolerated and could be safely applied for treating severe ocular inflammatory conditions, thereby reducing the systemic side effects of corticotherapy [113]. The same system and drug were used to assess the efficacy in three subjects with acute corneal graft rejection. The subjects were given

Solu-Medrol through iontophoresis at 1.5 mA for 3 min, once a day, as a supplement to the topical dexamethasone drops. This treatment was found to be tolerable with no side effects. Moreover, visual acuity was also improved after the second treatment [114]. A similar study was conducted by Behar-Cohen et al. in which 18 subjects with acute corneal graft rejection were treated with Solu-Medrol iontophoresis at 1.5 mA for 4 min, once a day, for 3 consecutive days. Even this study presented similar results, with absolutely no side effects and 88% subjects showing complete reversal of rejection processes [55, 115].

Parkinson et al. reported the ocular tolerance of saline iontophoresis using the transscleral OcuPhor™ hydrogel drug delivery applicator. Different current intensities ranging from 0 to 4 mA were used for 20 or 40 min. The results of the study revealed that applicator and iontophoresis were well tolerated. However, when 4 mA of current was applied, half of the subjects experienced burning sensation, which resolved after 22 h [116]. Another study evaluating iodide iontophoresis for the treatment of dry eye was conducted by Horwath-Winter et al. The study investigated the efficacy of coulomb-controlled annular applicator in the treatment of moderate to severe dry-eye patients. After 10 days of treatment, improvement in vital staining and certain tear function parameters was reported [117].

A single-center, randomized, phase I study has been conducted by EyeGate Pharmaceuticals, Inc. using escalating iontophoretic doses of buffer solution. A 100 mM citrate buffer solution was administered through EyeGate® II delivery system (EGDS), and its safety and tolerability in 105 healthy human volunteers were evaluated (clinical trial identifier, NCT00698425). This study aimed to define the current dose as well as maximum tolerable iontophoresis current levels and/or iontophoresis treatment times. No dose-limiting effects were observed at the maximal levels studied (7 mA of iontophoresis current levels, 10-min treatment times, and 20 mA-min current doses). This study could not identify the maximum tolerated treatment parameters for the EGDS, as all subjects successfully completed the treatment [118]. Recently, a multicenter, randomized double-masked, phase II trial was completed by EyeGate Pharmaceuticals using the iontophoretic dexamethasone phosphate ophthalmic solution, EGP-437 (clinical trial identifier, NCT01602068). The study was conducted in patients undergoing cataract surgery with an implanted posterior intraocular lens, and the dexamethasone solution was delivered using EyeGate® II Delivery System (EGDS). The safety and efficacy of the solution were evaluated and compared to that of an iontophoretic placebo (sodium citrate buffer solution). EGP-437 treatment (4.0 mA-min at 1.5 mA) was applied 1 day prior to surgery to avoid placing the delivery device directly on a postsurgery open wound. This study has been completed; however, the results were not posted in the public domain. Despite a few decades of research in transscleral iontophoresis, very few clinical trials have been conducted in this area. The possible reasons for this could be the differences in results observed between in vivo and ex vivo pharmacokinetic studies. In addition, the need for repeated and long-duration iontophoresis in many cases increases the risk of toxicity, which eventually impedes medical progress [119] .

Delivery of Nanocarrier Systems Using Transscleral Iontophoresis

Over the last decade, there has been a surge in the use of nanocarriers in combination with iontophoresis for fast and sustained delivery of high drug concentrations in specific ocular tissues [103]. Studies have been carried out to evaluate the potential of iontophoresis in driving charged nanocarrier systems across the sclera for maintaining therapeutic drug concentrations in the eye over a prolonged time. Moreover, short-duration transscleral iontophoresis might fail to deliver drugs directly to the back of the eye due to the long path length from the site of application and other dynamic barriers in the eye [103]. Consequently, the use of sustained-release nanocarrier systems for transscleral iontophoresis might be a desirable approach. The use of charged nanocarriers, in combination with iontophoresis, allows delivery of drug molecules, regardless of a drug's ionic strength/diffusion properties, in ocular tissues and sustains the drug release at the target tissue for an extended time period. It could also make the delivery more site- and target-specific by altering the particle size and attaching specific antibodies to the nanoparticles [120].

The penetration of charged fluorescent nanoparticles dispersed in hydrogel into rabbit eyes was studied in the presence of iontophoresis [60]. The penetration efficiency of negatively charged nanoparticles, in comparison with positively charged nanoparticles, was also evaluated. This study concluded that negatively charged nanoparticles exhibited faster uptake (less than 30 min) into peripheral ocular tissues, followed by particle migration into the inner tissues up to 12 h posttreatment. However, positively charged nanoparticles demonstrated better penetration into inner ocular tissues compared to the negatively charged particles (Figs. 14.8 and 14.9). A mixed micellar system of egg lecithin and taurocholate in a 1:1 ratio was used to encapsulate dexamethasone, triamcinolone acetonide, and β-estradiol to enhance drug solubility and penetration through human sclera. The results showed that mixed micelles enhanced the solubility of drugs and effectively transported the drug across human retinal layers during cathodal iontophoresis. In addition, the application resulted in higher drug loading and showed sustained drug release into the sclera, which could be attributed to the ionic accumulation. The drug was found to release in a sustained manner from mixed micelles in the sclera after iontophoretic delivery, due to ionic accumulation. The application of iontophoresis to micellar systems resulted in higher drug loading into the sclera and prolonged release of the drug from the sclera [103].

Wu et al. investigated the penetration efficiency of chitosan nanoparticles loaded with bovine serum albumin (BSA) to the posterior eye segment in vitro and in vivo using transscleral iontophoresis. The study reported higher penetration efficacy of BSA inside chitosan nanoparticles with anodal and cathodal iontophoresis. The penetration under cathodal iontophoretic was enhanced over twofold, and the distribution profile of nanoparticles revealed a quick adhesion/uptake by the outer ocular tissues. This was followed by a slow migration into the inner tissues up to 12 h post-

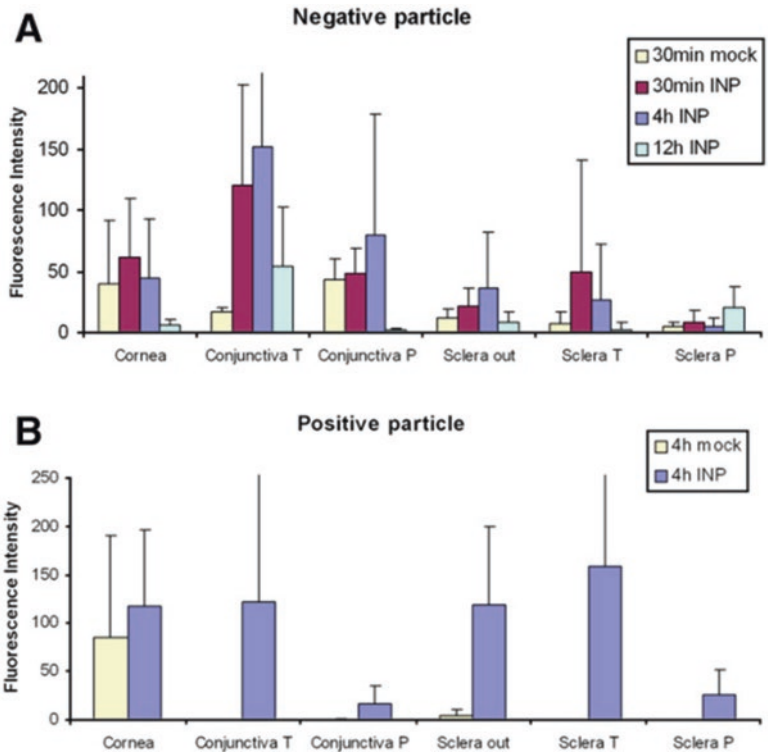

Fig. 14.8 Mean fluorescence intensity in different outer ocular tissues. (**A**) 30 min, 4 h, and 12 h after negatively charged nanoparticle iontophoresis and (**B**) 4 h after positively charged nanoparticle iontophoresis, compared to mock application. (Reproduced with permission from Eljarrat-Binstock et al. [120])

treatment [121]. So far, very limited research is available on the use of nanocarriers for transscleral iontophoresis. Further studies are required to evaluate the efficacy and safety of this combination approach.

Toxicity of Transscleral Iontophoresis

Iontophoresis is a noninvasive method that is usually accompanied by minimal discomfort. However, recent studies have mentioned certain complications associated with the procedure, including epithelial edema, decrease in endothelial cells, inflammatory infiltration, and burns. The magnitude of ocular toxicities observed was found to depend on the application site, current intensity, and duration of application [21]. Minor toxicities at the application site are observed due to the low-voltage nature of the induced current [122]. As compared to transcorneal complications, which could instantly affect the vision and induce discomfort, transscleral route toxicities could be more subtle yet equally detrimental [21].

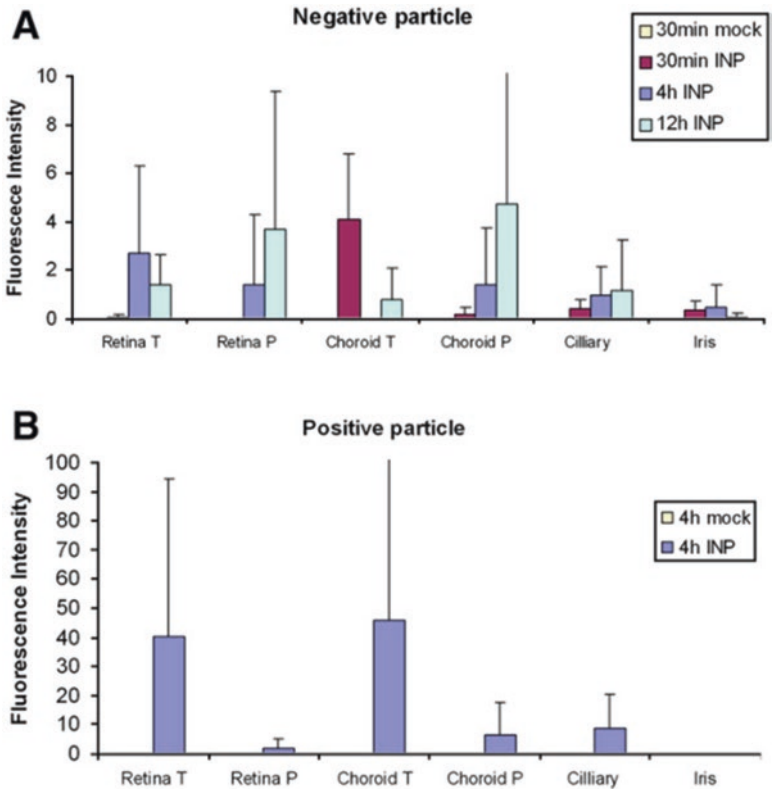

Fig. 14.9 Mean fluorescence intensity in different inner ocular tissues, (**A**) 30 min, 4 h, and 12 h after negatively charged nanoparticle iontophoresis and (**B**) 4 h after positively charged nanoparticle iontophoresis, compared to mock application. (Reproduced with permission from Eljarrat-Binstock et al. [120])

Many of the electrode arrangements used for initial studies were tubular, with a reduced area of contact with the sclera. This resulted in a very high current density, leading to increased drug concentration in the vitreous chamber. However, small burns were observed over areas where the current was applied, and the increase in drug concentration was attributed to facilitated diffusion of the molecules through the ruptured tissue barriers [44]. With transscleral iontophoresis, some amount of retinal damage is inevitable. A variety of toxicities have been observed post-application, namely, edema beginning after seconds of application, deformation of the retina, necrosis, fibrosis, and RPE hyperplasia, all of which worsen after repeated applications [98, 123]. The rupture of the retinal layers and the inflammation of choroid have been reported following post-iontophoretic delivery of fluorescein [124]. Maurice et al. reported damage to retinal layers and disruption of the choroid following transscleral iontophoresis of fluorescein (127–254 mA/cm^2 current density for 2–5 min) [41]. In a case study by Barza's group [42], even after 3h of induction, the animals displayed no unease at the tested current densities (254.6, 127.0,

and 101.9 mA/cm^2 for 5 or 10 min and voltage of 150 V) for gentamicin and cefazolin iontophoresis. Incidentally, necrosis, inflammation, and infiltration of polymorphonuclear cells into the retina, choroid, and ciliary body were observed with histopathological findings at 254.6 and 127 mA/cm^2 current intensities.

In another publication by the same group [125], it was reported that transscleral iontophoresis using extensively high current density (765.3 mA/cm^2) for 10 min in monkeys for gentamicin delivery resulted in retinal burns. Yoshizumi et al. studied the ocular toxicity in rabbits treated with iontophoretic foscarnet. Indirect ophthalmoscopy revealed retinal and choroidal burns of about 1–3mm in diameter. Moreover, microscopic studies showed focal retinal, retinal pigment epithelial, and choroidal necrosis at the site of iontophoresis [123]. An experiment to study retinal toxicity by iontophoresis was conducted by Lam et al. They studied retinal lesions in rabbits until 14 days after transscleral gentamicin iontophoretic application (1.5 mA) for a duration of 2–25 min. The authors noted an increase in size and severity of lesions with increase in duration of application. No retinal lesions were observed up to 1 min; however, well-circumscribed chorioretinal lesions appeared after 2-min applications. After 5 min of iontophoresis, the edematous retina exhibited necrotic RPE, loss of outer segments, and thinning of the inner and outer nuclear layers. In the following 5 days, macrophages and simultaneously proliferating RPE cells were observed. After 14 days, the retina was converted to a glial membrane. An overall loss of the inner and outer segments of the retina and a diminishing of outer nuclear layers were seen [126] (Fig. 14.10).

Contradictory findings were also reported in the literature on the occurrence of side effects with transscleral iontophoresis. A study was performed to compare transscleral and transcorneal iontophoretic delivery of gentamicin with subconjunctival injection. The authors reported no significant differences in the toxicities before and after the study [127]. In a different study on three monkeys, only four of six retinal burns were observed at the site of contact of the electrode while attempting to deliver gentamicin sulfate via transscleral iontophoresis. This work was determined to be safe to repeat with humans [125]. Behar-Cohen reported the absence of histological damage to the cornea and sclera after application (1.2 mA/cm^2 current for 4 min, 6 mm diameter of eyecup) in a rat. Also, with 4 mA/cm^2 for 10 min, no lesions or histological anomalies were reported after a day of using the annular transscleral device [51]. Voigt [53] and Kralinger [101] investigated single and multiple iontophoresis of aspirin using a current density of 5.0 mA/cm^2 for 10 min by slit-lamp biomicroscopy and ophthalmoscopy. Normal histopathology and the absence of retinal detachment or other abnormalities were reported in both the anterior and posterior segments of the eye. Moreover, after multiple applications for a week at 24 h intervals, no change in retinal membranes, rods, and cones or nuclear layers was observed.

Coulomb-controlled iontophoresis has shown fewer ocular complications and irregularities as it is carried out at a constant current density (in milliamps per square centimeter) and a suction of 5 mmHg for the duration. This has proved to yield effective transport of drugs following iontophoretic delivery. CCI appears to be a safer option with respect to patient discomfort and ocular damage among other

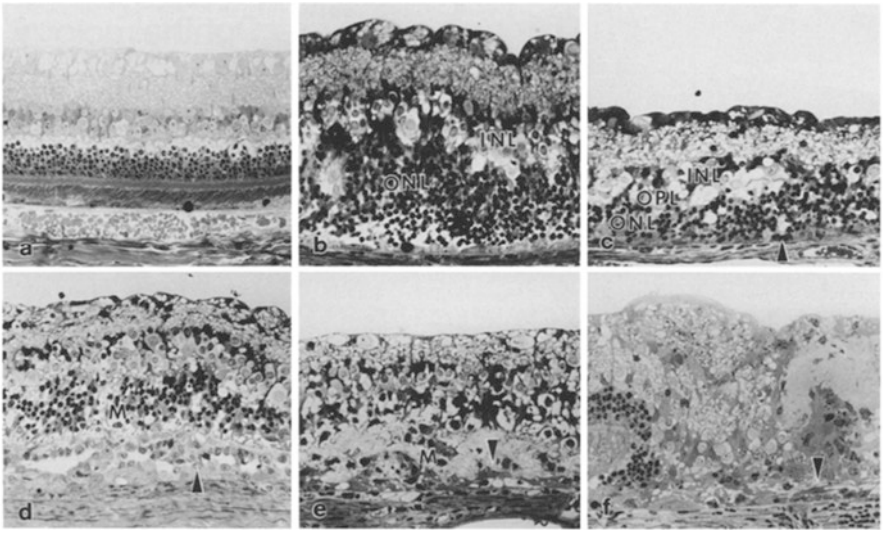

Fig. 14.10 Increasing morphologic changes in the retina with iontophoresis application time. A current of 1.5 mA was applied for 1, 2, 3, 4, 5, and 15 min, and retinal lesions were examined 5 days after the procedure, (**a**) 1 min: the retina appears intact, x210. (**b**) 2 min: loss of outer and inner segments, disorganized outer nuclear layer (ONL), and irregular thinning of inner layers are visible, x220. (**c**) 3 min: focally absent RPE (arrowhead) and irregular thinning of the outer nuclear (ONL), inner nuclear (INL), and outer plexiform layers (OPL) can be seen, x210. At (**d**) 4 min and (**e**) 5 min, reactive proliferation of RPE (arrowhead) and macrophages (M) is visible, x210. (**f**) 15 min: the necrotic retina is infiltrated with macrophages. Actively proliferating RPE cells are visible on Bruch's membrane (arrowhead). x210. (Reproduced with permission from Lam et al. [126])

kinds of iontophoretic technique variants. Nonetheless, the maximum level of safe current density has to be determined, and the irritation potential of individual molecules under the influence of electric current needs to be evaluated. In addition, it would be desirable to have built-in safety mechanisms that could stop the flow of the current above a threshold voltage [128].

Conclusion

Transscleral iontophoresis appears to be a promising strategy for treating disorders in the back of the eye. Its full potential for use in ophthalmology is yet to be realized. Research in this area is necessary for better understanding the interactions between the ocular tissues and the application of electric current. Further assessment of devices and probes is required to determine the optimal conditions for the safe and therapeutic use of iontophoresis. A rapid clearance of the drug into systemic circulation through episcleral vessels and conjunctival lymphatic system has been observed with transscleral iontophoresis. This necessitates repeated

iontophoresis administration, especially in chronic diseases. This problem could be overcome by loading drugs into charged nanocarriers such as microemulsions, nanosuspensions, liposomes, dendrimers, niosomes, cubosomes, nanoparticles, polymeric micelles, and solid lipid nanoparticles. A combination therapy involving charged nanocarriers and iontophoresis is quite possible in the near future. Further, such technologies should be fully evaluated for long-term safety in humans. In addition, ocular iontophoretic studies, in conjunction with magnetic resonance imaging (MRI), need to be explored, as it provides real-time data about charged drug movement without the need of dissection or fluid sampling.

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Part IV
Implant Formulation for Posterior Eye Segment

Chapter 15
Biodegradable Polymeric Implants for Retina and Posterior Segment Disease

Aditi Pandhare, Priyanka Bhatt, Hardeep Singh Saluja, and Yashwant V. Pathak

Abstract Drug delivery to the retina and posterior segment of the eye is challenging as the traditional procedures involve frequent clinical visits and administration of medications, drugs, and other injections that could potentially increase chances of infection and intraocular hazards.

Biodegradable implants are explored to overcome these limitations, and these implants are formulated from biocompatible polymers which can achieve a sustained release of therapeutic agent in ocular target site such as the retina or posterior segment of the eye with minimal side effects. These polymers are not toxic and can be broken down via enzymatic activity as well as hydrolysis within our body in months or years, and therefore, they have sufficient biocompatibility, especially when incorporated in systems for posterior eye disorders. This chapter discusses about examples of such implants, their formulation, material used, advantages, disadvantages, and possible toxicity profile as well as use of biodegradable polymeric implants in drug delivery for the retina and posterior segment of the eye.

Introduction

Use of Nanotechnology

Nanotechnology can alter biological structures and affect physiological processes at the molecular level. This involves the utilization of nanoparticles (NPs), which is one of the areas of concentration in our study of treating ocular diseases [1]. The more pharmaceutical NPs may enclose active pharmacological ingredient (API) and are comprised of numerous molecules as small as 5 nm to as large as 300 nm, relatively speaking, and come in various configurations such as crystalline and needles [1].

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Further research and innovation in nanotechnology have brought more utilization of biodegradable microspheres that hold ocular pharmacological agents and thus provide better intravitreal delivery of many medications [2].

Posterior Segment Disease Drug Delivery Overview

Many disorders in the posterior segment of the eye are strongly associated with visual impairment [3]. Age-related macular degeneration (AMD), diabetic retinopathy, diabetic macular edema (DME), retinal vein occlusion (RVO), uveitis, and endophthalmitis are some posterior segment eye diseases that severely affect vision and may cause further visual impairment and/or blindness [2]. In order to treat such conditions, topical, systemic, transsceral, and intravitreal methods involving certain drugs have been implemented in pharmaceuticals, laboratory, and clinical research [2]. Topical drug delivery has mainly been utilized for treating anterior segment eye diseases [3]. Retinal diseases have been treated by corticosteroids because they obtain antiangiogenic and anti-inflammatory traits [4]. Also, intravitreal steroids have shown to enable higher drug efficacy and lower risk of systemic consequences [4]. Furthermore, intraocular implants have helped extend treatment with controlled drug release, direct drug rate transfer to the intended site of activity, increasing a drug's half-life, and evading side effects related to recurring intravitreal injections [4]. Methods such as polymeric-controlled release injections and implants, nanoparticulates, microencapsulated cells, iontophoresis, and gene medicines have been developed to provide a more sustained release as well as better retention and a reduction in intravitreal injections [3].

Ocular Disease Treatment Overview with Drug Delivery Systems

The posterior segment of the eye can be treated via various methods and therapeutics when blighted with certain diseases. Treating ophthalmic pathologies can involve frequent clinical visits and administration of medications, drugs, and other injections that could potentially increase chances of infection and intraocular hazards [5]. Application of drugs by traditional forms of dosage is specifically pertinent to the eye, not to mention that therapeutic drug concentrations that are in certain tissues are not retained in the eye for long [6].

Generally, topical applied drugs may not completely touch the posterior segment of the eye; thus, periocular or intraocular systemic drug injections may be utilized in clinical therapeutics [6]. The eye's distinct shape and functions incorporate defensive barriers to hinder drugs from reaching the target tissues [6]. Some of the drugs that have been considered or utilized in posterior drug delivery systems in clinical trials include dexamethasone, fluocinolone acetonide (FA), ganciclovir, verteporfin, triamcinolone acetonide (TA), etc. [6].

Treatment of ocular diseases such as age-related macular degeneration (AMD) and diabetic retinopathy can be implemented via the use of novel drug delivery systems that involve biodegradable implants. Such methods also include topical, systemic, and intravitreal pathways [7]. Biodegradable polymer systems are being studied for the treatment of chronic vitreoretinal diseases [6]. An example of a biodegradable intraocular therapy for retinal diseases is Ozurdex [6].

Topical Approaches to Treating Posterior Segment Diseases

The utilization of topical permeation-boosting liposomes and emulsions has been some of the advances in treatments for ocular diseases [2]. Although topical drug administration mainly targets diseases that occur in the anterior segment of the eye, there are many ways that alter the processes and routes of topical agents in order to yield more penetration to the posterior segment [2]. For instance, cyclodextrins, prodrug formulations, permeability enhancers, transcorneal diffusion, and transconjunctival or transscleral penetration can be utilized in particular methods to accomplish this [2]. One research study found that the vitreous and the retina of rabbit eyes showed high levels of topically applied dexamethasone-cyclodextrin complexes, implying that such methods could be beneficial in treating vitreoretinal diseases that need chronic drug administration [2].

Direct administration of topical agents might not produce an adequate drug concentration in the posterior segment [2]. Therefore, in order to increase drug absorption, certain drugs and liposome permeability enhancers may need to be combined and work together [2]. Liposomes are used in topical administration because they are spherical lipid bilayer structures that envelope and contain pharmaceutical agents, can enable gradual drug release, and can bind to the cornea [2]. It has been exhibited in rabbit corneas that positively charged liposomes could bring in four times more penicillin G than would controls [2].

Overall, administering drugs via the periocular route and using permeability enhancers may decrease the limitations of topical routes to the posterior segment of the eye [2].

Biodegradable Implants

Implantation and extraction of nonbiodegradable materials have exhibited some detrimental outcomes such as vitreous hemorrhage and endophthalmitis [4]. The utilization of nonbiodegradable implants may involve additional removal and application of a new device in the posterior portion of the eye [4]. Thus, more studies have shifted the focus toward biodegradable implants, which have been proven to be soluble and don't require more reimplantation [4]. Biodegradable implants ensure a more lasting sustained drug release [8].

Drugs placed into the biodegradable implants are formulated into polymers such as poly(glycolic acid) (PGA) and poly(lactic-co-glycolic acid) (PLGA) [4]. These polymers are not toxic and can be broken down via enzymatic activity as well as hydrolysis within months or years [4]. Therefore, they have sufficient biocompatibility, especially when incorporated in systems for posterior eye disorders.

Triamcinolone Acetonide (TA) Biodegradable Implant

TA is commercially presented and accessible as an ester and is one of the most commonly utilized steroids for treating retinal disorders [4]. TA has anti-inflammatory properties that are five times higher than those of hydrocortisone [4]. It has been noted that TA could be therapeutic for about 3 months after a 4 mg intravitreal injection and that it potentially remains in effect for the greatest time period of 140 days [4].

There are three TA implants that are used for intravitreal injections: Kenalog®, Trivaris®, and Triesence® [9]. For the treatment of diabetic macular edema (DME), TA can be delivered via a subtenon or intravitreal application [9]. Let's take for example, a study of a (TA) biodegradable intrascleral implant. It was created with a heavy poly(lactic acid) (PLA) polymer as a one-sided coating and 6.4 mg of TA [7]. This enabled the drug to get absorbed in a unidirectional form into the sclera [7]. Next, researchers conducted an in vivo study using 20 rabbit eyes in order to evaluate the efficacy of the TA biodegradable implant in touching the vitreous [7]. Consequently, there was a significant amount of TA detected in the aqueous humor as well as in the retina-choroid, until 4 and 8 weeks post-implantation, respectively [7]. The implant was present throughout the vitreous during the full 12-week span [7]. This indicates a plausible sustained release and retention of the implant. More research is being conducted to determine whether this implant will prove to be just as effective in human eyes.

Another application is an implant that contains an aggregation of drugs to treat proliferative retinopathy (PVR). The implant was measured at 7 mm long and 0.8 mm in diameter [7]. This consisted of a PLGA matrix polymer for the drugs 5-fluorouridine, TA, and tissue plasminogen activator (t-PA) [7]. Each of these drugs was placed in three cylindrical components accordingly, and the experiment was proceeded with in vitro trials for 2 weeks [7]. Even though this implant was effective for PVR, it has yet to undergo in vivo trials [7].

There was limited damage or unwanted consequences after interpreting the efficacy of the TA implant. Such studies do present a plausible use for such an implant; however further investigation must be conducted in order to confirm how competent it will be in human subjects.

Another investigation involved the use of TA in experimental uveitis in a rabbit model [10]. A TA implant formed with poly(D, L-lactide) (PLA) was embedded within the sclera of the eyes of eight albino rabbits after they had been injected with tuberculin antigen [10]. On the other hand, eight control animals were given sham devices [10]. In order to prompt uveitis, all eyes were injected again with tuberculin

antigen and then intravitreal antigen on the 14th day [10]. Protein concentrations were recorded, inflammation of the anterior chamber (AC) and vitreous opacity were measured and clinically scored, and retinal functions were examined electroretinographically and histologically [10]. After 1 month of observation, anterior chamber cells and vitreous opacity scores showed incredibly less inflammation in the experimental eyes than in the control eyes [10]. Furthermore, according to histopathologic examinations, there was less inflammation and tissue disorder in the experimental eyes [10]. It was concluded that the intrascleral TA implant effectively inhibited the inflammation stimulated by experimental uveitis in rabbits for a month [10].

Dexamethasone (Ozurdex) Biodegradable Implant

Dexamethasone is used in a biodegradable implant in order to treat DME, neovascular AMD, and posterior uveitis [11]. The treatment for DME may be functional for about 6 months with the use of Ozurdex, an implant that includes the sustained release of dexamethasone [11]. There has been research that exhibits how this implant can reduce cataract progression [11]. Dexamethasone can instantly alleviate inflammation in uveitis and other retinal diseases by steady release without the need to extract the implanted device [11]. However, one drawback of this particular approach with Ozurdex is that it might not function for a longer period of time [11]. The half-life of dexamethasone in an intravitreal injection in the vitreous humor is 5.5 h [9].

Ozurdex is an intravitreal implant that includes dexamethasone that is covered by poly(lactic-co-glycolic acid) (PLGA) [12]. This particular implant is a rod that is inserted into the vitreous with a 22-gauge needle device [12]. It releases high doses of dexamethasone for 2 months and then a lower dosage for the next 4 months [12]. Dexamethasone has a shorter half-life than triamcinolone and fluocinolone and is 5 and 20 times stronger than them, respectively [12].

For the treatment of DME, dexamethasone has been utilized to counteract the short half-life of corticosteroid solutions and repeated intravitreal injections [13]. In a research study involving two large-scale Phase III randomized trials for 1048 subjects, a dexamethasone implant (0.35 mg and 0.7 mg) was delivered in at least 6-month intervals and benefitted best-corrected acuity (BCVA) and improved conditions of macular edema [13]. Anatomical evaluations depicted a decrease in macular edema and a reduced rate of retinopathy progression [13]. Less than 2% of injection-related complications were infrequent in this study, however [13]. Many Phase II and Phase III studies showed the effectiveness of the dexamethasone intravitreal implant in subjects with DME [13]. A 6-month Phase II study consisting of incisional vitreous placement of the dexamethasone implant in 171 DME patients indicated a potential dose-response relationship [13]. This included more patients accomplishing at least a 10- and at least a 15-letter word gain in BCVA with a 0.7 mg implant than with a 0.35 mg implant at months 2, 3, and 6 [13]. More prominent changes occurred in macular thickness and retinal capillary leakage at 6 months

in patients with the dexamethasone implants than did the controls, or the subjects that did not receive the implant [13]. When it came to the visual acuity findings, anatomic reactions were more evident with the 0.7 mg implant than with the 0.35 mg implant [13]. The dexamethasone implant was also found to be effective in focal, cystoid, and diffuse DME in further examination of the Phase II study [13]. This intravitreal implant can prove beneficial for the treatment for DME, particularly in persistent cases [13]. It can also be utilized to treat retinal vein occlusion [13].

Other researches incorporate dexamethasone used in diabetic patients. In a retrospective study, three injections of Ozurdex were administered to 165 type 2 diabetes patients [14]. Levels of glycated hemoglobin (HbA1c), total cholesterol, and triglycerides were examined 15 months after the drug implant was applied to the subjects [14]. The average values of HbA1c, total cholesterol, and triglycerides before the treatment were 7.1%, 176.7, and 125.6 mg/dl, respectively [14]. After the treatment, the values of these variables were recorded, accordingly of the Wilcoxon test p values: 0.68, 0.06, and 0.33, respectively [14]. From the data analysis, this indicated that the values were similar [14]. The average low-density lipoprotein (LDL) cholesterol levels somewhat rose after the Ozurdex treatment, but it returned to normal levels after the 15-month follow-up [14]. However, in 24 bilaterally treated patients, there was notable increase in LDL cholesterol levels [14]. Moreover, 8 of these patients experienced a rise in baseline LDL levels by more than 20%, but then a decline after the 15-month follow-up [14]. Overall, in this study, Ozurdex didn't affect HbA1c, and changes in lipid profile were not too significant [14]. On the other hand, it did increase LDL levels in patients who received frequent bilateral injections [14]. More investigation into lipid levels could be implemented in order to examine how this treatment could affect patients with a history of heart problems.

Verisome Biodegradable Implant

Verisome is a drug delivery system that gives promising therapeutic effects [5]. With the use of a 30-gauge needle, Verisome merges into a spherical structure that gradually breaks down as medication is liberated [5]. This drug delivery system can transfer peptides, proteins, and other molecules [5].

One study demonstrated a drug delivery system in which TA was formulated with Verisome. It exhibited better development for improving chronic cystoid macular edema (CME) [5]. In the first clinical trial, ten patients with CME due to retinal vein occlusion (RVO) – a common retinal vascular disorder – received a single dose of the drug containing the combination of TA and Verisome [15]. The subjects were studied for a year [15]. The first and foremost end point of this study was at day 60, when safety and tolerability were measured. Further end points involved measures of visual acuity and retinal thickness from optical coherence tomography (OCT) [15]. The drug system was administered via intravitreal injection with 30-gauge needles [15]. Patients of average age of 73 years were divided into cohorts of equal numbers, the first receiving a lower dosage than the latter [15]. During the study,

there was no record or occurrence of uveitis, endophthalmitis, or injection-related adverse events [15]. Cohort 2 displayed a greater average retinal thickness than did cohort 1 [15]. The researchers inferred that this drug system of TA and Verisome was accepted well by patients without any relevant side effects [15]. The formulation showed controlled release efficacy for low dosage for patients with CME due to RVO, and the higher dosage showed more efficacy [15].

Prednisolone Acetate Microfilm Drug Delivery System Biodegradable Implant in the Rat Keratoplasty Model

There has been an extensive use of topical corticosteroids as a treatment following corneal transplantation in order to stop graft rejection [16]. Nevertheless, therapeutic drug levels are not met from the utilization of the topical eye drops [16]. Thus, in a particular study, a biodegradable drug delivery system of prednisolone acetate (PA) microfilm was implemented in a rat keratoplasty model. There were five groups total in this study. In group 1, the drug release profiles of the microfilm were identified [16]. Forty-eight penetrating keratoplasty (PK) were implemented in four groups, syngeneic control grafts (group 2), allogeneic control grafts (group 3), allogeneic grafts with subconjunctivally implanted PA microfilm (group 4), and allogeneic grafts with PA eye drops (group 5), and each group contained a sample size of 12, or $n = 12$ [16]. The PA microfilms maintained steady sustained release for 12 weeks, and PA concentrations in the aqueous humor of PA microfilm or the PA eye drop group were stable and coherent in follow-up duration lasting 4 weeks [16]. In the histopathological analysis, PA microfilms or PA eye drop groups exhibited much lower occurrence of stromal edema and cellular infiltration [16]. When compared to allogeneic controls, PA microfilm implantation in corneal transplants demonstrated significantly more extended rejection-free graft survival [16]. It has been found that sustained PA-filled microfilm enables further corneal allograft survival [16].

Prednisolone Acetate (PA) Biodegradable Subconjunctival Implant in a Nonhuman Primate Model

Implants consisting of poly(L-lactide-co-caprolactone) (PLC) and 40-wt% PA were formulated, 8 and 16 mm long [17]. This study incorporated 12 monkeys, which were separated into two groups: one group of six monkeys that received the PA implants and another six monkeys that received blank microrods [17]. The toxicity and plasma toxicokinetic (TK) profile were examined from the drug release during 12 weeks of the study [17]. The greatest plasma concentration was 17.2% lower in animals treated with 40-wt% PA 16-mm microrods compared to those with 8-mm microrods [17]. The PA release presented minimal toxicity (Liu YC). There was no substantial difference in the amount of time it took to reach maximum concentration

between the 8- and 16-mm microrod groups (7.33 and 8 h, respectively; $P = 0.421$) [17]. According to the results from clinical evaluation, hematology, and histopathology, there were no reported adverse systemic or ocular side effects [17]. The PA biodegradable implant(s) demonstrated safe toxicokinetics even with 16 mm one containing more PA concentrations [17]. This PA implant may be regarded as a safe alternative to the topical administration of PA eye drops [17].

PA-Loaded Subconjunctival Implant Treatment for Uveitis in Rabbit Eyes

In a randomized control study, PA biodegradable microfilms were delivered into rabbit eyes that had uveitis [18]. The uveitis was created via a unilateral intravitreal injection of *Mycobacterium tuberculosis* H37Ra antigen in some rabbits [18]. The subconjunctival implant(s) also included ten PLC microfilms and six blank microfilms [18]. An in vivo PA release was measured from remaining PA concentrations in microfilms [18]. The rabbit eyes were observed clinically for 28 days for ocular symptoms, mainly inflammation [18]. Previous in vitro studies demonstrate that sandwich PA-loaded microfilms (60–40–60%) perform higher release of the drug than do homogenous PA-loaded microfilms [18]. In this study, the in vivo release was about 0.12 mg/day PA [18]. According to histological findings, experimental rabbits containing the PA microfilms showed little to no inflammation in comparison to the control rabbits [18]. Therefore, PA-loaded biodegradable microfilms were effective in inhibiting inflammation in the rabbit model with uveitis [18]. Further investigation may be required to analyze the extent to which such a PA-loaded implant can therapeutically treat ocular inflammation.

Timolol Maleate Implant for Intraocular Pressure

Glaucoma is an eye disease that affects the optic nerve and can lead to visual field loss [19]. It affects millions of people globally and is declared the second leading cause of blindness [19]. This disease is highly characterized by intraocular pressure (IOP) [19]. Timolol maleate (TM) is used to treat IOP in patients who have glaucoma [19]. It is a beta-adrenergic receptor antagonist that decreases IOP by lowering the amount of aqueous humor created [19]. Nonetheless, the TM treatment is also based on patient compliance [19]. Although the topical method of applying this drug is via eye drops, it is not the most favorable [19]. Additionally, partial control of the IOP results in advancement of the disease and can cause blindness [19]. Treatment of IOP via eye drops has proven to be suboptimal due to low bioavailability of TM from quick termination within the pre-corneal area [19].

In a research study, a subconjunctival TM microfilm was created to execute sustained and fixed ocular administration of the drug with the use of the biodegradable polymer, PLC [19]. The development of TM drug delivery has been continual and includes hydrogels, nano-fiber, microspheres, etc. [19]. The particular study

developed a TM system in the subconjunctiva and examined TM release from copolymer microfilms with the aim of establishing a daily dosage of approximately 2.5 microliters for 3 months [19]. This was a much less invasive method that might ensure extensive sustained drug delivery as of yet [19].

To confirm the functioning of the implant between in vitro and in vivo data, it was placed into the subconjunctival space of primate eyes and demonstrated good efficacy for up to 5 months. This study utilizes a method that consists of multilayering and blending with poly(ethylene glycol) (PEG) copolymers in order to produce a TM-incorporated biodegradable film that can administer TM therapeutically for 90 days in vitro [19]. This, as well as the utilization of the base form of TM to increase lipophilicity and a sandwich film, enabled a longer sustained release [19]. It was inferred from the results of a diseased primate model that there was sufficient decrease in IOP for 5 months due to one TM implant and its adequate biocompatibility and partial degradation [19]. These findings indicate that this specific implantation could provide a crucial change in treating IOP in glaucoma [19].

Fluocinolone Acetonide (FA) (Iluvien) Biodegradable Implant

An Iluvien implant consisting of FA was used to treat eyes with DME and vitrectomy in a research study. In this retrospective study of six European centers, a 0.2 μg/day FAc implant was delivered to 26 vitrectomized eyes from 25 patients with DME and a prior vitrectomy [20]. Vitrectomy is utilized to treat DME and enables release of vitreomacular traction, raising oxygen levels of the retina and inhibiting the diffusion of pro-permeability factors such as VEGF [20]. Prior research in therapies for posterior eye segment diseases includes anit-VEGF systems and steroids [20]. The eyes underwent pars plana vitrectomy (PPV) in order to test for vitreoretinal interface, diabetic retinopathy, and vitreous hemorrhage. With the use of the early treatment diabetic retinopathy study (ETDRS), efficacy data was recorded from the letter scores [20].

Nine of the 26 eyes were examined prospectively with the decimal scale and then retrospectively altered to the closest value of the ETDRS scale [20]. The remaining 17 eyes were prospectively studied with the ETDRS scale and retrospectively recorded also [20]. The average time between the PPV and the delivery of the implant was 24.2 months [20]. There was an average follow-up of 8.5 months after administration of the FAc implant [20]. Fifty-four percent of the 25 patients showed at least one sign of the PPV [20]. As a result, the average differences in BCVA, central foveal thickness (CFT), and IOP were + 11.7 ETDRS, −233.5 μm, +1.4 mm Hg, respectively [20]. According to the data, even though eight eyes were in need of continual use of IOP decreasing medications, the 0.2 μg/day FAc implant is beneficial for vitrectomized patients of a decent safety profile [20]. Nonetheless, more research is needed to analyze the effects of the implant over a longer duration and time of follow-up after drug delivery [20].

Bimatoprost SR for Glaucoma

In a Phase I/II clinical trial, Bimatoprost SR (sustained release) was administered intracamerally to 75 open-angle glaucoma patients, in the experimental eye [21]. The other eye received topical bimatoprost 0.03% on a daily basis [21]. This study was conducted to investigate how Bimatoprost SR would impact the IOP and effectively reduce it in a 2-year Phase I/II study [21]. Observations were recorded for 6 months, and it was found that the Bimatoprost SR implant reduced IOP efficiently [21]. The average IOP decrease from baseline during week 16 in experimental eyes was 7.2, 7.4, 8.1, and 9.5 mm Hg with the 6-μg, 10-μg, 15-μg, and 20-μg dosages of the Bimatoprost SR implant, respectively [21]. On the other hand, the fellow eyes with the topical bimatoprost had undergone a reduction of 8.4 mm Hg from baseline, and no data was revealed for the rescue topical IOP-lowering medication or a single repeat treatment of the Bimatoprost SR implant [21]. Conjunctival hyperemia including onset after 2 days following the implant delivery was more present within eyes that were given topical bimatoprost as compared to the eyes with the implant [21]. The implant had good efficacy and was sufficiently secure for 6 months [21]. A single delivery of the implant reduced IOP in most of the patients [21]. Note however, the implant formulation was enhanced to adjust the rate of the polymer degradation as well as the drug release [21]. So far, results up to 6 months of observation for the safety and efficacy of the implant were reported [21].

Open-angle glaucoma is a chronic vision-impairing disorder that affects more than 40 million people globally [21]. Originally, the preferred treatment for open-angle glaucoma is often topical ocular hypotensive medication to decrease IOP [21]. Data based on pharmaceutical claims have suggested that adherence to topical IOP-reducing medication is worse than adherence to oral prescriptions and treatments for hypertension and diabetes [21]. The data has indicated that patients attained prostaglandin or prostamide glaucoma medication for dosage 37% of all 365 days [21]. Some complications to abiding by topical glaucoma administration are imprecise dosing frequency, inaccurate information about the eye disease, etc. [21].

Bimatoprost is a prostaglandin analogue (PGA) that lowers IOP in topical delivery [21]. Bimatoprost SR is a biodegradable implant made to respond to the nonadherence in glaucoma and open-angle glaucoma patients who cannot withstand or can't utilize topical administration of drugs specified to glaucoma [21].

Biodegradable Nanoparticles

Nanospheres have been applied to retinal pigment epithelium (RPE) [4]. In a past study by Kim et al., human serum albumin nanoparticles were used to see how injected nanoparticles moved intravitreally [4]. Anionic nanoparticles showed more potential than cationic nanoparticles for RPE because they were able to travel across

the vitreous more freely [4]. Corticosteroids included in nanoparticles have been demonstrated to impede the presence of vascular endothelial growth factor (VEGF) in vitro, even in a RPE cell line [4].

Biodegradable nanoparticles (NPs) provide strong biocompatibility and suitable sustained releases for numerous drugs and compounds in several medicinal applications [22].

TA Poly(Beta-Amino Ester) Nanoparticles

Previous research on ocular drug delivery including polymeric mucoadhesive nanoparticles has implied that there is better retention and permeation of nanoparticles [23]. In a particular study, novel polymer, poly(β-amino ester), was used to formulate triamcinolone acetonide-loaded nanoparticles via the use of an altered emulsification/solvent diffusion method [23]. The nanoparticles were characterized using in vitro drug release, X-ray powder diffraction, scanning electron microscopy, and differential scanning calorimetry [23]. In the rabbit eye model, uveitis was produced by injecting it intravitreally with a lipopolysaccharide (Sabzevari). Thirty-six hours after the injection, samples were taken from the aqueous humor, and levels of protein, nitric oxide, and prostaglandin E2 were recorded [23]. Nanoparticles of an average size of 178 nm and drug loading of 5.3% were created and utilized for in vivo studies of rabbits that had uveitis [23]. In polymeric nanoparticles of TA, there was a larger anti-inflammatory effect than in microparticles of prednisolone acetate and TA [23]. Nonetheless, compared with a subconjunctival injection of TA, the polymeric nanoparticles of TA also suppressed inflammatory factors [23]. Thus, in conclusion, the polymeric nanoparticles of TA proved effective in inhibiting inflammation and could be used in further research for alleviating posterior segment eye disease.

Biodegradable NPs for Retinal Pigment Epithelium (RPE)

Sakurai et al. investigated the intraocular kinetics of nanospheres and discovered that polystyrene nanospheres with fluorescein can be retained in the vitreous, retina, and trabecular network for over a month after the intravitreal injection in rabbit eyes [7]. Shah et al. demonstrated how the intravitreal injection of rhodamine-loaded (6G and Nile Red) polylactide nanoparticles was feasibly maintained in the retina for 4 months after intravitreal injection [7]. Other nanoparticles that were used or are in development to treat RPE include human serum albumin and anionic [7]. It has also been reported by Gaudana et al. that certain ligands, for example, folate and biotin, are capable of raising the uptake of steroidal nanoparticles due to the RPE [7]. In another study by Kompella et al., VEGF expression in vitro in a RPE cell line could be hindered by nanoparticles and microparticles that included corticosteroids called budesonide [7].

Utilization of Biodegradable Polymers Within Ocular Drug Delivery Method

Overall Structure of Biodegradable Polymers

Biodegradable polymers that include drugs are fashioned into one of two systems: a matrix or reservoir [7]. For nanospheres, drugs and polymers are mingled in a matrix system, and the drug is discharged from it while the polymer is disintegrating [7]. On the other hand, the reservoir system consists of drug encapsulation by specific polymers, which is more utilized in biodegradable nanocapsules [7].

Biodegradable Polymers

Characteristics of Biodegradable Polymers

Biodegradable polymers can be broken down within the body after the drug is released and do not require surgical removal because the device degrades [11]. This avoids the use of surgical extraction [11]. Biodegradable polymers may also be constructed into many different forms [11]. Many factors of biodegradable polymers such as molecular weight, hydrophobicity, water absorption, degradation, and material chemistry can impact the characteristics of biodegradable biomaterials (9).

Examples of Biodegradable Polymers

Liposomes

One clinical example is liposomes (verteporfin and ganciclovir), which can be created from lipids and phospholipids [11]. Liposomes are vesicles that have an aqueous zone and at least one lipid bilayer in which lipophilic active substances reside [11]. They have been used to treat cytomegalovirus (CMV) with the use and inclusion of the drug ganciclovir [11]. For AMD, liposomes have been constructed with verteporfin [11]. Liposomes in a nanosized form have been effective to the posterior region of the eye after topical instillation [24]. Many physiochemical characteristics such as lipid composition, particle size, and surface modification have influenced the intraocular behaviors of the drug as well as the carrier [24].

PLGA

PLGA is another biodegradable polymer that has been used in ocular disease treatment. It is placed into the eye via pars plana insertion and is utilized for the formation of rods; many drugs have been used with PLGA such as dexamethasone,

aciclovir, and retinoic acid for uveitis, herpes, and proliferative retinopathy, respectively [11]. Aliphatic PLGA NPs set by emulsion solvent diffusion (ESD) proved to be appropriate drug carriers [24].

Moreover, PLGA was used to administer albumin and doxycycline in a study that interpreted its efficacy [25]. PLGA microspheres were administered in subconjunctival injections in a rodent model in order to examine an inflammatory response as well as safety measures [25]. A single injection of PLGA-doxycycline (a metalloproteinase inhibitor) was delivered to examine its efficacy before applying it onto a desiccating stress (DS) model in six standard laboratory mice for 5 days [25]. The PLGA microspheres were formed via a double-emulsion solvent extraction method involving oil and water [25]. Selected drugs were successfully extracted by the PLGA-based microspheres over a certain amount of time [25]. It was also feasible to adjust the drug elution rates and delivery times by changing specific polymers and measures of synthesis [25]. PLGA-doxycycline effectively prevented DS-induced corneal barrier from disrupting [25]. To properly execute and analyze drug release, PLGA microparticles were filled with FITC-albumin at 37 degrees Celsius for 20 days and sampled in a timely manner and then measured for attaining an in vitro elution assay [25]. The encapsulated drugs showed plausible elution continually even after 2 weeks within in vitro studies [25]. The in vivo study of the dry eye murine model involved the delivery of subconjunctival injections of BSS-loaded PLGA to control subjects [25]. After 5 days of DS, a corneal barrier disruption occurred [25].

Nonetheless, this disruption was prevented via PLGA-doxycycline [25]. This suggests that doxycycline can be used as a topical treatment in a dry, experimental eye [25]. PLGA-based drug implants are advantageous because modifying the molecular weight of the polymer, the proportion of lactic acid to glycolic acid, as well as the size of the particles can alter drug release rates [25]. This study demonstrated a sufficient sustained drug release system because one injection of PLGA microspheres containing doxycycline enhanced corneal barrier function in mice before the implementation of DS [25]. PLGA-based implants have remarkable biocompatibility, and they have proven to be effective in drug release in both in vivo and in vitro trials [25]. Similar to a daily dose of doxycycline, PLGA-doxycycline prevented DS-induced corneal barrier disruption in the study [25]. The use of PLGA can improve the function of many drug delivery systems and offer more application of microparticles with biopharmaceuticals for further research.

PLC

Another example is PLC. It's a new biodegradable copolymer that can break down into lactic and caproic acids, which are not lethal and can be eradicated in the tricarboxylic acid cycle [16]. PLC is more hydrophobic than PLGA and reaches longer release profiles due to slow degradation [16]. Degradation rates are influenced by the type of crystalline structure of the polymers [16]. For instance, PLGA breaks down more readily because it has an amorphous whereas PLC has a semicrystalline

arrangement [16]. It has been noted in previous research that PLC microfilms break down more gradually than PLGA microfilms in vitro as well as in vivo [16]. Additionally, PLGA copolymers are harder in structure due to attaining a greater glass transition temperature than PLC copolymers [16].

Polyanhydrides

Additionally, there are polyanhydrides, which can break down from surface erosion and become biocompatible monomers that are then eliminated [26]. This way, there will be a more sustained release than if polyanhydrides were to be degraded by bulk erosion [26]. There are many types of polyanhydrides including aliphatic, unsaturated, and aromatic [26]. Aliphatic polyanhydrides degrade faster than aromatic ones [26]. Such properties as degradation rates can indicate and give more information about how a proper drug delivery system can be developed to give efficient and more desirable drug release [26]. The copolymer of 1,3-bis(carboxyphenoxypropane) (PCPP) and sebacic acid (SA) is utilized most often [26]. SA is aliphatic and hydrophilic and has a much lesser longevity than does PCPP [26]. Such copolymerization has been studied in the administration of 5-fluorouracil, taxol, and etoposide for experimental glaucoma filtration surgery in a primate model [26]. In this particular investigation, filtration surgery was performed in order to ascertain whether bioerodible polyanhydride disks with 5-fluorouridine (5-FUR) extended the success of operation [27].

Primarily, in vitro studies showed that 5-fluorouridine was released from these disks for duration of at least 16 days in a bioactive form that hindered fibroblast propagation [27]. Then, an initial sequence of six eyes indicated that the utilization of disks with 5-fluorouridine prolonged the intraocular pressure-lowering effect of filtration surgery [27]. This observation was confirmed in more trials: eight extra eyes of four animals, which incorporated one eye containing a disk with 5-fluorouridine and the other, a disk with no drug [27]. In the eyes that received polyanhydride with 5-fluorouridine, the operation was more successful and lasted longer than it did in the eye without the drug [27]. The success of the clinical trials was confirmed by histologic findings [27].

Furthermore, another study that focused on glaucoma filtration surgery utilized bioerodible polymers and 5-fluorouracil. In a randomized and placebo-controlled experiment, a bioerodible polyanhydride made up of bis(p-carboxyphenoxy) propane and sebacic acid administered 5-fluorouracil to18 rabbits [28]. The drug and polymer were condensed into small disks [28]. They were then delivered to one eye, while a blank polymer was delivered to the other associated eye [28]. The findings demonstrated that intraocular pressure (IOP) decreased in the experimental eyes for 12 days, even though both eyes reverted to preoperative levels in the end [28].

Eventually, the biodegradable implant did disintegrate after IOP levels increased [28]. Therefore, the surgery was not successful because filtrations blebs were not completely functional either [28]. Nevertheless, it was evident that the implant did reduce IOP pressure to a certain degree. With further research into this drug implant, it could be promising in lowering IOP more efficiently and substantially.

Polycaprolactone (PCL)

Polycaprolactone (PCL) is a biodegradable polymer that is semicrystalline as well as hydrophobic [26]. When placed in water, PCL is left with pores, enabling an extensive sustained release of over a year [26]. From previous research, it has been noted that intravitreal PCL implants can administer dexamethasone for at least 55 weeks [26]. At 55 weeks, 79 percent of dexamethasone was sustained in the implant [26]. In rabbit eyes, there was sustained release of the PCL implant containing dexamethasone with no inflammation [26]. Also, a PCL implant with triamcinolone acetate was implanted in the subretinal space of rabbit eyes and proved to have an effective sustained release for at least 4 weeks with no signs of inflammation [26]. In most cases, PCL can be mixed with more hydrophilic polymers to create polymers that break down more quickly [26]. Such mixtures of polymers have been utilized for delivery drugs such as cyclosporine and tacrolimus [26].

Nanomaterial Toxicity in the Human Body

Nanomaterials (NM) can impact the human body at even the molecular level, thus in turn, influencing some physiological characteristics and mechanisms. There is a complex interaction between NM and biological molecules such as macromolecules [1]. Such interactions could lead to toxicological effects from the NM [1]. NM could deteriorate membranes, DNA, and mitochondria [1]. They could penetrate and puncture cells, thus accessing and bringing toxicity to the cell organelles as well as causing leaks of intracellular fluid into the cell environment [1]. This could also potentially cause cell death [1].

Impact of Nanotoxicity on Central Nervous System

NP can enter the brain after inhalation through the trans-synaptic transport and uptake via the blood-brain barrier [1]. The chemical properties of the NP have exhibited damaging effects to the blood-brain integrity [1]. Therefore, further studies should be examined, and more investigation should be taken into consideration when referring to the influence of NP and biodegradable NP on the nervous system.

Implantation of Drug Delivery Treatment Systems

Some new intravitreal systems are in development for the treatment of AMD and macular edema, including anti-VEGF drugs such as pegaptanib, ranibizumab, and bevacizumab [7]. As of now, intravitreal injection is the most successful approach to

handling vitreoretinal disease [7]. This way, the drug can directly be applied to the posterior segment of the eye, therefore eradicating obstacles associated with topical and systemic administration [7]. Subsequently, a higher concentration of the drug can get to the posterior site and effectively treat the disorders [7]. Even though TA is applied intravitreally to treat AMD and macular edema, several injections may be needed due to the limited half-life of numerous compounds in the vitreous [7]. Nonetheless, from this, there is higher risk of cataract, retinal detachment, hemorrhage, and endophthalmitis [7].

Intravitreal Drug Delivery for Vitreous Inflammation

Treating vitreous inflammation can involve extensive medication just to alleviate and manage symptoms [5]. Intravitreal injection may be administered more frequently (10). There could even be side effects to accessing and permeating the posterior segment of the eye with topical agents [5]. Nonetheless, high intravitreal drug concentration may be needed to treat ocular diseases in the posterior segment of the eye [2].

Moreover, corticosteroids have been used to treat posterior segment eye diseases due to their anti-inflammatory and antiangiogenic properties. Corticosteroids have exhibited control of gene expression of inflammatory moderators [4]. This in turn affects the expression of vascular endothelial growth factor (VEGF), impedes pro-inflammatory genes such as tumor necrosis factor-alpha (TNF-α) as well as other inflammatory chemokines, and stimulates the expression of anti-inflammatory factors such as pigment-derived growth factor (PEDF) [4].

It has been noted that topical steroids don't completely go through to the posterior segment of the eye [4]. However, it has also been reported in previous research that therapeutic dosage of steroids can approach the posterior segment of the eye by transscleral absorption along with periocular dispensation [4]. Therefore, subconjunctival, subtenon, and posterior juxtascleral infusions were investigated [4].

Intraocular Delivery, Function, and Barriers of Corticosteroids

Commonly used corticosteroids such as dexamethasone, TA, and FA are formidable agonists at the glucocorticoid receptor [13]. Subsequently, a steroid-receptor complex is formed, and then it can suppress gene transcription, mRNA, as well as protein synthesis [13].

Pertaining to DME, corticosteroids are antiangiogenic as they inhibit angiogenic factors such as VEGF [13]. They also stabilize the retinal vasculature [13]. By suppressing the main pro-inflammatory transcription factors, the corticosteroids interrupt the pro-inflammatory feedback loop of macular edema [13]. Corticosteroids suppress phospholipase A2, which has been demonstrated in a study involving a

streptozotocin-induced diabetic rat [13]. Moreover they can stimulate the expression of anti-inflammatory proteins such as adenosine, IL-10, and IκBα [13]. In fact, in a diabetic rat model, dexamethasone has been shown to lower eukostasis and vascular endothelial ICAM-1 expression [13]. Corticosteroids can reduce retinal vascular permeability and foster retinal fluid clearance via influencing transcellular aquaporin-4 (AQP4) and potassium channels [13].

Systemic and intravitreal corticosteroids suppress leukocyte adhesion by inhibiting ICAM-1 gene expression, lowering protein levels, and suppressing the degradation of blood-retinal barrier (BRB) via the reduction in VEGF levels [9]. TA is a crystalline steroid that is usually used for treating retinal diseases and is not as water soluble, but is injected in a suspension form [9]. The intravitreal injection of TA has demonstrated more efficacy than the subtenon injection has [9]. But it must be noted that as an ocular side effect, TA is affiliated with more cataract progression as well as an increase in IOP [9]. Dexamethasone has a stronger binding affinity to its glucocorticoid receptor than TA and FA have to theirs [13]. Nevertheless, TA has more lipophilicity than does FA and dexamethasone [13]. Furthermore, dexamethasone is five times more powerful than TA [9]. When it comes to treating retinal disorders, effective and sustained release of drugs while limiting undesired consequences on other ocular components are main priorities and challenges that need to be addressed [13].

Certain drug systems may not be able to penetrate ocular structures completely. For drugs that may cross the BRB, blood volume may dilute, and this may require the utilization of big systemic doses even though the rapid flow rate in the choroidal and retinal circulation reduces the period of time of drug exposure [13]. Drug exposure might be hindered by ocular surface tear mechanisms, whereas a drug that permeates through the sclera and corneal epithelia may be eradicated by lymph flow, the choroidal circulation, and the transcellular xenobiotic efflux transporters [13]. Topical drugs may exhibit very low intravitreal bioavailability, considering they have to move through several ocular tissue layers to get to the posterior region of the eye [13].

Intravitreal injection poses risks such as endophthalmitis, retinal hemorrhage, or tear [13]. Additionally, the duration of activity of the intravitreal drug that is delivered is delayed by drug clearance mechanisms, particularly the transretinal and anterior aqueous humor elimination pathways [13]. For the common corticosteroids, the intravitreal half-life of the dissolved drug fraction is 2–3 h in monkey and rabbit eyes [13]. Nonetheless, biodegradable systems such as liposomes, nanoparticles, and nanoemulsions are in development in order for the vitreous humor to have the ability to retain the drug for an extended period of time [13]. Corticosteroid systems that are used for sustained drug release include Ozurdex®, Kenalog®, and Trivaris®, just to name a few [13]. Ozurdex® and Iluvien® have been developed for treating DME [13].

Moreover, factors that can affect drug release include drug solubility in the vitreous humor, the distance of the drug implant to the primary elimination pathway, and the chemical qualities of the implant [13]. Implants of the posterior segment of the eye enhance drug administration to the retina and lessen drug exposure in the lens and trabecular meshwork [13].

Although delivery drug systems to the posterior segment of the eye may be complex, there is a necessity to alter and alleviate the progression of detrimental diseases such as AMD and DME. Thus, drug delivery is augmented by more therapeutic aspects such as oligonucleotides and antibodies as well as requirements for chronic therapy [29]. Repeated intravitreal drug delivery can trigger retinal detachment, endophthalmitis, and higher IOP [29]. Additionally, although it has been noted that the periocular route is a beneficial one due to a big surface area and increased permeability of the sclera, the BRB and efflux transporters hinder the transfer of remedial components to the retina [29]. As such, this confirms some of the obstacles of posterior eye drug delivery. Nevertheless, there are several methods that may help overcome them as they lead to better recovery from posterior eye segment diseases. Intravitreal drug delivery of biodegradable implants provides a more promising approach for therapeutic effects and treating retinal and posterior segment eye disorders.

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Chapter 16
Nanomedicine-Based Gene Delivery for the Retina and Posterior Segment Diseases

Anita Lalwani, Pragna Shelat, and Jayvadan K. Patel

Abstract The eye is an immune-privileged site and is more amenable to genetic therapy. A number of diseases in the posterior segment of the eye have genetic origin. The posterior segment of the eye is difficult to access. But routes to deliver drugs and genes have been worked upon. The genetic therapy ensures long-term treatment of the disease in the eye. The genes that are to be delivered to their proper site can be given through viral and non-viral vectors. Viral vectors offer a number of advantages when it comes to transfection efficiency and gene expression but are associated with immunity issues and cannot be loaded with genes which are more than 5 kb. Over the years non-viral gene vectors are becoming increasingly popular because of their higher gene loading capacity and number of distinct advantages over the viral vectors. But the genes that are to be delivered to the nucleus have to escape a number of degradative mechanisms outside and inside the cell to reach the nucleus. Lipoplexes and polyplexes have been successfully used in the treatment in nonclinical studies, but the clinical applications have still to see the light of the day.

Introduction

The expected number of people with visual disability in the world is 285 million, 39 million blind and 246 million having low vision; 65% of people visually impaired and 82% of all blind are 50 years and older. Posterior segment diseases are a foremost cause of visual impairment globally and likely to become more prominent, with the rapid growth of the aging population. The fraction of the total visual impairment and blindness from age-related macular degeneration, glaucoma, and diabetic retinopathy is at present greater than from infective causes such as trachoma and corneal opacities (http://www.who.int/blindness/causes/priority/en/index8.html).

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Gene therapy deals with the modification of individual's genes so as to correct their expression or correction of abnormal gene. This involves the administration of a specific DNA (or RNA). Inherited eye diseases are attractive targets for gene therapy for several reasons. Inherited eye retinal degenerations have been studied intensively, and the mutations leading to death of photoreceptor cells have been described in over 200 genes (https://sph.uth.edu/retnet/home.htm), and so the targets are known. The retina is accessible from outside the body, and injections may be viewed through the lens. The thickness of the retinal layers and integrity of photoreceptors can be measured through clear cornea and lens in the living eye. The genetic disorders in the eye like diabetic retinopathy, glaucoma, and age-related macular degeneration require long-term treatment that may be approachable by gene therapy. [6].

Vectors that encapsulate and deliver foreign genetic materials into specific cell types need to be efficient and nontoxic. These carriers may be viral in origin or may be non-viral, i.e., lipid-based or polymer-based. Viral vectors like adeno-associated virus (AAV) have high gene transduction capability, but the issues with their toxicity still need to be addressed. The size of viral vectors, which restricts the inclusion of genes to <5 kb, is another constraint [5]. Non-viral vectors offer promising platform for delivering plasmid DNA for gene therapy because of their safety and higher payload capacity [8].

The Eye as a Therapeutic Target [9]

The eye can be separated in two anatomical segments: the anterior segment, where the cornea, lens, and conjunctiva are the most significant structures, and the posterior segment, where the retina plays an important role in image attainment (Fig. 16.1). The anterior segment concentrates light onto the photoreceptor cells in the retina, while photoreceptors translate the light image to electrical signals and transmit it to the brain through the optic nerve to enable vision.

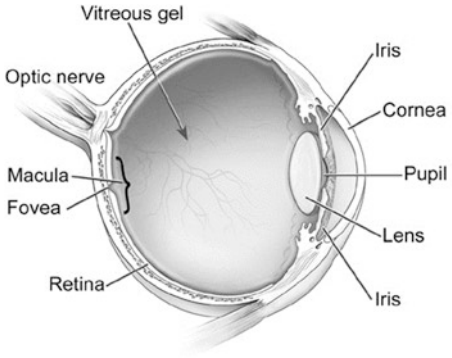

Fig. 16.1 Anatomy of the eye

The posterior segment of the eye is made up of three layers, the sclera, choroid, and retina, which surround the vitreous cavity filled by the vitreous, a transparent gel made of water, collagen, hyaluronic acid (HA), and proteoglycans. The vitreous supports the contour of the eye by keeping the retina flat aligned with Bruch's membrane. The sclera is a strong external layer and is largely composed of connective tissue. The choroid is a vascular layer and endows oxygen and sustenance to the outer layers of the retina. The retinal pigment epithelium (RPE) is a monolayer of pigmented epithelial cells that outlines the back of the retina and is responsible for the protection of the neural retina and vision. Further the tight junctions between RPE cells comprise the blood-retinal barrier (BRB) which grants the eye its immune-privileged status.

Ocular Disorders [1]

Retinitis Pigmentosa

Retinitis pigmentosa (RP) is a cluster of clinically and genetically diverse disorders. RP has a worldwide prevalence of 1 in 3000 people. This is characterized by night blindness (tunnel vision) at the onset because of the loss of rod cells in the periphery of the retina. As the disease advances, cone cells begin to degenerate, which leads to complete blindness of the patient. The reason behind the loss of cone cells in RP cases is less understood, although rod cells possess defective genes. In this context, it is hypothesized that these cone cell dystrophies might be the result of loss of rod cell-based supporting factors. Numerous genetic inheritance patterns have been found in RP patients. These genetic inheritance patterns can be *autosomal recessive (*arRP), *autosomal dominant* (adRP), *or x-linked.* In general, RP is divided into two categories: one is nonsyndromic, which is limited to the eye, and the other one is syndromic, which also affects other organs and tissues in the body. RP is linked to more than 100 mutations in the different regions of the rhodopsin gene that accounts for close to 30–40% of adRP. Mutation in the rhodopsin gene can affect the rod cell functions at variable severity levels. The mutation in the Phosphodiesterase 6b gene is responsible for the arRP in humans. This earliest and most harsh form of the disease contributes to 5% of all arRP cases.

Usher Syndrome

Usher syndrome (USH) is a genetically diverse group of autosomal recessive genes that influence both hearing and vision, along with occasional loss of balance. Clinically, there are three types of Usher syndrome (USH1, USH2, and USH3). Regarding the onset of hearing loss, USH1 is the most severe followed by USH2 in

terms of severity. In USH3 cases, the child is born with usual hearing and balance, but there is loss of hearing by adolescence. There are five USH1 genes that codify known products, and these are myosin VIIA, cadherin 23, protocadherin 15, scaffold protein containing ankyrin repeats and sam domain, and calcium- and integrin-binding protein 2. The USH2 (usherin, VLGR1, and whirlin) and USH3 (clarin-1) proteins are also found in the same sections of photoreceptor cells in the mouse retina. Mutations in these genes can result in the loss of protein functions that finally lead to loss in hearing and defects in the photoreceptor cells of retina.

Stargardt's Disease

Stargardt's is an autosomal recessive juvenile disorder which mainly occurs in children between the ages of 6 and 16 years, with a prevalence of 1 in 8000–10,000 patients. This common genetic macular disorder presents throughout the world, because of the mutation in a gene that encodes a PR ATP-binding cassette (ABC) lipid transporter protein (more commonly known as ABCA4).

Leber Congenital Amaurosis

Leber congenital amaurosis (LCA) is an autosomal recessive inheritance pattern. So far, 14 genes have been evaluated that are associated with this severe form of congenital blindness that presents in early childhood. The worldwide incidence of this disease is 1 of 30,000 cases, 20% of all inborn blindness and 5% of all innate retinal dystrophies. One of the most important mutations is the RPE65 gene that encodes RPE65 (RPE-specific 65 KDa) protein in RPE cells. This has led investigators to hypothesize that restoration of function of RPE65, which is involved in photoreceptor cell cycling, might prevent progression of degeneration and may allow restoration of visual function.

Diabetic Retinopathy

Diabetic retinopathy (DR) affects 93 million people worldwide. Diabetes might cause damage to the retinal blood vessels that nourish the retina. Blood and other fluids that leak from these vessels can cause thickening and inflammation of the retina. Fluid that builds up because of chronic high blood sugar levels causes blurred vision. DR can sometimes be restricted if the blood glucose level is stabilized. DR associated with gene alteration, however, may not always be controlled by the long-term management of blood sugar by using insulin therapy.

Age-Related Macular Degeneration

Symptoms of age-related macular degeneration (AMD) usually present around age 60 and are caused by deterioration of the macula. AMD manifests in two forms: one is "dry" (non-neovascular) and the other is "wet" (neovascular) AMD. The dry form of the disease is more common than the wet form. Dry AMD is related with the deposition of yellowish lipid proteins known as drusen beneath the retina that build up slowly and cause detachment of the retina. All these events lead to steady loss in central vision. Dry AMD can evolve to geographic atrophy or the more destructive wet form. In wet AMD, an atypical angiogenesis rapidly leads to the choroidal neovascularization (CNV) within the retina and deteriorates PR cells in the macula. Ninety percent of AMD-related blindness have been attributed to this angiogenesis. Though the pathophysiology of AMD is complicated and multifactorial, the core of treatment for the neovascular form is inhibition of vascular endothelial growth factor (VEGF), which requires monthly assessment and repeat intravitreal administrations. Additionally, pigment epithelium-derived factor and placental growth factor and new molecules such as endostatin and angiostatin modify the permeability of the retinal and choroidal vasculature and represent interesting targets for gene therapy.

Gene Therapy in the Eye

Gene therapy in the eye can be divided into three categories [2]:

1. Gene replacement.
2. Knockdown technology.
3. Treatments for neurodegenerative disorders (glaucoma, age-related macular degeneration) that do not have a monogenic cause.

Gene delivery for LCA arising from mutations in the RPE65 gene is the most successful example of gene therapy in the eye. RPE65 encodes the 65-kDa RPE-specific isomerase which is important for recycling 11-cis retinal, the chromophore of rod and cone opsins. rAAV vector-mediated gene replacement has led to the rescue of vision in the Swedish Briard dog, a spontaneous RPE65-null model, and stable vision improvement has continued for over 8 years after a single rAAV vector administration. These results, in addition to the absence of side effects after rAAV vector subretinal delivery in nonhuman primates, have cemented the way to three ongoing clinical trials using rAAV2/2 vectors for RPE65 gene replacement in patients affected by LCA due to mutations in RPE65.

Many of the common disease-causing mutations in the retina are dominant, gain-of-function mutations. In these cases, gene replacement alone is not a viable treatment option. Researchers have confirmed the ability of small interfering RNAs contained in AAV-2 vectors to knock down cotransduced reporter gene expression in retinal ganglion cells[39]. Researchers were able to specifically knockdown mouse

rhodopsin expression (in cultured retinal explants) using short hairpin RNAs and concomitantly express (at ~90% of wild-type levels) a co-transfected mouse rhodopsin with silent mutations in the shRNA recognition sequence.

There are diseases which are not monogenetic, i.e., they do not involve single genetic mutation. Diseases like glaucoma and age-related macular degeneration cannot be related to a single genetic component. Efforts are being directed to evaluate the expression of neurotrophic or antiapoptotic factors in the eye for the suppression of angiogenic factors.

Gene Delivery Routes [4]

The posterior segment of the eye can be targeted through topical, systemic, intravitreal, and periocular routes. Some of the limitations of the topical route to target posterior segment include rapid drainage through the nasolacrimal ducts, poor permeability of the corneal epithelium, blood aqueous barrier, and systemic absorption. Diffusion of the drugs which are administered through systemic route is hindered by blood-retinal barrier (BRB).

Intravitreal injections have gained considerable impetus during the last two decades. In this method drug solution is injected directly into vitreous through pars plana using a 30 G needle. Intravitreal injection gives higher drug concentrations in vitreous and retina than the other routes of administration. Intravitreal injection offers high concentrations of drugs in the retina, but it is associated with various short-term complications such as retinal detachment, endophthalmitis, and intravitreal hemorrhages. This mandates that the patients be carefully monitored following intravitreal injections. The inner limiting membrane (ILM) and the BRB are the main biological barriers that hamper drug transport from the vitreous to the retina. The ILM forms a frame between the vitreous humor and the retina and is the major barrier to drug diffusion to the retina. The endothelial cells of retinal blood vessels and retinal pigmented epithelium (RPE) form the BRB which acts as a secondary barrier hindering the transport of the drugs to the inner retinal cells.

Drugs from the vitreous humor are eliminated through the anterior chamber or across the retinal surface. Passive diffusion through the BRB leads to rapid clearance of lipophilic drugs administered into the vitreous. Further, the molecules that are substrates for the innate active transport mechanisms are rapidly eliminated by the efflux transporters in the endothelium.

Periocular Routes

The periocular route has an equal merit for administering drugs to posterior eye segment. Periocular refers to the region surrounding the eye. Periocular pathways used for the delivery of drugs to the posterior tissues of the eye include the

retrobulbar, peribulbar, subtenon, and subconjunctival routes. Drug solutions are placed close to the sclera which results in high retinal and vitreal concentrations. Sclera is made up of fibrous tissue and offers less resistance to permeability of drugs. The periocular route of drug delivery enables the deposition of molecules against the external surface of the sclera, thereby minimizing the risk of endophthalmitis and retinal damage associated with the intravitreal route of administration**.** It is considered to be the least painful and the most efficient route of drug delivery to the posterior eye.

The direct penetration pathway is the main route in achieving high concentrations of a drug in the vitreous following subtenon injections. The sclera, with its large surface area (16.3 cm^2), is less resistant to permeation of molecules and has lower protease activity compared to the cornea. Nevertheless, only minute concentrations of a drug administered via the transscleral route end up in the vitreous. This low bioavailability can be attributed to the loss of the drug from the periocular space, BRB, choroidal circulation, and the binding of drugs to tissue proteins as well as efflux transporters.

Drugs may be conveyed to the posterior ocular segment through the systemic blood circulation. Drugs that have high permeability can cross the blood-retina barrier to reach the retina and vitreous. The choroid is easily accessible owing to the extensive blood flow and leaky vasculature in this tissue. High doses are required, and systemic undesirable effects are common (e.g., systemic treatment of glaucoma with carbonic anhydrase inhibitors) through this route since only a very small fraction of the blood circulates through the posterior ocular segment. Such approach is not feasible for drugs that have small dose and narrow therapeutic index.

Viral Vectors

Viral vectors include adenovirus (Ad), AAV, retrovirus (RV), and lentivirus (LV). These viral vectors are disabled genetically so that they do not cause disease in the infected target cell. AAV and LV are the more commonly used. AAV is a member of the parvovirus family and is non-enveloped, replication-defective virus having a size of 18 to 26 nm. LV is a member of Retroviridae family and tends to be larger in size (80–120 nm) which can infect nondividing cells. AAV has a smaller size than LV, so less genetic material can be packaged into AAV. AAV can incorporate up to 4.7 kb of genetic material, while LV can accommodate up to 10 kb. However, the smaller size of AAV makes it a more adaptable option for delivery to the outer retina and photoreceptors. Further AAV does not integrate its genome into the host cell genome. Transgenic material exists as an episome in the case of AAV, while LV vectors integrate genetic material into host chromosomes which may cause mutagenesis. Table 16.1 lists clinical trials that are in progress or have been completed. Viral vectors seem to be the most used gene delivery systems for ocular diseases as shown by these clinical trials.

Table 16.1 Clinical trials of gene therapy for ocular diseases using naked plasmid or viral vectors

Indication	Phase	Gene	Vector	Administration	Trial	Date
AMD	I	PDGF	Adenovirus	Intraocular	USA	2001
MD	II	CNTF	Naked/plasmid DNA	Intraocular	USA	2005
Choroideremia	I	REP1	AAV	Subretinal	Canada	2014
LCA	I	hRPE65	AAV	Subretinal	Israel	2009
Retinitis pigmentosa	I	CNTF	Naked/plasmid DNA	Intraocular	USA	2003
RPE65 mutations	I	RPE65	AAV	Subretinal	USA	2004

Abbreviations: AAV adeno-associated virus, *AMD* age-related macular degeneration, *MD* macular degeneration, *REP1* Rab escort protein 1, *LCA* Lebers congenital amaurosis, *PDGF* platelet-derived growth factor, *CNTF* ciliary neurotrophic factor

Improvement of Cellular and Nuclear Uptake of Non-viral Vectors [11]

Well-organized uptake of non-viral vectors in the target cell is imperative for the success of the gene therapy. The uptake of DNA plasmids is a Herculean task because, when they reach the cell membrane, there are several barriers the particle has to overcome to reach the nucleus and attain expression. The overall effectiveness of a gene delivery system is dependent on three key factors: (1) cellular uptake of NPs, (2) escape of NPs from endosomal vesicles into the cytosol, and (3) transfer of the plasmid DNA to the nucleus (Fig. 16.2).

Cellular Uptake

The plasma membrane is the first barrier that is to be overcome to get into the cell. RPE cells have a high rate of phagocytosis. Particles in their environment are therefore readily taken up. Photoreceptors do not exhibit phagocytosis and so are difficult to transfect. Non-phagocytic cells, however, require a number of proteins to maintain their function, and these pathways can be exploited for the delivery of transgene.

Clathrin-Coated Pits

Clathrin-coated pits are formed on the surface of plasma membrane when a ligand binds to its specific receptor forming a complex. This complex is internalized by endocytosis where it fuses with cytoplasmic organelles for further processing. Small receptor ligands like transferin and hyaluronic acid have been used to increase the uptake of nanoparticles. Hyaluronic acid targets the CD44 receptor that is overexpressed in RPE cell layer and retinal glia cells. When uptake occurs using CD44 pathway, it decreases the intracellular degradation of nanoparticle as well.

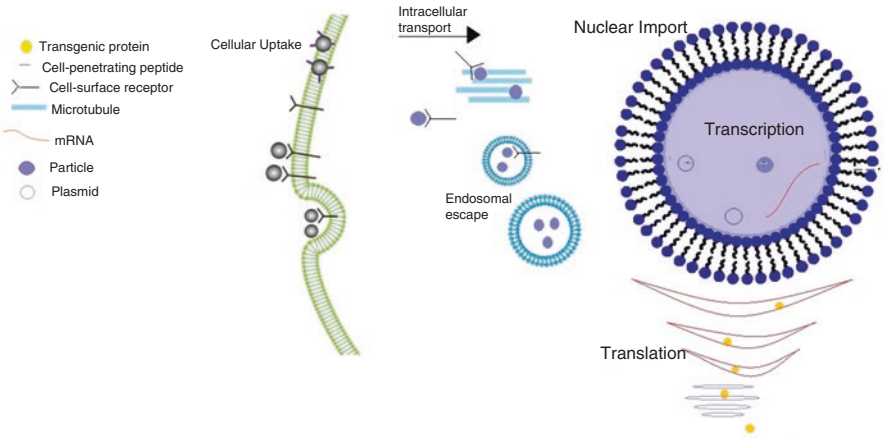

Fig. 16.2 Expression from non-viral gene vectors

Cell-Penetrating Peptides (CPPs)

Proteins such as Tat (human immunodeficiency virus) and VP22 (herpes simplex virus) can pass through intact biological membranes and are called cell-penetrating peptides. These CPPs can be coated on the surface of nanoparticles to affect their internalization. Once inside the cell, the delivery system has to survive the endosomal uptake so that the plasmid remains intact and enters the nucleus. The mechanism of survival depends on the type of non-viral vector and will be discussed at relevant place.

For the transgenic DNA to be transcribed, it is imperative that it enters the nucleus. It is easy for a plasmid DNA to enter into the nucleus when the cell is in a state of active division as nuclear envelope splits down during mitosis. But for cells like photoreceptors, postmitotic cells, the entry into the nucleus is possible only through the nuclear pores of 9 nm in diameter, and entry of molecules with molecular weight greater than 50 kDa is thus restricted. Nuclear localization sequence from simian virus 40 (SV40), a single seven-amino stretch (PKKKKRKV) [141], has been found to increase the transfection efficiency. These sequences combine with different proteins like nuclear pore transport proteins in the cell and lead to the active translocation of proteins into the nucleus. These NLS sequences can be attached to the surface of non-viral vectors to facilitate their nuclear entry.

Vector Engineering

Bacterial plasmid DNA has been the building block for non-viral gene therapy. Although plasmids have been very useful for non-viral gene transfer (especially when coupled with efficient compaction), there exist problems like gene silencing

and transient expression because of the episomal nature of the plasmid and the bacterial elements required for propagation. Recent work has therefore focused on incorporating additional DNA elements. Enchancers, insulators, S/MARs, inverted terminal repeats (ITRs), and CpG depletion are some of the various techniques used to engineer vectors to increase the transgene expression and its longevity. Such complex vectors containing additional DNA elements require delivery tools that can compact them. Luckily, the availability of non-viral packaging methods which can deliver larger DNA payloads has made this possible.

Cationic Lipid/DNA Complexes (or Lipoplexes) [10]

Plasmid DNA can be coated with lipids in an organized structure of a liposome. When the organized structure is complexed with DNA, it is termed as *lipoplex*. The liposomes contain two components: a cationic lipid and a neutral lipid, also called helper lipid. Cationic lipids are amphiphilic molecules that contain a positively charged polar head group that is linked via an anchor to a hydrophobic domain comprising of two alkyl chains. The length and degree of nonsaturation of the alkyl chains contribute to the structural variations in the hydrophobic domain of cationic lipids. Amine group with different degrees of substitution, amidine, guanidium, and pyridinium form the positive charge of the cationic lipids. The size and charge of the cationic head group plays a more decisive role in transfection than that of alkyl chains. Synthetic lipids that are commercially available include DOTAP and DC-Chol with monovalent head groups and DOSPA with a multivalent head group. Lipopolyamines not only bind to DNA but also compact it. DOPE and cholesterol are used as neutral lipids.

Lipoplexes may be prepared by methods that are used of liposomes like hydration of a lipid film, dehydration-rehydration, ethanolic injection, reverse-phase evaporation, or the detergent analysis technique. The cationic liposomes are then complexed with DNA, forming lipoplex particles by spontaneous self-assembly. The concentration, temperature, environment, and kinetics of mixing are important for transfection efficiency and should be considered when forming lipoplexes. Lipoplexes with a net positive charge interact more efficiently with the negatively charged cell surface and therefore have higher transfection efficiency. At high positive- or negative-charge ratios, homogeneous, relatively small aggregates of lipoplexes are formed which may also contribute to better transfection efficiency.

After endocytosis by cells, DNA needs to be released from lipoplexes. Spermidine3+ and spermidine4+, the biogenic polycations, are known to be present in the cytosol during the cell cycle. These cations remove DNA from the lipoplexes to form hexagonally packed DNA-polyamine particles in cytosol. The positively charged lipids confer protection to the DNA against degradation by cellular nucleases, and therefore lipoplexes show better transfection.

Polymeric Nanoparticles: Polyplexes

Cationic polymers can form particulate complex with DNA, and the complexes so formed are smaller than that of cationic liposomes. Small particle size favors the transfection. The most potent polyplex formulations have accomplished efficiencies of viral vectors, although more number of particles per cell are required for successful transfection.

Cationic polymers include natural DNA-binding proteins like histones, synthetic polypeptides, cationic dendrimers, or carbohydrate-based polymers. Since most of these are synthetic in nature, the molecular weight can be tailored, and ligands can be attached to their surface. Poly(l-lysine) (PLL) and PEI are among the most widely studied polymers for gene delivery.

Cationic polymers do not contain a hydrophobic domain and so cannot destabilize the endosome by direct interaction with the endosomal membrane as cationic lipids. Actually the first-generation cationic polymers, such as polylysine or polyarginine, were quite ineffective in terms of endosomal escape and transfection abilities. Second-generation cationic polymers, such as polyethylenimine and polyamidoamine dendrimers (PAMAM), act as proton sponge and so can mediate endosome disruption.

Pollard et al. [7] projected the "proton sponge" hypothesis to clarify the high transfection efficiency of PEI-based polyplexes. The hypothesis suggests that at physiological pH only 1–6 nitrogen atoms of PEI-based polyplexes gets protonated. Upon lowering the pH, e.g., in endosomes, the proportion of protonated nitrogen increases and generates a charge gradient that causes a Cl^- influx. The increase in Cl^- concentration aggravates the water influx and, ultimately, osmotic swelling and rupture of endosome.

PEI-based gene delivery has been studied in the eye. Intravitreal delivery was not very successful; however, results from topical treatment (for some corneal conditions) have been more positive. Its capability to transfect RPE cells has been mostly tested in vitro, and the capacity of these polymers to generate panretinal transfection and treat RPE disease models has not been established.

Chitosan particles have been studied for RPE transfection in vitro, and experiments have demonstrated efficient transfection, biocompatibility, and cell viability after treatment signifying positive outcomes in future in vivo studies if toxicity concerns can be answered.

Positively charged polylysine peptides (CK30) have been used to compact negatively charged DNA or RNA into nanoparticles, but these tend to aggregate and become unstable. However, conjugation of the CK30 to PEG (CK30PEG) has proved to facilitate improved gene expression. These particles have a diameter of <25 nm with the smallest ones having diameters in the range of 8–11 nm. Particle size and homogeneity are important advantages of the CK30PEG compacted DNA nanoparticles as compared to those of PEI and chitosan polyplexes. As opposed to AAVs, CK30PEG nanoparticles have a huge capacity, tested up to

20 kbp in the lung and 14 kbp in the eye and so can deliver large genes. These nanoparticles have been shown to bind to lipid raft-associated cell surface nucleolins, although it is not clear if the same pathway is responsible for RPE uptake. The nucleolin-NP complex is internalized by the nucleus, although the small size of these nanoparticles may allow transport through nuclear pores. Transfecting of both mitotic and postmitotic cells thus becomes possible, a benefit for RPE therapy. Additionally these nanoparticles have been shown to be nontoxic and non-immunogenic.

CK30PEG nanoparticles have been used in vivo to drive gene expression and mediate therapeutic rescue in the lung epithelium and nasal mucosa, brain, retina, and retinal pigment epithelium. Retinal expression was several folds higher than that achieved with naked DNA and on the same scale as that observed with AAVs. In adult mice at 2 days posttreatment, authors demonstrated that a single subretinal injection of CK30PEG nanoparticles yielded reporter gene expression in 55% percent of RPE cells. Further this expression and therapeutic rescue was sustained in the RPE for 2 years or more when the reporter gene plasmid contained a scaffold/matrix attachment region. Subretinal delivery of these particles was demonstrated as safe and nontoxic after delivery to both normal and diseased eyes. Combined, these features make CK30PEG nanoparticles exciting tools for RPE-based gene therapy.

Conclusion

The main driving force for translational research is the commercial ophthalmic market. The ultimate marker of success is whether the treatment makes it to the clinic and thereby becomes available to treat illness. There is a lot of growth potential in this largely untapped market; 55% of all debilitating ocular diseases are posterior segment diseases, yet only 5% of ophthalmic pharmaceutical sales in 2007 were for treating posterior segment diseases. The predictions of the future prevalence rates of posterior segment diseases issued in epidemiologic studies should be considered; increasing aging of populations throughout the world, paired with other factors, including an increase in the prevalence of age-related diseases and sedentary lifestyles, will ultimately lead to a far greater worldwide prevalence of many diverse ophthalmic diseases, in addition to those related to only aging and obesity [3]. Gene therapy holds lot of promise for the treatment of many genetic retinal diseases. This approach directly attacks the root of the disease, rather than treating symptoms, and is therefore supposedly the closest approach to a cure.

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Part V
Prodrug Strategies for Retina and Posterior Segment Disease

Chapter 17
Transporter-Targeted Prodrug Approach for Retina and Posterior Segment Disease

Andrea Burgess, Tanjm Azad, Nandish Pathak, Vipul Amin, and Sheeba Varghese Gupta

Abstract Prodrug strategy is used to increase the bioavailability of drugs either by increasing the passive absorption by improving physicochemical properties of the parent drug or making them substrates of active transporter. There have been numerous reports and clinical applications of various prodrugs for oral delivery. Transporter-mediated prodrug approach is still a new and not completely explored area in the case of ocular delivery. This chapter gives a brief snapshot of the transporter-mediated prodrug approach for ocular delivery of drugs. There is also brief account of other types of prodrugs used for improving ocular bioavailability.

Introduction

Topical administration is the most commonly used route of drug delivery to the eye, but it is plagued with disadvantages such as poor penetration and rapid loss of therapeutic agents [1]. Formulations such as solutions, ointments, gels, nanoparticle, dendrimers, and liposomes are used to improve the bioavailability after topical administration of therapeutic agents. In addition to these formulation approaches, chemical modification such as prodrugs has been utilized to improve physicochemical and pharmacokinetic properties of therapeutic agents for enhancing its ocular bioavailability [2].

Prodrugs have many uses in improving therapeutic actions of the drug. Prodrugs are therapeutically inactive and require activation in vivo either enzymatically or nonenzymatically to active drug. Prodrugs can be administered orally, topically, or parenterally. The main purpose of the prodrug strategy is to enhance the bioavailability of the therapeutic agent and/or improve pharmacokinetic properties [3].

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Most prodrugs are derivative of some sort so the parent drug must be susceptible to chemical derivatization with having specific functional groups [3]. In the case of ocular delivery, prodrugs are designed to target a specific enzyme for drug productivity. Intravitreal (IVT) injection is a highly efficient but equally invasive technique for drug delivery to the posterior segment. Delivery using prodrug strategy can offer a less invasive option to deliver drugs to the posterior segment.

Drug Delivery to the Retina and Posterior Segment

The human eye is composed of two parts, the anterior segment and the posterior segment. The anterior segment has the cornea, iris, lens, ciliary body, and sclera. The posterior segment extends to the back of the eye. It consists of retina and optic disk and is filled with vitreous. The complexity of the anatomy of the eye makes it challenging for drug delivery to the posterior segments [4, 5]. Topical methods of drug delivery are sometimes used for the eye. Although it is not invasive, it isn't efficient because there is low drug absorption to the eye. It doesn't penetrate deep enough like some of the other techniques.

Drug delivery to the posterior segment is usually done with intravitreal delivery. Depending on the condition and disease in the eye, different drug delivery techniques are used. Intravitreal injection to the posterior of the eye is the most common method of treatment for vitreoretinal diseases. This technique eliminates the problems of topical drug application because it goes directly into the eye and targets the right areas [5]. Although this method is very effective, there are drawbacks to using it such as retinal detachment and hemorrhages.

Transscleral diffusion is another method of drug delivery. This is an attractive method because it targets adjacent to the choroid and retinal pigment epithelium. This falls under the periocular drug delivery method. Unlike intravitreal injections, this method is far safer because it is less invasive [6]. This is a form of periocular drug delivery and it includes subconjunctival. The drug is injected outside the globe of the eye and the drug is diffused through the sclera of the eye [6]. Because of the high surface area of the sclera, permeability is high, and drugs can be passively diffused through the layers.

Types of Prodrugs and Their Examples for Delivery to the Retina and Posterior Segments

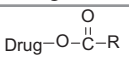
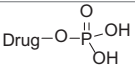
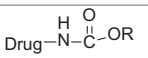
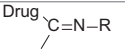
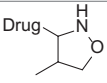
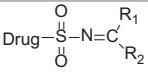
As stated earlier, prodrugs are created to overcome any physical or chemical complication with the parent drug. Prodrugs relating to the eye must have certain characteristics to make it effective, such as chemical stability and aqueous solubility. Prodrugs also need to have a long shelf life so it does not expire on the shelf of a store. There are two classes of prodrugs that are most frequently used. One of them

is carrier-linked prodrugs, which means that the active molecule is linked covalently to the carrier. In the body the prodrug goes through transformation which releases the parent drug into the system. The other class is bioprecursor prodrugs, which means that the prodrug is transformed chemically from hydration [7].

Many of the approaches include transporter-targeted prodrugs. This approach takes advantage of the nutrient transport systems which the prodrug attacks to penetrate into the cell [8]. There are a lot of nutrient carriers that are on the retinal endothelial cells, which can be targeted by prodrugs [8].

Common functional groups that are used for the ophthalmic prodrugs are usually carboxyl, hydroxyl, amine, and carbonyl group. These functional groups are modified to create ophthalmic prodrugs [2]. Prodrugs that contain esters in it are derived from carboxylic acids or alcohol. This is done because esters have been more lipophilic which favor drug permeability, unlike carboxylic acids and alcohol which can be easily ionized and are hydrophilic. Phosphate ester prodrugs are made specifically for poorly soluble drugs. This can include many topical drugs that need to be absorbed into the aqueous layer [2]. Drugs that lack functional groups such as hydroxyl, amine, or carboxyl use oxime prodrugs which are a derivate of ketones. It hydrolyzes in the body and releases the drug. Table 17.1 offers summary of commonly seen functional groups in ocular prodrugs.

Table 17.1 Common functional groups of ocular prodrugs [2]

Prodrug	Functional groups	Prodrug linkage	Drugs	Hydrolyzing enzymes	Effect(s) due to prodrug formation
Ester	Drug-OHdrug-COOH		Antimetabolites Prostaglandins	Esterases	Enhanced corneal permeation and IOP reduction. Chemical instability in aqueous solution
Phosphate ester	Drug-OH		Vidarabine Cannabinoids	Phosphatases	Enhanced aqueous solubility
Carbamate ester	Drug-NH_2		Timolol	Esterases	Prolonged IOP reduction and improved therapeutic index
Oxime			β-Adrenergic blockers	Oxime hydrolase Ketone reductase	Prolonged IOP reduction and improved therapeutic index
Oxazolidine			Phenylephrine		Enhanced corneal penetration, improved therapeutic index
N-sulfonyl imidates	Drug-SO_2NH_2		Carbonic anhydrase inhibitors		Enhanced aqueous solubility, slow hydrolytic rate

Prodrugs for the Retina and Posterior Segment

Prodrugs can be classified into two main classes based on the types of carrier molecules attached to the active drug: carrier-linked prodrugs [2] and bioprecursor prodrugs [9]. In carrier-linked prodrugs, the drug is linked to a carrier molecule (also known as a promoiety) that when upon entry to the body is bio-activated via hydrolytic cleavage of the carrier [10]. The ideal prodrug yields a high active drug recovery with its promoiety being nontoxic, stable in dosage form, and non-immunogenic. Modified functional groups such as esters, amines, carboxyl, hydroxyl, and oximes are frequently used in ophthalmic prodrug design as a means of reducing physiochemical barriers to the absorption and activation of the active drug [2].

Carrier-linked prodrugs can be further divided into subclasses that include bipartite, tripartite, polymeric, and mutual prodrugs. Bipartite prodrugs contain carrier molecules that are directly linked to the active drug, in contrast to the carrier molecules of tripartite prodrugs that are indirectly linked to the active drug via a connector [11]. Majority of prodrugs are bipartite by default, but in certain cases prodrugs are made tripartite if the carrier-active drug bond affects the transport of the drug.

One of the more popular routes of drug delivery for the treatment of posterior segment diseases involves an intravitreal injection or formulation insert into the eye. The bipartite prodrug valganciclovir is used in the treatment of cytomegalovirus (CMV) retinitis in immunocompromised patients and is administered through slow-release insert injected into the vitreous humor of the eye [12]. Valganciclovir is hydrolyzed by esterase to generate the active drug ganciclovir in vivo by esterases (Fig. 17.1).

Polymeric prodrugs utilize polymers as the carrier molecule such as polyethylene glycol (PEG) and polylactide-*co*-glycolide (PLGA). An example of a polymeric prodrug is pegaptanib sodium, which consists of a conjunction of branched PEG and an anti-VEGF aptamer that is utilized in the treatment of neovascular age-related macular degeneration (AMD). Pegaptanib sodium is also administered with an intravitreal injection. The popularity of this method stems from its ability to transport the active drug into the posterior region of the eye by bypassing the barriers of the anterior eyes such as the cornea and sclera [13].

Another popular method to overcome the anatomical, physiological, and distal barriers employs a transporter-targeted prodrug approach. One advantage of this method is that the prodrug can be administered orally, intravenously, intravitreal,

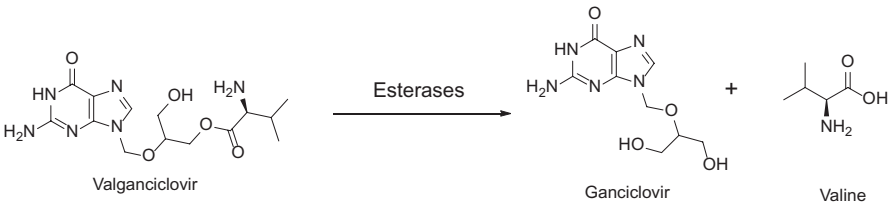


Fig. 17.1 Bioactivation of valganciclovir

etc. as a means of reaching the specific transporters of the eye in contrast to just intravitreal injections. This method ameliorates the absorption of the active drug by reducing the prodrug's affinity to efflux transporters (P-glycoprotein/multidrug resistance proteins) and increasing its affinity to the influx transporters (GLUT1, ASCT1) [2]. The prodrug valacyclovir is administered and then is symmetrically converted into acyclovir through the targeting of amino acid and/or peptide transporters like LAT1, LAT2, and PepT1 of the body [14, 15]. The drug acyclovir is an antiviral applied in the treatment of the herpes simplex virus of the eye.

Transporter-Targeted Prodrug Approach

Active transporters in the body are instrumental in facilitating the transport of nutrients and other vital endogenous biomolecules; they are also known to transport exogenous molecules structurally similar to the natural substrates of the transporters [16]. Influx transporters can also be utilized to increase the ocular bioavailability of drugs. At the same time, the efflux transporters in the ocular region can decrease the bioavailability of the drugs. The prodrugs are designed in such a way that the prodrugs can have either low affinity toward efflux as in case of the quinidine prodrugs, where amino acid prodrugs of quinidine were shown to have decreased affinity for efflux transporter, P-glycoprotein [17]. Similarly, amino acid prodrugs of acyclovir (valacyclovir) [14] (see Fig. 17.2 for valacyclovir hydrolysis) and ganciclovir [18] have shown to have increased ocular bioavailability due to active transport by peptide transporter PepT1 [19].

In transporter-targeted prodrug approach a promoiety, drug targeting to specific receptor using carrier-mediated absorption is considered as an important and emerging approach for improving delivery for ocular drugs. Receptors relevant for ocular delivery are listed in Table 17.2. Endogenous receptors are responsible for internalizing nutrients, vitamins, and transferrin. The importance of the transporters in maintaining the normal function of the ocular makes them an attractive mode for the ocular delivery and targeting of the drugs. Folate receptor has been extensively studied for tumor-targeted drug delivery; however, their potential in ocular delivery still needs to be evaluated.

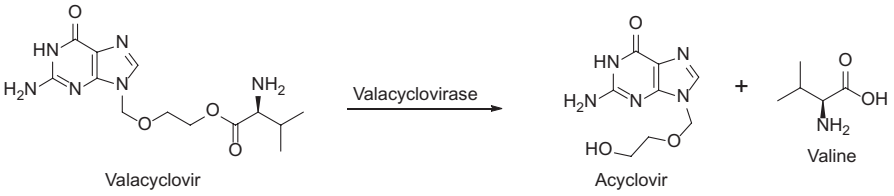


Fig. 17.2 Hydrolysis of valacyclovir

Table 17.2 Transporter and receptors in human ocular tissues [2]

Transporters	Substrates	Receptor location				
		Cornea	Conjunctiva	Retina	Retinal pigment epithelium (RPE)	Blood retinal barrier (BRB)
Uptake transporters						
GLUT1	Glucose	√	√	√	√	√
ENT1	Nucleoside			√	√	
MCTs	Monocarboxylate				√	
SVCT2	Vitamin C (ascorbic acid)				√	
SMVT	Biotin				√	
Riboflavin	Riboflavin (vitamin B2)			√	√	
LAT1, LAT2	Large neutral amino acids	√			√	
ASCT1	Neutral amino acids		√			
B (0, +)	Neutral and cationic amino acids	√	√			
Reduced-folate transporter (RFT)	Reduced folate				√	
Transferrin	Transferrin	√	√		√	√
Efflux transporters						
P-glycoprotein (MDR1)	Various drugs	√	√		√	
Multidrug resistance-associated proteins (MRPs)	Various drugs	√	√		√	
Breast cancer resistance protein (BCRP)	Various drugs	√			√	

Prodrugs Currently on the Market

In recent years, the amount of prodrugs available for purchase has steadily increased as new strategies for stabilizing and formulating new prodrugs arise. As an indication of that change, it was estimated that almost 15% of the 100 best-selling drugs of small molecular mass in 2009 [19] were prodrugs [10]. The annual sale of prodrugs can range from hundreds of millions of dollars (USD) and is employed to remedy a variety of eye diseases. This section will focus on some of the prodrugs presently being sold for consumer purchase and the ophthalmic conditions it treats with an emphasis being placed on retina and posterior segment disease (Table 17.3).

Table 17.3 Examples of ophthalmic prodrugs

Prodrug (active drug)	Disease state
Prednisone [12] (prednisolone)	Uveitis, conjunctivitis
Dipivefrin (epinephrine)	Open-angle glaucoma
[a]Pegaptanib sodium (pegaptanib sodium)	Neovascular age-related macular degeneration (AMD)
[a]Timolol (timolol)	Open-angle glaucoma
Fluocinolone acetonide (fluocinonide)	Uveitis
[a]Ranibizumab [10] (ranibizumab)	Neovascular AMD, diabetic retinopathy
OPPH 088 (cyclosporine A)	Dry eye syndrome
Valganciclovir (ganciclovir)	Cytomegalovirus (CMV) retinitis

[a]Selected drugs are considered bioprecursor prodrugs and are metabolized in vivo without a carrier molecule.

Future Directions

The next step in the prodrug design would be to address the challenges of the transporter-targeted approach for ophthalmic diseases, especially for retina and posterior segment disease. The discovery of more transporters of interest, development of stronger prodrugs, and locating alternative routes to the posterior segment of the eye can assist in the creation of new drug delivery systems. In the case of intravitreous injections, the frequency of drug administration can lead to ophthalmic complications for patients [12], so the generation of stronger concentrated prodrugs or slow-release prodrugs might reduce amounts of injections and reduce likelihood of secondary complications. In conclusion, there have been advancements in ophthalmic drug delivery, but the unique barriers to the posterior regions of the eye still impact disease treatment.

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Chapter 18
Lipid Prodrug Approach for Retina and Posterior Segment Disease

Dasharath M. Patel and Jayvadan K. Patel

Abstract An inch or less. That is the short distance an ocular drug must travel to reach a target site at the back of the eye. Research has traveled a long way over the past four decades in efforts to make this inch-long journey more successful. Delivering ocular drugs to their target tissues often requires them to traverse the fat–water–fat structure of the corneal barrier while ensuring minimum wastage through tear washout and systemic absorption. This is why delivering drugs effectively to the posterior of the eye is a challenge that many companies have worked to overcome. Treatment of diseases of the retina and posterior segment of the eye, such as age-related macular degeneration, cytomegalovirus retinitis, diabetic retinopathy, posterior uveitis, and retinitis pigmentosa, requires novel drug delivery systems for efficacious delivery of therapeutic drugs. This challenge has prompted the development of biodegradable and nonbiodegradable sustained release systems for injection or transplantation into the vitreous as well as drug-loaded nanoparticles, microspheres, and liposomes. These drug delivery systems utilize topical, systemic, subconjunctival, intravitreal, transscleral, and iontophoretic routes of administration. The focus of research has been the development of methods that will increase the efficacy of spatiotemporal drug application, resulting in more successful therapy for patients with posterior segment diseases. This chapter summarizes recent advances in the research and development of drug delivery methods of the posterior chamber of the eye, with special emphasis on the use of lipid prodrug approach.

Keywords Lipid prodrug approach · Retina · Posterior segment disease

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Introduction

Drug delivery, in its simplest, most common form, is comprised of fast-acting chemical compounds that are dispensed either topically or systemically. Depending on the method of application, the drugs are distributed locally, regionally, or systemically and may lead to various undesired side effects, such as drug accumulation and toxicity. While the topical and systemic forms of drug delivery are useful in certain disease processes, they pose many limitations to the treatment of diseases of the posterior eye, including age-related macular degeneration (AMD), diabetic macular edema (DME), endophthalmitis, and retinitis pigmentosa. Huge literature is published in the last two decades focusing on ocular drug delivery techniques most suitable for drug delivery to the retina and posterior segment of the eye [1–31]. This chapter emphasizes on lipid prodrug approach.

Lipid Prodrugs

Molecules can cross cell membranes through passive diffusion. In the eye, drug absorption takes place either through corneal route (cornea–aqueous humor–intraocular tissues) or noncorneal route (conjunctiva–sclera–choroid/RPE) [32]. Due to lipophilic nature of the cornea and other intraocular tissues, both hydrophilic and hydrophobic drugs take transcellular pathway to cross the ocular membrane. In addition to these, sustained drug delivery for prolonged periods of time at the target site is required especially for the treatment of eye diseases at the posterior segment of the eye like the vitreous, retina, and choroid. In order to improve lipophilicity of hydrophilic drug molecules and hence to improve corneal permeation, the lipid prodrug approach has been developed.

Lipid prodrugs are chemical entities where a drug molecule is covalently bound to a lipid moiety, such as fatty acid, diglyceride, or phosphoglyceride. Lipid prodrugs diffuse readily across the cell membrane by facilitated diffusion thereby resulting in improved cellular absorption. It also shows sustained delivery of parent drug molecule at the site of action [33, 34]. Lipid molecules are a vital component of the cellular makeup of living cells. Conjugation of a drug molecule with a lipid graft would change the pharmacokinetic/pharmacodynamics [PK-PD] profile [35]. Manipulation of factors, such as the lipid chain length, the configuration of the double bonds, and the nature of the lipid–drug linkage, would aid in the design of a variety of lipid prodrug systems [35, 36].

However, high lipophilicity of molecules can result in limited permeability as it will stick inside the lipid membrane of the cornea. Chein and Schoenwald reported a parabolic relationship between the lipophilicity and permeability of drug molecules across the rabbit cornea. Maximal permeability is observed for prodrugs with log P value of about 2–4 where P is defined as octanol/pH 7.4 buffer partition coefficients [37]. So depending on the hydrophilicity of each drug molecule, lipid chain

length needs to be adjusted in order to get maximum permeability across the cornea. Intraocular permeation can be further enhanced by conjugating a targeting moiety (receptor/transporter) at one end of lipid prodrug, which is being currently explored.

Lipid–Conjugated Transporter–Targeted Prodrug Approach

Gokulgandhi et al. [38] worked on a transporter-targeted lipid prodrug approach. Cidofovir is used in the treatment of cytomegalovirus (CMV) retinitis. The molecule, however, has low oral bioavailability. Previously, researchers had synthesized lipid conjugates of cyclic cidofovir (CDV) to improve its oral bioavailability [39, 40]. Cheng et al. [41] studied the intravitreal pharmacokinetics of crystalline octadecyloxyethylcyclic-CDV (ODE-cCDV) in rabbit eyes. An intravitreal injection produced 2–3 weeks of constant drug levels in the retina–choroid. The crystalline drug ODE-cCDV forms a depot in the vitreous, slowly releasing the drug into the vitreous humor for a prolonged period of time. This free ODE-cCDV is then metabolized in the retinal membrane by the phosphatases releasing CDV [41]. The prodrug depot in the vitreous humor resulted in toxicity and the formation of retinal floaters which could affect patient compliance [42]. Ma et al. [43] used lipid-derivatized CDV, hexadecyloxypropyl-cidofovir (HDP-CDV), to form micelles in water without the addition of any other therapeutic components. HDP-CDV demonstrated much superior protection and treatment efficacy compared to CDV in an equimolar dose in a herpes simplex virus-1 (HSV-1) retinitis model in rabbits. HDP-CDV showed a vitreous half-life of approximately 6 days, which would provide approximately 40 days of vitreous residence if six half-lives would clear 95% of the drug from the vitreous humor. A long vitreous half-life can act as a promising marker for prolonged therapeutic effect, as demonstrated by a 9-week prophylaxis success [43]. Ganciclovir is an acyclic 2α-deoxyguanosine analog used by immunocompromised patients in the treatment of human CMV [44]. Janoria et al. [45] studied the effect of a sodium-dependent multivitamin transporter (SMVT) on biotin-conjugated ganciclovir uptake in a human retinal pigmented epithelium cell line as well as in rabbit retina. They observed that SMVT recognized the biotinylated ganciclovir and also that the prodrug had a better pharmacokinetic profile in comparison with ganciclovir [45]. Cholkar et al. [44] synthesized long-chain acyl ester lipid-conjugated prodrugs of ganciclovir based on the hypothesis that conjugation of long carbon chain esters would provide sustained ganciclovir levels due to the slow hydrolysis of its ester linkages. The in vitro toxicity of ganciclovir-conjugated long-chain lipids was studied in the human retinal pigmented epithelium cell line (ARPE-19), showing that the prodrugs were nontoxic and well tolerated [44]. Gokulgandhi et al. [38] hypothesized a synergistic strategy to improve the permeability of cyclic CDV by attaching a lipid raft to the biotinylated drug. The lipid raft would enhance the prodrug–membrane interaction, whereas the vitamin conjugation would improve SMVT targeting. The hypothesis was that the enhanced lipophilicity of the prodrug system could improve melanin binding in the retina and

aid in the formation of a drug depot for prolonged activity. An in vitro uptake study was conducted to trace the affinity of the transporter (SMVT) for the biotinylated lipid prodrug. The results indicated that prodrugs showed strong interaction with the SMVT and that the elongation of the lipid chain increased interaction with the cell membrane (B-C12–cCDV > B-C6–cCDV > B-C2–cCDV). The authors suggest that cellular uptake of both the biotinylated and nonbiotinylated lipid prodrug was similar, where the lipid raft enhanced prodrug–membrane protein interaction which resulted in the docking of biotin into the SMVT [38]. As with ganciclovir, both the lipid prodrug approach and the transporter-targeted approach have been studied for acyclovir. Vadlapudi et al. [46] worked on the biotinylated lipid–drug conjugation strategy for acyclovir (Fig. 18.1) and synthesized biotin–ricinoleic acid–acyclovir (BRACV) and biotin–12-hydroxystearic acid–acyclovir (B-12HS-ACV). Biotinylated lipid prodrugs showed an enhanced in vitro cellular uptake in human corneal epithelial cells (HCECs) in comparison with just the biotinylated prodrugs of acyclovir, which enhanced lipophilicity and SMVT targeting [46]. Lipid rafting also improved the stability of these prodrugs in the cornea, iris–ciliary bodies, and lens, probably due to slower enzymatic hydrolysis [47].

Cytarabine Crystalline Lipid Prodrug

Cytarabine is a chemotherapeutic agent that inhibits cellular proliferation by inhibiting DNA synthesis. It demonstrates better in vitro antiproliferative efficacy in retinal pigment epithelium than 5-fluorouracil (5-FU) [48]. Cytarabine has an intravitreal half-life of approximately 12 h, whereas cytarabine encapsulated in a

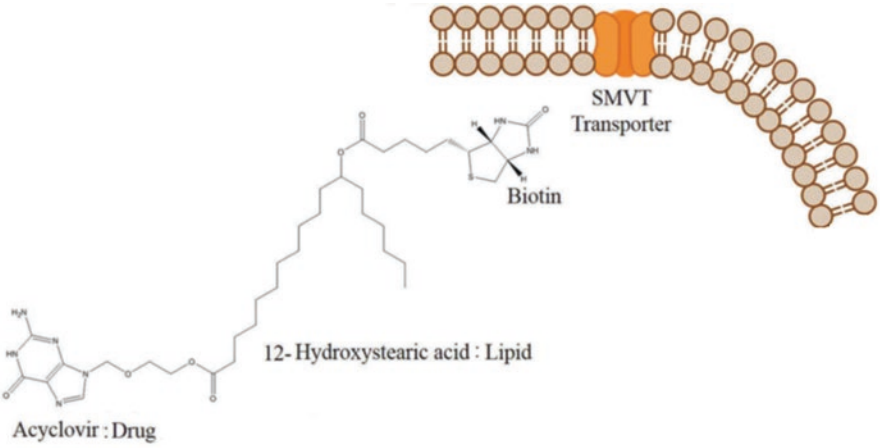

Fig. 18.1 Biotinylated lipid prodrugs: biotin–12-hydroxystearic acid–acyclovir. The drug, acyclovir, is conjugated to the biotinylated lipid, 12-hydroxystearic acid. The lipid enhanced prodrug–membrane protein interaction, and the biotin conjugation improved the SMVT targeting

liposomal formulation has a half-life of a few days [49]. Kim et al. [50] synthesized two prodrugs of cytarabine, HDP-P-Ara-C (hexadecyloxypropyl cytarabine 5′-monophosphate) and HDP-cP-Ara-C (hexadecyloxypropyl cytarabine 3′,5′-cyclic monophosphate), which, following an intravitreal injection, release small amounts of drug over a prolonged period of time. In vitro simulation studies revealed that the noncyclic prodrug formed micelles and was rapidly cleared (by day 6), whereas the cyclic analog HDP-cP-Ara-C did not form micelles and showed peak release at around day 10, with the compound still detectable at the end of the experiment (day 36). In vitro cytotoxicity studies determined that both compounds possess more potent antiproliferative activity than unmodified cytarabine [50].

Fluorouracil Lipid Prodrugs

5-Fluorouracil (5-FU) is an antimetabolite which has failed to exhibit significant benefit to intraocular cell proliferation, resulting from many vision-threating vitreoretinal diseases as it has short vitreous half-life after perioperative infusion [51]. Therefore, 5-FU implant is required in proliferative vitreoretinopathy [52]. Recently, Cheng et al. has reported two lipid derivatives of 5-fluorouracil nucleoside analog, 2α-deoxy-5-fluorouridine (5-F-2dUrd) in order to achieve sustained intravitreal drug release, thereby achieving drug delivery by simple intravitreal injection (Fig. 18.2). An alkoxyalkyl phospholipid residue is covalently anchored to 5-F-2dUrd to obtain hexadecyloxypropyl 5-fluoro-2α-deoxyuridine5α-monophosphate (HDP-P-5F-2dUrd) and hexadecyloxypropyl 5-fluoro-2α-deoxyuridine 3α, 5α-cyclic monophosphate (HDP-cP-5-F-2dUrd). Compared to 5-FU, both lipid prodrugs exhibit longer vitreous half-life with higher nontoxic dose. In addition, the potency of these prodrugs against cell proliferation jumped 11.6 times and 3.5 times for HDP-P-5F-2dUrd and HDP-cP-5-F-2dUrd, respectively [53].

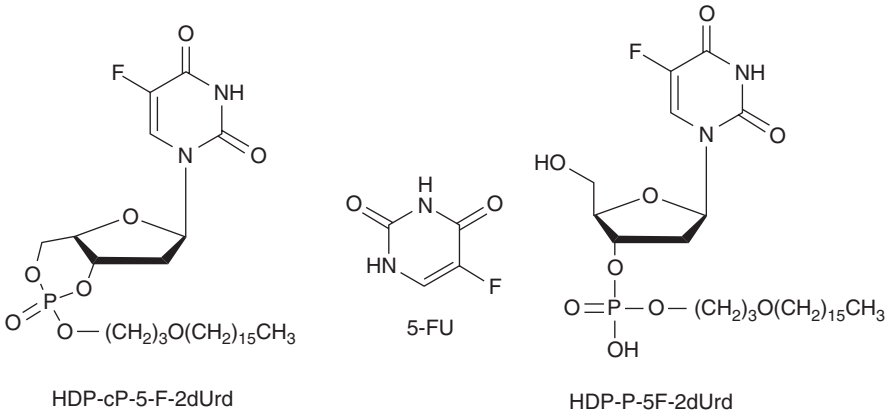

Fig. 18.2 Chemical structure of lipid prodrugs of 5-fluorouracil

Conjugated lipid chain with 5-F-2dUrd enhances cellular uptake of lipid prodrugs by the inner limiting membrane. Inside the cells, this lipophilic nucleotide converts back to the corresponding nucleoside triphosphate which exhibits antiproliferative activity.

Biotinylated Lipid Prodrug of Acyclovir

Recently, Vadlapudi and co-workers have developed a novel targeted lipid prodrug strategy to improve cellular absorption of acyclovir (ACV) for the treatment of herpetic keratitis [54]. They have used approach that combines both the lipid and transporter-targeted delivery to generate synergistic effect. Targeted lipid prodrugs of ACV exhibited higher affinity for sodium-dependent multivitamin transporter (SMVT). Biotinylated lipid prodrugs of ACV demonstrated synergistic improvement in cellular uptake presumably due to recognition of the prodrugs by SMVT on the cornea and lipid-mediated transcellular diffusion. Biotin–12-hydroxystearic acid–acyclovir (B-12HS-ACV) (Fig. 18.1) was selected as a model prodrug because of its enhanced binding affinity toward SMVT, higher cellular uptake, lack of cytotoxicity, and excellent in vitro antiviral activity against HSV-1 and HSV-2. It was evident by the EC50 values that B-12HS-ACV displayed 34-fold and 60-fold increase in antiviral efficacy against HSV-1 and HSV-2, respectively.

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Chapter 19
Injectable Pro-drugs Approach for Retina and Posterior Segment Disease

Anita Patel, Jayvadan K. Patel, and Yashwant V. Pathak

Abstract Drug delivery to the posterior segment of the eye is an area of intense research and massive prospective. Because of the anatomical and protecting structure of the eye, drug delivery to the interior parts of the eye still remains bothersome. Regardless of the emergence of effectual drugs to treat various retinal diseases, doctors still wrestle with how best to administer these sight-saving drugs. An ideal route of administration would deliver therapeutic levels of drug to targeted parts in a remarkably protected way at the same time as providing negligible interruption to the patient's quality of life. Promising innovative ocular drug delivery such as an injectable pro-drug strategy has been, and is being, employed for this purpose. This novel pro-drug approach offers manifold benefits over the parent compound as they enhance the membrane permeability, site specificity, transporter targeting ability, and aqueous solubility. In this chapter, we have discussed a range of pro-drug strategies, for instance, functional group approach, polymer and lipid conjugation with the drug moiety to impart lipophilicity or hydrophilicity or else to target nutrient transporters by conjugation with transporter-specific moieties, which have been extensively functional for improving drug penetration into the ocular tissues, in addition to overall ocular bioavailability, with minimal disruption of the ocular diffusion barriers. We have also discussed an update on the use of injectable pro-drug concept in ocular drug delivery and highlighted continuing academic and industrial research and progress in terms of ocular pro-drug design as well as delivery.

Keywords Injectable pro-drugs · Increased lipophilicity · Enhanced permeability · Transporter targeting · Posterior segment disease

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The Need of New Drug Delivery Systems to Target Posterior Segment

For pharmaceutical scientists, the inimitable anatomy and physiology of the eye make ocular drug delivery a major challenge. To design an effectual treatment predominantly for the posterior segment diseases has been considered as a redoubtable task. More important, drug delivery to the posterior segment of the eye poses even stiff challenges, including the site of origin and development of different sight-threatening diseases, for example, age-related macular degeneration, cytomegalovirus retinitis, diabetic macular edema, diabetic retinopathy, and proliferative vitreoretinopathy, form most of the cases of irretrievable loss of sight. Treatment of posterior segment diseases still remains a herculean task for the formulation scientists. Tight junctions of blood-retinal barrier (BRB) limit the entry of drugs administered systemically into the retina [48]. Latest treatments are being investigated and built up. At present, the therapy of posterior segment diseases is restricted by the complexity in delivering effectual drug concentrations to the target tissues to the posterior choroids, retina, or vitreous. In the treatment of posterior segment diseases, elevated vitreal drug levels are requisite, which can be made feasible merely with the local administration by intravitreal injections/implants or periocular injections.

Posterior segment diseases necessitate site-specific drug delivery systems which specifically target the choroid, retinal pigment epithelium (RPE), and vitreous cavity [48]. Conventional topical route of administration for ocular drug delivery does not maintain therapeutic drug concentrations in the posterior tissues for a long period due to different anatomical and physiological barriers. After administration of topical eye drops, pre-corneal factors, for example, blinking, solution drainage, tear turnover rate, and nonproductive absorption by nearby tissues like conjunctiva and lachrymal gland, considerably decrease pre-corneal drug level. For this reason, for retinal drug delivery, topical drug instillation is often insufficient, notwithstanding high patient acceptance as well as compliance. Therapeutic agents, when administered systemically, it shows restricted absorption into the retina and vitreous. In some cases, systemic administration works, although the higher doses required for therapeutic effect are often related with significant side effects [40].

Intravitreal and periocular injections are other delivery routes to the posterior segment tissue. Intravitreal administration is required in serious disease conditions, which involve direct injection into the vitreous humor, escaping all the barriers. Periocular injections refer to introducing the drug in the surrounding region of the eye. Intravitreal injections and sustained-release vitreal implants being incredibly intrusive are used for posterior segment diseases [41, 105].

Albeit intravitreal, subconjunctival, and intravenous injections can attain elevated drug levels in the neural retina, possible risks, for example, cataract, retinal detachment, or endophthalmitis from repetitive injections, vitreous hemorrhage, and other retinal toxicities because of elevated levels upon bolus dose administration, may possibly give rise to poor patient acceptance and compliance [30, 85,

105]. As a result, the therapy of posterior segment diseases through conventional drugs is not so resourceful [45, 85, 105].

The present confront facing drug delivery scientists and ophthalmic pharmacologists are to deliver medicinal agents to target tissues by efficiently circumventing the inimitable anatomical barriers of the eye, without causing any patient discomfort or disruption in defensive physiologic mechanisms. In this regard, targeting transporters on ocular barriers using an injectable pro-drug strategy offers much promise. A pro-drug approach includes modification of the active moiety into a range of derivatives in a manner that imparts several advantages, for example, membrane permeability, site specificity, transporter targeting, and better aqueous solubility, over the parent drug molecule. The applicability of pro-drug approach offers an alternative for improving drug penetration into the ocular tissues and overall ocular bioavailability, with minimal disturbance of the ocular diffusion barriers [97]. Pro-drugs improve drug permeability by altering the hydrophilicity or lipophilicity of the drug. The process comprises modification of chemical structure of the drug molecule, consequently making it selective, site-specific, and a safe and sound ocular drug delivery system. A number of the pro-drug approaches involve polymer and lipid conjugation with the parent drug molecule to impart hydrophilicity or lipophilicity, otherwise to target nutrient transporters by conjugation with transporter-specific molecules.

Essential Considerations for Ocular Pro-drug Design

The pro-drug theory has turned out to be an imperative part of the ocular drug design and delivery. Designing pro-drugs which achieve most if not all necessities of an ideal formulation is very demanding. Essential considerations while synthesizing ophthalmic pro-drugs are [13, 64, 68, 99]:

- Parent drug molecule must have functional group liable to chemical derivatization.
- At the functional group site, chemical modification of parent drug must be reversible in nature.
- Parent drug, pro-drug, and the pro-moiety attached to parent molecule must be harmless. Pro-moiety should exert rapid elimination from the body. In general amino acid, small peptide or vitamins, has been utilized as a pro-moiety which is very safe and sound and with no trouble eliminating natural body substrates.
- In vivo pro-drug bioreversion must be governed by functionally active biological enzymes like esterase and peptidase, and the bioreversion rate should be optimized to circumvent pro-moiety detachment and parent drug release at nontarget site.
- The final pro-drug formulation must acquire satisfactory shelf life as well as stability.

- Most ocular formulations are delivered in the form of liquid, and so, aqueous solubility of pro-drug is a vital factor to think about when parent drug is lipophilic and owns low water solubility.
- Pro-drug must also have optimal lipophilicity so as to achieve higher diffusion across lipophilic ocular barriers.
- The final pro-drug formulation must have the ability to avoid inauspicious physicochemical and biopharmaceutical properties of a parent drug molecule. As well to resolve formulation problems related with drug, pro-drug should also show higher affinity with site-specific delivery of parent drug molecule. These characteristics will not only conquer side effects connected with parent drug molecules, but it will also assist minimizing dose of final formulation.

Injectable Pro–drug Approach: The Concept

A pro-drug is defined as conversion of an active drug into an inactive species by chemical modification of the which, when administered, will release an active drug effectively in a single step (i.e., enzymatic conversion). Pro-drug strategy is one of the most hopeful and effectual strategies at present being investigated for ocular drug delivery. Exploring the innate drug metabolism capacity of ocular tissues is one of the most relevant features of pro-drug design. A pro-drug derivative can increase the bioavailability of an active drug. Generally, majority of ophthalmic pro-drugs are lipophilic esters or diesters with improved permeability than the parent drug. Certainly, the pro-drug uptake by and the diffusion across the lipophilic membranes (which act as a barrier to the hydrophilic drugs) are facilitated by increased lipophilicity. Mandel et al. [75] were the first to officially bring in the idea of pro-drugs in the late 1970s with the testing of a pro-drug of epinephrine – dipivefrin – for enhancement of epinephrine corneal penetration [75].

In this approach, modification of drug molecule is done chemically by attaching it to pro-moiety to get better physicochemical characteristics, such that high drug absorption into tissue can be obtained. A pro-drug is designed to be therapeutically inactive species until in vivo activation to produce to parent drug. The compounds are synthesized by connecting a suitable chemical moiety to the parent drug, generally linked by an amide or an ester bond. The pro-drug will be subjected to enzymatic hydrolysis (bioreversion by esterases/peptidases) to free the active parent drug molecule upon absorption into the tissue (Fig. 19.1).

A variety of factors influence the rate of bioreversion, counting affinity of the pro-drug association toward hydrolyzing enzyme(s), the capability as well as turnover rate of the enzyme, etc. Far and wide all biological fluids and tissues are containing the enzymes responsible for hydrolysis of pro-drug. Such as, esterases are articulated all over the body and can be used in the hydrolysis of an ester functional group. The esterase activity in ocular tissues has been found to be the maximum in iris-ciliary body after that cornea and aqueous humor. Ester linkages containing drugs and pro-drugs can undergo changeable extents of esterase-mediated hydrolysis as permeating the cornea/conjunctiva and upon entering aqueous humor, ciliary body, and iris.

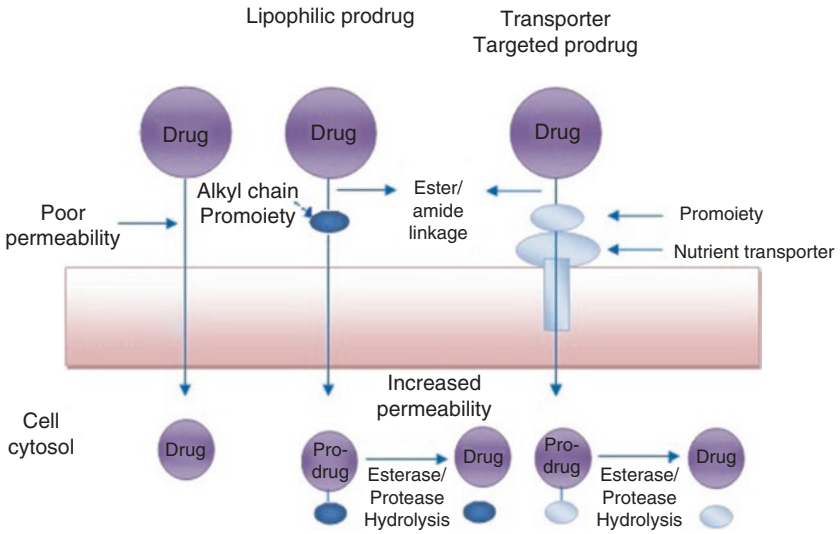

Fig. 19.1 Schematic: Lipophilic and transporter-targeted pro-drug design. (Modified from Talluri et al. [94])

Proteases or peptidases are principally accountable for hydrolysis of amide linkage in peptide-based pro-drugs. These enzymes are classified as either "endo-" or "exo-" based on whether they cleave external or internal peptide bonds [94].

In order to avoid the nonspecific absorption of drugs into nontargeted tissues and to avoid systemic toxicity, injectable such as intravitreal administration of pro-drugs may be justified. As well, subconjunctival administration can be used to deliver pro-drugs targeted to specific transporters which expressed on the basolateral side of the RPE. After subconjunctival injection, the pro-drug first diffuses into the sclera and after that into the choroidal circulation, where it interrelates with specific transporters expressed on the RPE. These specific transporters will bring the pro-drug into the retinal tissue, where it is cleaved into the parent drug. A sustained delivery of drug to the retina as well as vitreous layers might be practicable, if the drug is built in a polymeric vehicle which manages the release of the pro-drug [79].

Different Pro–drug Strategies for Ocular Drug Delivery

Pro-drugs are bioreversible derivatives of drug molecule which undergo enzymatic or chemical transformation in vivo to deliver active parent drug molecule, which can then bring out desired pharmacodynamic response [89]. The main purpose for designing pro-drugs is to get better biopharmaceutical, physicochemical, and pharmacokinetic properties of pharmacologically active ophthalmic drugs. In particular most pro-drugs are designed to raise solubility, improve drug shelf-life or stability both chemically and metabolically so this conjugate can reach at their physiological target, and reduce the side effects and help in formulation [5].

Functional Group Approach

Carboxylic, hydroxyl, amine, and carbonyl groups are the most common functional groups used in ophthalmic pro-drug design. Modification of these functional groups, which comprises esters [35, 36, 71], carbamates [3], phosphates [51, 52], and oximes [34, 91], results in ophthalmic pro-drugs.

Ester Pro–drugs

The majority of ophthalmic pro-drugs developed as far as this are esters derived from either COOH or OH functional group present in the drug molecules. Under physiological conditions, generally COOH or OH functional group in drug molecules is present in ionized form which does not favor passage of drug through the lipid membrane, resultant in insufficient bioavailability of drug. Proper esterification of active drug molecules with other pro-moieties is able to produce ester derivatives with enviable hydrophilicity and lipophilicity along with in vivo lability [50]. Generally, Steglich esterification reaction conditions have been applied to produce ester pro-drugs with coupling reagent *N,N′*-dicyclohexylcarbodiimide (DCC) or ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and as a catalyst 4-dimethylaminopyridine (DMAP) [81].

Conversion of ester pro-drugs into active parent drugs can be done by esterases present in the ocular tissue. In the eye, esterases appear to be concentrated in the iris-ciliary body, corneal epithelium, retina, and optic nerve. For simplistic conversion of the ester pro-drugs to parent drugs, different classes of esterases specifically acetylcholine esterase, butyrylcholineesterase, carboxylesterases, and pseudocholinesterase are accountable [33].

Phosphate Ester Pro–drugs

Phosphate ester pro-drugs are typically designed for hydroxyl functionalities of poorly water-soluble drugs. Aqueous solubility of the parent drugs is improved by the presence of dianionic phosphate pro-moiety in phosphate pro-drugs [51, 52]. These types of compound demonstrate appropriate to outstanding chemical stability which establishes the option of developing topical eyedrop formulations [89]. Phosphate ester pro-drugs are capable of quickly hydrolyzing to parent drug molecules by alkaline phosphatases present in the eye [78].

Carbamate Pro-drugs

Amine and carboxyl functionalities are utilized for formulation of carbamate pro-drugs. Even though amines can be simply acylated, carbamate pro-drugs are not often used in ophthalmic delivery due to the moderately high in vivo enzymatic

stability [90]. Nevertheless, the problem can be conquered by inserting an enzymatically labile ester group in the structure of carbamate that results in N-(acyloxy)alkyl carbamates, which are very stable in aqueous solutions [3] and also show enhanced in vitro corneal penetration [67]. N-(acyloxy)alkyl carbamates demonstrate higher vulnerability to enzymatic bioreversion to the active parent molecule. An unstable (acyloxy)alkyl carbonyl intermediate is generated by esterase-catalyzed hydrolysis of terminal ester linkage in these derivatives, which undergo impulsive decomposition to parent amine through a labile carbamic acid [42]. However such a pro-drug strategy has restricted applicability to primary amines since N-(acyloxy)alkyl carbonyl derivatives of primary amines undergo intramolecular acyl transfer reaction resulting in the formation of stable N-acylated derivatives [92].

Oxime Pro–drugs

Oximes are derivatives of ketones, which provide an opportunity to modify drug molecules, lacking hydroxyl, amine or carboxyl functionalities. Some oxime or methoxime derivatives of well-known β-adrenergic blockers such as propranolol [76] and timolol [18] have been produced and considered as prospective antiglaucoma agents. Production of these derivatives entails oxidation of secondary hydroxyl functional groups which is present in the original β-blocker alcohols, by activated dimethyl sulfoxide (Pfitzner-Moffat oxidation) after that coupling of resultant ketone by addition of either hydroxylamine or methoxyamine in the same reaction mixture. Oxime or methoxime derivatives are present in alternative Z (syn) or E (anti) configuration. These compounds exhibit significant and long lasting intraocular pressure (IOP) lowering activity with improved therapeutic index. Oxime pro-drugs undergo sequential hydrolysis to parent β-blockers. The activation process includes initial hydrolysis by oxime hydrolase of oxime derivatives into ketone which further undergo enzymatic hydrolysis by ketone reductase to parent β-blockers (S−(−) stereoisomer). Oxime hydrolase and ketone reductases ocular distribution is mainly restricted to iris-ciliary body [17].

Transporter-Targeted Pro-drug Approach

Across lipid bilayers of epithelial cells to transfer hydrophilic molecules, i.e., nutrients and xenobiotics, mammalian cells express various types of transport systems, which are requisite to regulate the supply of vital ingredients into cells for maintaining integrity of cell functions. To take advantage of the nutrient transport systems, the parent drug may well be conjugated covalently to the nutrient moiety by means of an enzymatically cleavable bond producing a pro-drug [44].

Absorption of poorly permeating parent drug can be appreciably improved by designing pro-drug to target these nutrient transporters. This can be attained by chemical conjugation of a parent drug with the particular substrate in such a way

that the conjugate can be transported through the carrier system across the cell membrane. On different body membranes, specific nutrient transporter proteins are located which identify the nutrient and drug conjugates those resulting in translocation of the conjugate across the membrane. Following biotransformation of pro-drug would split in drug and nutrient. Pro-drugs mostly designed to bear a resemblance to various nutrients structurally are predictable to be absorbed by specific carrier. Present approach of aiming the membrane transporters holds incredible prospective for improved drug delivery through biological barriers [88].

Particular attentions in ocular drug delivery are efflux and influx transporters [28, 38, 77]. Efflux transporters fit into the superfamily of ATP-binding cassette, while influx transporters belong to the solute carrier protein superfamily. Efflux transporters result in low bioavailability as they bring molecules out of the cell membrane and cytoplasm. Main efflux proteins recognized on different ocular tissues comprise P-glycoprotein, multidrug resistance protein (MRP), and also breast cancer resistance protein. P-glycoprotein expels lipophilic compounds from both normal and malignant cells and is involved in the appearance of drug resistance. P-glycoprotein's functional as well as molecular aspects have been distinguished on different ocular cell lines and tissues, for instance, RPE [24, 32, 62]. MRP as well works in a same manner however effluxes organic anions and conjugated compounds. From nine isoforms of the MRP family, three isoforms have been known on ocular tissues. MRP1 is mostly expressed on RPE [10], while MRP2 and MRP5 have been identified on corneal epithelium [56, 57]. On the corneal epithelium, the molecular existence of breast cancer resistance protein was as well stated [55]. The breast cancer resistance protein-mediated transfer takes place in conjunction with nonpolar substrates in the lipid bilayer and is able to function as a drug flippase, transporting drugs from the inner to the outer part of the membrane. In cells, the expression levels along with patterns of these transporter proteins might be different depending on its origin as well as culture conditions. Conversely, influx transporters are involved in transporting vital nutrients and xenobiotics, for example, amino acids, glucose, lactate, nucleobases, and vitamins into cells. Influx transporters are frequently targets for pro-drug delivery for the reason that these derivatives can get better absorption of poorly diffusing parent drugs. Amino acid, peptide, and vitamins transporters are influx transporters frequently targeted for ophthalmic drug delivery.

Some examples of nutrient transporters are carriers for amino acids, folates, peptides, monocarboxylic acids, nucleosides, and organic anions and which can be generally targeted for drug delivery [65]. Recent literature reports depict a host of such nutrient carriers being articulated more specifically on the retina, and also on the RPE as well as retinal endothelial cells, which sequentially be able to utilize for targeting and delivering drugs to the vitreoretinal spaces [31, 37, 39, 53, 54]. These transporters can be targeted by different routes of administration, i.e., systemic, intravitreal, and periocular. Transporters expressed on the RPE can play a significant role in the translocation of the prodrug and thereby elevate the concentration of the prodrug/ drug in the retina following systemic and periocular administration. Intravitreally administered pro-drugs can be known by the nutrient transporters

articulated on the retinal endothelial cells and in that way translocation of pro-drugs across neural retina across the RPE. Afterward, pro-drugs are undergoing enzymatic cleavage at the target site to deliver the parent drug, which brings out the desired therapeutic action. Although a number of nutrient transporters on the retina have been identified and characterized and are discussed as follows.

Amino Acid Transporter–Targeted Pro–drugs

In the protein synthesis of all living cells, amino acids are essential. Attributable to their hydrophilicity, membrane proteins are present to transport vital amino acids across lipoidal membranes. Amino acid transporters comprise a big family of membrane transporters which are categorized depending on their substrate affinity, substrate specificity, sodium dependence, as well as pH dependence [23, 96]. Some of these transporters are present on a variety of ocular tissues such as conjunctiva, retinal pigment epithelium. It has been also reported that existence of oligopeptide transporter in rabbit corneal epithelium [8]. These proteins are generally categorized as anionic, cationic, and neutral amino acid transporters and are additionally subclassified into sodium-dependent as well as sodium-independent transporters. System L, a large amino acid transporter, system y^+, a cationic amino acid transporter, and system $b^{0,+}$, a cationic and neutral amino acid transporter, belong to sodium-independent transporters while system X^-, an anionic transporter; system A, B^0, $B^{0,+}$, ASC (alanine-serine-cystein) and, ß-amino acid transporters belong to the sodium dependent category [19, 27, 61, 86, 87, 100]. A large neutral amino acid transport (LAT) system presents in two isoforms, LAT1 and LAT2, that differ in their substrate specificity. LAT1 is mostly engaged in the large neutral amino acids transport, for example, His, Ile, Leu, Met, Phe, Trp, Tyr, and Val, and LAT2 involves in transport of both small and large neutral amino acids. In rabbit corneal epithelial cells, the presence of sodium-independent systems, like LAT1, and sodium-dependent systems, for example, $B^{0,+}$, and ASCT1 transporters, have been reported [46, 47, 59, 104].

A few of the substrates of amino acid transporters are drugs such as gabapentin, α-methyldopa, and L-dopa, which are expressed in various tissues, with the eye [104]. To explore the feasibility of improvement of ocular bioavailability of the antiviral agent acyclovir by designing amino acid pro-drugs targeted to the amino acid transporters on the rabbit cornea, Anand et al. [7] studied the transcorneal flux of two water-soluble ester pro-drugs of acyclovir glutamate as well as L-tyrosine. They reported in their study that amino acid pro-drug design appears to be a smart approach to improve the solubility of poorly aqueous soluble compounds and in addition to give targeted and probably improved drug delivery of the active drug compound [7]. Researchers reported the synthesis of a series of pro-drugs of acyclovir (ACV) targeting the amino acid transporter, for example, alanine-ACV, isoleucine-ACV, serine-ACV, γ-glutamate-ACV, and valine-ACV, with an aim to improve the corneal permeability and in turn ocular bioavailability of acyclovir [58]. Serine-ACV exhibited a better pharmacokinetic profile relative to the parent

drug resulting in an appreciably high area under concentration-time curve (AUC), Cmax, and Clast values, because of transporter-mediated translocation across corneal epithelium [58]. Molecular evidence and functional activity of LAT2 (sodium independent) have been made known in ARPE-19 cells (human retinal pigment epithelial cells) in the posterior segment [37].

Other carriers for amino acids in the RPE and retinal endothelial cells are at present under research. Ultimately, amino acid transporters can be used for targeting pro-drugs to improve absorption of poorly permeable drugs across ocular barriers following systemic, periocular, and intravitreal routes of administration.

Peptide Transporter–Targeted Pro–drug

For the translocation of dipeptides, tripeptides, and peptidomimetic drugs across epithelia, peptide transporters are liable [1]. Peptide transporters are proton-coupled and are mostly categorized into PepT1, PepT2, and peptide/histidine transporters (PHT1 and PHT2), different to some extent in their affinity, localization, tissue distribution, and capacity to transport substrates. In the current years, these transporters have attained interest for targeted drug delivery due to their reasonably higher capability to carry molecules across lipoidal membranes together with a low structural specificity requisite compared with other well-known transporters. By utilizing these transporters, various structurally different drugs with diverse pharmacologic activities can be delivered to cells. Designing of peptidomimetic prodrugs of ceftibutan, fosinopril, antiviral compounds (acyclovir, ganciclovir and valacyclovir) and beta lactam antibiotics with PepT1 and PepT2 substrates have shown improved drug delivery [82, 83].

In a variety of ocular tissues, for example, cornea, blood-aqueous barrier (BAB), and retina, these peptide transporters have also been found to be expressed [8, 9, 53]. Because of dipeptide conjugation to the parent drug, corneal permeation of acyclovir and ganciclovir was considerably high resulting in improved therapeutic activity against herpes viruses [8, 43]. On BAB, existence of a peptide transporter was also reported. Significant transport of a model (glycylsarcosine) PepT substrate, subsequent systemic administration provides the initial observation that PepT may be expressed on BAB [9]. Across the blood-ocular barrier, a time as well as concentration- dependent, carrier-mediated uptake of glycylsarcosine was described. In the aqueous humor, elevated levels of pro-drugs of acyclovir showed following systemic administration compared to the parent moiety [29].

More latest work suggested that significantly higher levels of acyclovir following systemic delivery of stereoisomeric dipeptide pro-drug of acyclovir (L-val-D-valacyclovir) on the cornea, as compared to the parent drug, provided further proof for the existence of PepT on BAB [93]. The presence of PepT has been reported on RPE as well as retinal endothelial cells in the retina. The transporter present in the neural retina, facing the vitreous humor, might be targeted for drug delivery by intravitreal route of administration, while transporter on the RPE could be targeted subsequent systemic and periocular administration. The expression of PHT1 on human and bovine RPE cells and neural retina has been reported in current study.

PepT1 expression was not identified, while PepT2 and PHT2 are identified to be expressed on bovine as well as human retina [84].

Latest study confirmed that the oligopeptide transport system on the RPE leads to the minimum twofold raise in the permeation of PepT-targeted pro-drugs of ganciclovir (valine-GCV, valine-valine-GCV, and glycine-valine-GCV) across the retina-choroid-sclera comparative to the parent moiety [53]. Following translocation by PepT, these pro-drugs are then cleaved by means of enzymes, predominantly the aminopeptidases, cholinesterases, and dipeptidases, found in the retina and vitreous. To summarize, peptide transporters are tremendously adaptable and are important targets for drug delivery to ocular tissues.

Nucleoside Transporter–Targeted Pro–drugs

In mammalian cells, transcellular flux of nucleobases and nucleoside has shown to be arbitrated explicit nucleobase as well as nucleoside transporters. In drug delivery, an example of nucleoside transporters' role is the movement of the anti-HIV drug 2,3-dideoxyinosine across blood-brain barrier [11].

Monocarboxylic Acid Transporter-Targeted Pro-drugs

The expression of monocarboxylic acid transporters by retinal pigmented epithelial cells might be targeted by monocarboxylate pro-drugs for improved uptake (e.g., acyclovir and ganciclovir) [66].

Folate Transporter–Targeted Pro–drugs

This receptor may be investigated for targeted drug delivery of anticancer drugs, as folate receptor is upregulated in cancer cells [70].

Receptor–Targeted Pro–drug Approach

As well as transporter-targeted delivery, drug targeting to specific receptor by means of carrier-mediated absorption is promising as a clinically important strategy. In different parts of the eye, receptors helpful for pro-drug targeting have been empathized. Folate, vitamin B12, and transferrin are receptors in charge for the internalization of nutrients. A number of researchers have studied the use of drug-receptor conjugation for drug delivery as well as drug targeting due to the importance of these receptors. Through receptor-mediated endocytosis, internalization of such conjugates has been attained fruitfully. To date, for tumor-targeted drug delivery,

folate receptor has been used as a perfect candidate; however, fewer considerations have been given to receptor theory for ocular drug delivery [26]. In recent times, Dal Pozzo and co-workers have started working on the extension of targeted pro-drug strategy by producing receptor-targeted pro-drugs for ocular drug delivery. Nevertheless, a lot of attention is desired to investigate and widen receptor-based pro-drug strategy.

Lipid Pro-drug

Through passive diffusion mechanism, drug molecules can cross cell membranes. Absorption of drug, specifically in the eye, happens either via corneal route (cornea-aqueous humor-intraocular tissues) or else through non-corneal route (conjunctiva-sclera-choroid/RPE) [38]. Both hydrophilic and hydrophobic drugs get transcellular pathway to cross ocular membrane attributable to lipophilic nature of cornea as well as other intraocular tissues. Besides these, sustained drug delivery for longer periods of time at the target site is necessary in particular for the management of eye diseases at posterior segment of the eye such as the choroid, retina, and vitreous. In lipid pro-drugs, a drug molecule is covalently bound to a lipid moiety, for example, diglyceride, fatty acid, or phosphoglyceride. Through facilitated diffusion, lipid pro-drugs diffuse freely across cell membrane and in that way bring about enhanced cellular absorption [16, 21]. Conversely higher lipophilicity of molecules can give rise to restricted membrane permeability because it will attach inside the lipid membrane of the cornea. Therefore, based on the hydrophilicity of each drug molecule, length of lipid chain necessitates to be attuned so as to obtain the greatest permeability across the cornea. By conjugating a targeting moiety, receptor/transporter at one end of lipid pro-drug intraocular permeation can be more improved.

Formulation Application of Injectable pro-drug for the Retina and Posterior Segment Diseases

The pro-drug approach has been attempted for considerable improvements in different properties such as solubility, stability, permeability and avoidance of efflux pump, and improvement of therapeutic effectiveness of various drug molecules.

Owing to the frightening obstruction created by the blood-retinal barrier and tight junctions of the retinal pigment epithelium, drug delivery to the choroid, retina, and vitreous is a difficult task. Only little fractions of drug administered through oral, intramuscular, or intravenous route [15] get to the target. So, administration of large and potentially lethal doses of drug is required. Another challenge to retinal drug delivery is that drug levels must be sustained for prolonged periods at the target site. If the half-life of the injected drug is very small, it is intricate to use intravitreal injections, as repeated injections would be needed. Cheng et al. [20] developed an intravitreal drug delivery system to make possible localized drug delivery to the

posterior segment by an injectable, sustained-release system, wherein a crystalline lipid pro-drug of ganciclovir (GCV), hexadecyloxypropyl-phospho-ganciclovir (HDP-P-GCV), is utilized. Researchers established that the unmodified crystalline lipid pro-drug of GCV obsessed a slow-release property after intravitreal injectable route. In their earlier study, they also reported herpes simplex virus-1 (HSV) infection of the retina prevented by HDP-P-GCV for 20 weeks following a single intravitreal injection, while a GCV single intravitreal injection showed less than 1 week of defense [20]. Cheng and co-workers confirmed that microfluidized small-particle HDP-P-GCV might have released a larger quantity of free drug into the vitreous fluid than the unmodified HDP-P-GCV large-particle formulation. In the large-particle group, AUCs in animals were found to be in the range of 60.7–155, while in the small-particle group, AUCs ranged from 140 to 351. The drug release was two-fold as high in the small-particle group. They consider that small particles have a large surface area which raises the contact surface with dissolution fluid, resulting in a high rate of dissolution relative to large particles. It is probable that awfully little quantities of crystalline pro-drug diffused to a remote part of the vitreous and be sampled into the vitreous tap, and this diffusion might be the reason of the fairly great difference in the vitreous fluid drug levels among individual animal eyes. Three animals per time point were used by researcher to obtain a mean value, as of which the data curve would demonstrate a clear trend and suitable information. At different time points, vitreous fluid was sampled by numerous vitreous taps. Although they sampled the vitreous fluid through the pars plana and they did not observe anterior or vitreous chamber fibrin formation, so it is feasible that numerous vitreous taps cause intraocular environmental change and influence the subsequent measurements of vitreous drug concentration. The local retinal toxicity resulting from drug depot and retinal contact because of gravitational effects and positioning could be eliminated by injecting lower drug concentration [20, 69]. In nonvitrectomized eyes, the present studies were conducted, and it was observed that the aggregation of drug and release profile could be fairly dissimilar if injected into a vitrectomized eye. Additional studies are necessary in vitrectomized eyes. Founded on their conclusion, they assume that by using mixtures of different sizes of crystalline drug in an intravitreal injection, controlled release could be attained. These mixtures could be specifically intended to have release profiles customized to care for different types of vitreoretinal diseases.

Cheng and co-workers first described this novel intraocular drug-delivery system using HDP-P-GCV as a prototype in their previous study [20]. They articulate that the hexadecyloxypropanol moiety might be attached to lots of nucleoside phosphates or phosphonates to produce solid hydrophobic crystals so as to dissolve gradually in water. Subsequently, the dissolved molecules go into the cells and are cleaved by phospholipase C into hexadecyloxypropanol and the parent drug intracellularly [22].

Intravitreal injection of 100 μg or lower doses of hexadecyloxypropyl-cyclic cidofovir (HDP-cCDV) crystals provided an ideal drug depot that floated in the inferior vitreous cavity without disturbing the visual axis. The vitreous somewhere else was clear, and no toxicity was caused in the eyes with 100 μg or lower doses. Through the contact of drug depot with the retina or lens, local toxicity was found

with higher doses, which was comparable to the local retinal toxicity induced by intravitreal high-dose HDP-P-GCV [20]. A larger drug depot in the vitreous was formed by higher dose, which has a tendency to make contact with intraocular tissues and resulting in toxicity. In the study of the eyes that received 100 μg or lower doses, decrease in an intraocular pressure (IOP) related with cidofovir (CDV) intravitreal injection was not observed. A gentle decline in IOP at the last time point showed in the eyes with higher doses (8.7 ± 2.1 vs. 11.3 ± 2.3 mm Hg, $P = 0.01$). Nonetheless, hypotony was not found. Hypotony (IOP of 5 mm Hg or lesser with allied retinal edema) is a renowned problem after local or systemic administration of cidofovir [12, 103]. The absence of hypotony may be because cyclic CDV and HDP-cCDV are not picked up keenly by organic anion transporters in the ciliary body.

Intravitreal pharmacokinetics exhibited that HDP-cCDV was still measurable at week 10 following a single intravitreal injection of 100 μg per eye. The predictable vitreous half-life for HDP-cCDV was 6.3 days, which positively compares to 10 h for cCDV or else 20 h for CDV [25]. At week 8, the measured concentration was 0.002 μM, which is higher than the IC50 for cytomegalovirus (CMV). They have revealed that the pro-drug can be metabolized by contribution of vitreous cells with HDP-P-GCV. When pro-drug was incubated with a heat-inactivated vitreous sample, slight parent drug was measurable; however, conversion was detected willingly by native vitreous which contains cells [22]. It has been identified that CDV is phosphorylated to cidofovir diphosphate, the active form of cidofovir which has long intracellular half-life [2].

Cheng and co-workers summarize that crystalline lipid pro-drug for intraocular drug delivery system has guarantee for demanding refractory chronic vitreoretinal disorders which need extended drug treatment. HDP-P-GCV small crystals have been shown to release more amount of drug over time relative to larger unmodified crystals, and the delivery system has been extensive to crystalline HDP-cCDV. This concept can be applicable to many compounds, including antiproliferative drugs such as phosphonomethoxyethylguanine (PMEG), arabinofuranosylguanine (Ara-G), and 5-fluoro-2′-deoxyuridine (5-FUdR) and other antiproliferative drugs. This pro-drug generated more drug exposure to the retina than the vitreous [21].

In the precorneal area or vitreous body to get better the retention time of tilisolol, Kawakami et al. [60] formulated liposomes containing the *O*-palmitoyl pro-drug of tilisolol. *O*-palmitoyl tilisolol was incorporated totally in the liposomes. A fairly lengthened retention of *O*-palmitoyl tilisolol in the vitreous body was found following intravitreal injection of *O*-palmitoyl tilisolol liposomes. At 24 and 48 h after intravitreal injection of *O*-palmitoyl tilisolol liposomes, the tilisolol concentration in the vitreous body was significantly higher compared with the concentration after intravitreal injection of tilisolol liposomes [60].

Dexamethasone is a widely used drug for posterior segment ailments. Barot et al. [14] developed transporter-targeted pro-drug therapy of dexamethasone, which can be recognized by peptide transporter present on the retina. They synthesized amino acid and dipeptide pro-drug of dexamethasone and confirmed by NMR and mass spectroscopy. The aqueous solubility of peptide pro-drug valine-valine-

dexamethasone 7.07 ± 1.86 mg/ml was significantly higher than parent drug 0.14 ± 0.09 mg/ml. Stability studies in ocular tissue homogenate revealed rapid bioreversion of the pro-drug into parent drug. The pro-drugs have shown pH-dependent stability. Peptide pro-drugs were highly stable at lower pH compared to higher pH. The cumulative amount of dexamethasone transported across sclera and reticulum cell sarcomas tissue preparation was found to be comparable for parent dexamethasone and its pro-drugs, indicating that pro-drug modification of dexamethasone had no negative effect on the permeability. Owning to their higher aqueous solubility, buffer stability, bioreversion property, and comparable permeability across ocular posterior segment tissues, amino acid and peptide pro-drugs of dexamethasone may improve overall ocular bioavailability. Transporter¯targeted pro-drug modification can be a promising strategy for transscleral delivery of dexamethasone in the treatment of posterior segment ocular diseases [14].

These attempts are highly effective in treating herpes simplex virus-1 (HSV-1) induced corneal epithelial and stromal keratitis [73]. Permeability values of valine-GCV, tyrosine-valine-GCV, and glycine-valine-GCV were found to be higher than parent GCV which was attributed to their interaction with oligopeptide transporter present on the retina [53]. Several dipeptide ester pro-drugs 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 pro-drugs appeared to permeate deeper into the retina after IVT administration relative to GCV [72]. Sustained release microsphere formulations of GCV and its lipophilic pro-drug GCV-monobutyrate, with higher entrapment efficiency, were developed using PLGA [49]. Ocular penetration of peptide pro-drug of ACV (valine-valine-ACV) was higher than the parent drug following systemic administration in rabbits. The pro-drugs appear to be less cytotoxic and highly water soluble with excellent in vivo activity against HSV-1 in rabbit epithelial and stromal keratitis [6]. Researchers developed a series of stereoisomeric valine-valine-based dipeptide ester pro-drugs of ACV. Pro-drugs including L-valine-L-valine-acyclovir (LLACV), L-valine-D-valine-acyclovir (LDACV), D-valine-L-valine acyclovir (DLACV), and D-valine-D-valine acyclovir (DDACV) were designed to make possible better residence time of intact pro-drug in the systemic circulation, as a result of facilitating targeting transporters on blood-ocular barriers subsequent to oral or systemic administration. Hydrolytic enzymes such as peptidases and esterases responsible for bioreversion of dipeptide pro-drugs 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 pro-drugs. Such incorporation enabled pro-drugs to be more stable in the systemic circulation and to facilitate recognition and translocation by the nutrient transporters at blood-ocular barriers. Among these pro-drugs, LLACV as well as LDACV hydrolyzed in Caco-2 cell homogenate; moreover, LDACV was moderately more stable of the two compounds. Incorporation of two D-valine moieties into a dipeptide moiety, however, enhanced the enzymatic stability but abolished the affinity of these pro-drugs (DDACV and DLACV) toward the peptide transporter [95].

Due to this pro-drug approach, incidence of systemic toxicity was found to be greatly diminished. Role of phase I and phase II ocular metabolic activities was also reviewed recently to rationalize the design of pro-drug and co-drug for ocular delivery by Al-Ghananeem et al. [4].

In another study, combretastatin A-4-phosphate, water-soluble pro-drug of combretastatin A-4, was found to suppress the development of VEGF-induced neovascularization in the retina and also block development of choroidal neovascularization [80].

From cytarabine (Ara-C), two types of lipid pro-drugs hexadecyloxypropyl cytarabine 5′- monophosphate (HDP-P-Ara-C) and hexadecyloxypropyl cytarabine 3′,5′-cyclic monophosphate (HDP-cP-Ara-C) were produced. Their vitreal clearance profile was simulated using a custom dissolution chamber, in vitro cytotoxicity was evaluated using cell proliferation assays, and in vivo ocular properties in rat and rabbit eyes were assessed using biomicroscopy, indirect ophthalmoscopy, tonometry, electroretinography, and histology. The cyclic monophosphate prodrug, HDP-cP-Ara-C, was found to have physiochemical properties better suitable for sustained delivery of cytarabine to posterior segments. These properties incorporated restricted aqueous solubility, in vitro antiproliferative activity, as well as excellent tolerability following injection into rabbit eyes [63].

Malik et al. [74] 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 pro-drugs of celecoxib: celecoxib succinamidic acid (CSA), celecoxib maleamidic 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, correspondingly. Eightfold higher transport for CSA than celecoxib has been established by in vitro transport studies transversely isolated bovine sclera and sclera-choroid-RPE. 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. In vivo delivery in pigmented brown Norway rats showed concentrations of total celecoxib (free + pro-drug) were significantly higher in the CSA group compared with the celecoxib group for all posterior eye tissues except choroid-RPE and periocular tissues. In this study, they demonstrated that the transscleral delivery of celecoxib can be significantly enhanced in vitro and in vivo through formation of pro-drugs that are more hydrophilic and exhibit less binding to melanin pigment. Such an approach might help translate transscleral drug delivery for clinical use in the long run [74].

Acyclovir diphosphate dimyristoylplycerol is a lipid pro-drug of acyclovir, forms liposomes, and provides considerable activity against acyclovir-resistant herpes simplex virus strains and herpes simplex virus, along with human cytomegalovirus. Taskintuna et al. [98] tested this hopeful new drug in a rabbit model of herpes simplex retinitis. An intravitreal injection in animals with acyclovir diphosphate dimyristoylglycerol proved retinitis that was less harsh relative to that animals injected

with acyclovir, buffer, and ganciclovir; variation in grading scores of the retinitis flanked by animals injected with acyclovir diphosphate dimyristoylglycerol as well as animals injected with buffer were statistically significant ($P = 0.0015$). In the rabbit model, acyclovir diphosphate dimyristoylglycerol had given away extended antiviral activity against herpes simplex virus-1 retinitis. This drug delivery system, modified to improve optical clarity, may allow long-acting intravitreal treatment of cytomegalovirus retinitis and other retinal diseases [98].

A novel pro-drug 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) that can be recognized by a specific sodium-dependent multivitamin transporter (SMVT). Lipophilic pro-drugs readily diffused across the cell membrane by facilitated diffusion, whereas transporter−/receptor-targeted pro-drugs 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 pro-drugs (biotin-ricinoleicacid-acyclovir (B-R-ACV) and biotin-12-hydroxystearicacid-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 human corneal epithelial cells [101]. The targeted lipid pro-drugs B-R-ACV and B-12HS-ACV exhibited much higher cellular accumulation than B-ACV, R-ACV, and 12HS-ACV. Both the targeted lipid pro-drugs 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 pro-drugs 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 pro-drug 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 [102].

Conclusion

The injectable pro-drug approaches outlined in this chapter may provide relevant information to researchers involved in designing appropriate and effective delivery systems for the treatment of posterior segment diseases. Injectable pro-drugs targeted toward membrane transporters expressed on epithelial cells are perhaps the most exciting of all the current drug delivery strategies. This approach achieved success in drug delivery enhancement through various epithelial barriers. Membrane transporter targeted drug delivery utilizing the nutrients linked compounds however is relatively unexplored particularly in case of ocular therapeutics. So far, not many

people have considered utilizing carrier-mediated transport mechanisms on the cornea, which is believed as a major route of absorption for topically applied drugs. Pro-drug design with water-soluble nutrient offers dual advantages. For example, on the one hand, pro-drug design with amino acids that targets amino acid transporters will increase the corneal permeability and thereby will provide increased accessibility of poorly absorbed drugs to posterior ocular tissues, while on the other hand, eliminating eyedrop formulation problem (water-soluble pro-drugs) as compared to conventional pro-drugs that are intended to enhance lipophilicity. The recent progress in the field of pro-drug design holds a promising future for ophthalmic drug delivery specifically to target posterior segment of the eye. Pro-drugs have become an integral part of the drug design and delivery process, as exemplified by the growing number of approved pro-drugs and patents. Growing utilization of coherent pro-drug approach at the initial phase of drug discovery will lead to the development of composite with improved physicochemical properties.

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Chapter 20
Stereoisomeric Dipeptide Prodrug Approach for Retina and Posterior Segment Disease

Shrushti Patil, Nandish Pathak, and Yashwant V. Pathak

Abstract Stereoisomeric dipeptide prodrug approach has overcome the barriers of the conventional drug delivery method. This approach offers a much more efficient release of the drug by becoming active after the drug is inside the body. Acyclovir (ACV) drug is mostly used to treat herpes, and it was modified by addition of stereospecific dipeptides, L-valine and D-valine, resulting in L-valine-D-valine-acyclovir. This prodrug disintegrates into the parent drug ACV inside the body. This chapter discusses the synthesis method of such drugs along with the types of diseases that could be treated. Moreover, various drug delivery methods are also mentioned, which further encapsulate these stereoisomeric dipeptide prodrugs like nanoparticles and solid lipid nanoparticles (SLN) for additional layer of protection to ensure better absorption. Although there are still some challenges that need to be considered including toxicity, error in absorption rate, and other side effects, by overcoming these issues we will be able to focus on the future applications of such a capable drug delivery method.

Introduction

The traditional treatments for ocular ailments include either oral or ocular mode of administration, which have been delivered inefficiently due to the poor bioavailability of the drug in the posterior eye [5]. Multiple physicochemical factors like high molecular weight, low aqueous solubility, and surface charge cause instability in the gastrointestinal tract causing limited absorption [1]. While diffusing through the anterior part of the eye to the retina, the drug's pharmacokinetics are negatively

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impacted due to the nature of the vitreous humor [6]. Other physiological processes comprising of tear production, blinking, metabolism, and binding of drug also hinder the chances of therapeutic dosage reaching the targeted sites [6]. Increasing the concentration of the drug to overcome these physiological barriers could foster drug accumulation as well as toxicity [6]. On the other hand, increasing the frequency of the dose to combat toxicity of high doses discourages patient to comply with such a rigorous regimen. All of the above issues result in inefficient treatment leading the ailment to worsen over time causing blindness. In order to overcome the problems of traditional treatments, prodrug approach has been introduced. The main objective of this approach is to ameliorate the drug's aqueous solubility, drug absorption, and membrane permeability [1]. In this chapter, we will be investigating stereoisomeric dipeptide prodrug approach in detail.

Stereoisomeric dipeptide prodrugs are among the pro-moieties used for the prodrug approach, which involves two amino acids in either L-isomer or D-isomer conformation linked to the parent drug via an ester bond. These prodrugs are innately inactive; however, their metabolites can be biologically active drugs obtained after disintegration of the prodrugs [1]. Additionally, this does not allow the parent drug to exhibit any undesirable side effects [1]. This method is the prime approached due to its quality of enhancing bioavailability as well as penetration at the targeted tissue [6]. Several ocular prodrugs contain carboxyl or hydroxyl functional groups, which can be further esterified into lipophilic ester prodrug [6]. Hydrolytic enzymes in the body, namely, phosphatases and esterases, are responsible for the breakdown of these prodrugs into the desired prodrug [6]. Stereoisomeric dipeptides successfully fulfill all the requirements for well-designed carrier-linked prodrugs as described above [1]. The typical treatment will be compared to the ideal treatment for analyzing the attributes of stereoisomeric dipeptide prodrug approach to ideal treatment. To understand the application of these stereoisomeric dipeptide prodrugs better, a few diseases will be discussed briefly, as well as pathogenesis caused by herpes simplex virus, types of antiviral drugs used in the treatment, and mechanisms used to counteract the effects of the virus by the prodrug. Moreover, the structures and synthesis of these prodrugs will also be examined in this chapter (Fig. 20.1).

Traditional Strategies Versus Prodrug Strategies

The traditional approach entails mainly of intravenous or oral administration for the treatment of ophthalmic disorders or diseases [2]. The most common issue regarding the oral administration is that most of the prodrug is metabolized quickly by the digestive tract before reaching the target intact [2]. The prodrug approach is an exemplary solution to this issue. The prodrug approach is a chemical approach that facilitates delivery of the drug inside the body improving the absorption efficiency [2]. This approach is essential to overcome the physiological and physiochemical barriers that hinder ocular bioavailability of prodrugs [2].

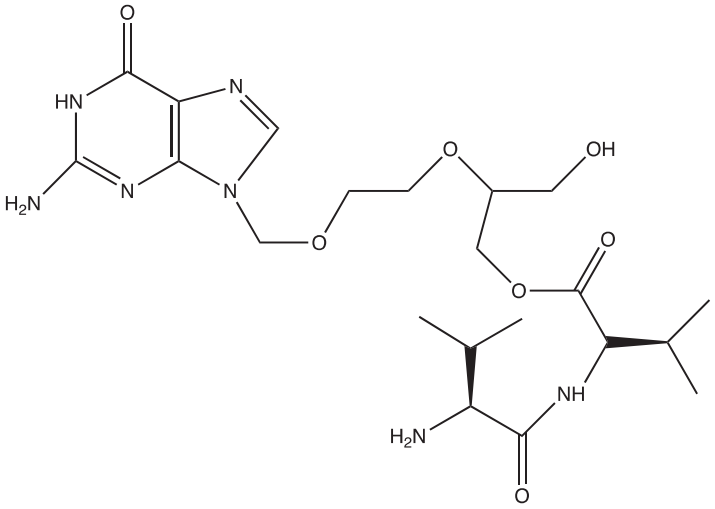

Fig. 20.1 This is an example of a stereoisomeric dipeptide prodrug, (L)-Val-(L)-Val-acyclovir that treats ocular herpes

Furthermore, there are various benefits to using the prodrug strategy compared to the traditional method. This strategy is mainly used to increase the shelf life, solubility, and stability of the drug. Furthermore, it has demonstrated a higher bioavailability of the drug and less severe side effects. The prodrug becomes more lipophilic facilitating permeability of cell membranes. There are several disadvantages to the intravitreal route of administration, which are caused due to its invasive nature especially if conducted frequently for chronic conditions. Some of the common adverse effects are inflammation, cataracts, retinal detachment, and even vitreous hemorrhages [3]. These consequences are the reasons why patients and their physicians deter from this procedure. Another treatment option is surgical implants to provide a more sustained delivery in addition to more therapeutic benefits [3]. However, the use of these implants is still limited due to the surgical procedure.

Ophthalmic prodrug designs commonly use functional groups like carboxylic, hydroxyl, amine, and carbonyl. Altering these functional groups creates ophthalmic drugs with esters, carbamates, phosphates, and oximes. The hydrolytic enzymes in the body play a vital role in breaking down the stereospecific dipeptide prodrugs and further have a higher affinity for L-isomers. Therefore, D-isomers are integrated into the pro-moiety to facilitate metabolism of the prodrug. This approach is meant to enhance the bioavailability of the intact drug by increasing its residence time in the bloodstream. The goal is to synthesize prodrugs that could be enzymatically stable, prevent initial hydrolysis, and increase ocular and oral bioavailability. Peptidases and esterases are also some of the hydrolytic enzymes that act toward bioreversion of dipeptide prodrugs, which are stereospecific and have a higher affinity for L-amino acids. All the dipeptides prodrugs analyzed to date have been based on L-amino acids since they are natural substrates for these hydrolytic enzymes (Table 20.1).

Table 20.1 displayed a comparison between the conventional system of drug delivery and the ideal system of drug delivery

Conventional system	Ideal system
Rapid loss of drug through tear turnover and blink reflex	Drug should have the ability to stay in the precorneal area for long periods
Eye drops need to be regulated frequently to attain ocular bioavailability	An ideal system has a more sustained release in a controlled manner
For example, ophthalmic inserts have possibility of causing discomfort and blurred vision. Solution (eye drops)	For example, colloidal nanocarriers made with biodegradable polymeric material designed to elevate penetration of the drug

Characterization of Novel Dipeptide Ester Prodrugs of Acyclovir

Acyclovir (ACV) is an antiviral medication, commonly used for the treatment of herpes virus [1]. It is a highly specific inhibitor of herpes virus proliferation due to its poor solubility in water as well as lipids [1]. Different drug delivery methods to efficiently administer the drug were developed including oral, intravenous, intramuscular, and ocular [1]. However, only intravenous and oral methods of delivery were successful due to low lipophilicity and aqueous solubility [1]. Another method of drug delivery involved the addition of amino acid moieties to ACV and development of a prodrug, which would augment drug delivery via ocular administration (eye drops) [1]. During the preparation of the eye drop formulation, the prodrug displayed low stability at physiological pH 7.4 as well as decreased proficiency in delivery [1]. PEP1, an oligopeptide transporter, is a crucial mammalian protein; it is present on the walls of the small intestine that functions to actively transport small peptides [1]. They are also present in the cornea and internal eye structures. The aim of the study is to ensure assimilation of the intact dipeptide prodrug via PEP1 transporter. Dipeptide prodrugs are able to exist in various conformers, which allows for experimentation of the dipeptide moiety conformation.

Previously during a study, a series of stereoisomeric drugs were manufactured by integration of D-isomers into dipeptide moieties at a certain location, in order to regulate the rate of metabolism [4]. The results from this study were compatible with the findings of other studies that also provided evidence that the integration of D-amino acid into dipeptide does not hinder its affinity for the PepT transporter [4]. Furthermore, it has depicted a higher stability against hydrolytic enzymes of the body, which could result in elevated cellular permeability [4]. It can be concluded that the incorporation of two D-amino acids into a dipeptide moiety not only increases the enzymatic stability but also concomitantly eliminates its affinity for PepT transporters [4].

Upon intravenous administration of L-valine-acyclovir (LACV), the results depicted a higher concentration of the parent drug, ACV, which signifies rapid hydrolysis in the body [4]. Additionally, L-valine-D-valine-acyclovir (LDACV) displayed not only increased oral bioavailability but also in vivo corneal uptake of the

parent drug (ACV) [4]. The concentration of LDACV was seen to be twice as high as LACV because of the increased hydrolysis rate of the former prodrug in the ocular tissue.

Synthesis of Dipeptide Ester Prodrug of Acyclovir

Synthesizing a prodrug is a difficult task, as it has to meet several requirements. Some of these requirements are as follows:

- Parent drug must entail the functional group necessary for chemical derivation.
- Chemical modification needs to be reversible.
- Parent drug, prodrug, and pro-moiety bound should be nontoxic and safe.
- Pro-moieties, like amino acids, small peptides, and vitamins, must be able to metabolize rapidly by the body.
- Prodrug must have a sufficient shelf life and be stable in its final formulation.
- Most of the ocular preparations must be delivered in a liquid form (aqueous form of drug is a vital parameter if the drug is lipophilic in nature).
- Final prodrug must have the ability to avoid unfavorable physicochemical and biopharmaceutical properties of the parent molecule [2].

Amino acids, valine and glycine, were utilized in different combinations to produce the most stable dipeptide moiety. N-(tert-Butoxycarbonyl)glycine (Boc-Gly-OH) and dicyclohexyl carbodiimide were dissolved into dry dimethylformamide under high-nitrogen condition. The end products have to go through extensive purification. It begins with a rinse using cold ethyl ether and involves thin-layer chromatography along with precipitation of the final products.

The stereoisomeric dipeptide prodrugs of ACV (DV-DV-ACV, LV-DV-ACV, and LV-LV-ACV) were produced using the above method and also additional minor changes. Resulting compounds are purified via silica gel column chromatography, and further deprotection is carried out using trifluoroacetic acid. Cold diethyl ester was utilized to recrystallize the newly synthesized drugs. Thin-layer chromatography as well as liquid chromatography monitored the progression of the aforementioned reaction. The structures of the compounds were verified by H-NMR spectroscopy and mass spectroscopy. Chemical shifts were measured in parts per million (ppm) against a standard, tetramethylsilane (TMS). A hybrid triple quadrupole-linear ion mass spectrometer carried out mass spectroscopy. An enhanced mass spectrum mode conformed the intermediates and the final products. Thin-layer chromatography is frequently used to monitor the progress of each reaction step during synthesis of these prodrugs along with liquid chromatography/mass spectroscopy [6]. This step is very crucial to ensure that all the reactants have completely reacted to form the desired compound [6]. This end product is then tested using H-NMR spectroscopy, in order to acquire accurate product. Steps involved into creating a dipeptide prodrug of acyclovir are shown in Fig. 20.2.

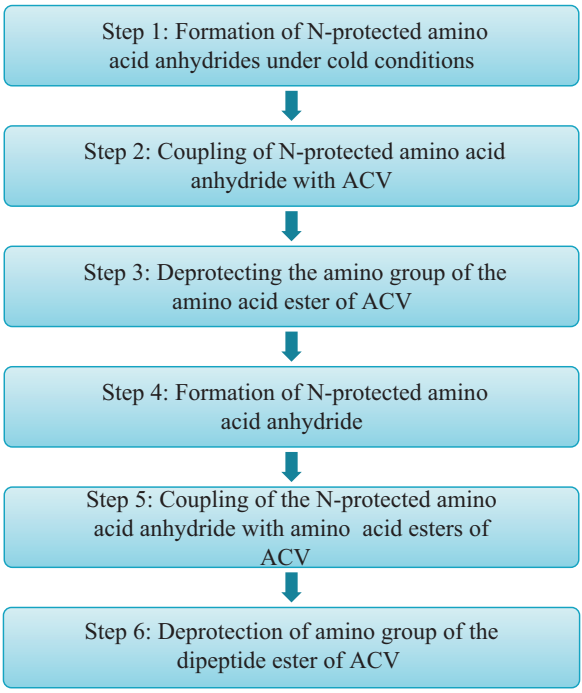

Fig. 20.2 Steps involved into creating a dipeptide prodrug of acyclovir

In Vivo Oral Absorption/Gastrointestinal Absorption

Based on the results of the prodrug metabolites, it depicted that Val-ACV disintegrated as ACV quickly, while dipeptide conjugates, Val-Val-ACV and Gly-Val-ACV, were hydrolyzed into parent drug gradually in Sprague-Dawley rats. Val-ACV was also efficiently absorbed by the gastrointestinal tract and converted to the parent drug and L-valine via hepatic metabolism. The metabolites from the portal and jugular vein were analyzed to attest the role of hepatic as well as intestinal metabolism. However, conjugate intermediates of tyrosine-ACV were not found, which could have been due to complete disintegration of the prodrug as well as the parent drug. This could be concluded since intermediate metabolites (GVACV and VVACV) were present in large amounts, in the form of the parent drug, after systemic absorption was compared to intestinal absorption. The overall absorption values for systemic circulation were significantly larger for the dipeptide ester prodrugs, in comparison with ACV. Therefore, the bioavailability of ACV is enhanced due to dipeptide prodrug approach. These drugs have also been shown to have higher affinity for PEPT1, an intestinal peptide transporter, which results in a better absorption in the intestinal tract compared to oral administration. Hence, the dipeptide drugs are considered a therapeutically superior in the treatment of oral as well as genital herpes infection.

Treating Various Diseases with Stereoisomeric Dipeptide Prodrugs

Treating Ocular Herpes with Acyclovir

Ocular herpes is one of the most common types of infectious disease in the United States caused by the herpes simplex virus (HSV-1). It is also a known cause for corneal blindness. Succeeding the primary ocular infection, HSV-1 is capable of staying latent for a lifetime into the host's trigeminal ganglia. The intermittent corneal infection could cause symptoms like corneal thinning, scarring and stromal opacity, and blindness. Previous studies have depicted a 50% probability of recurrence in patients infected with HSV-1.

Stereoisomeric dipeptide prodrug approach has been used successfully to treat ocular herpes by targeting oligopeptide transporter (PEPT). The drug was delivered most efficiently in the nanoparticle form, in order to increase corneal penetration. This approach displayed specificity toward the transporter and high permeability compared to the standard treatment of ocular herpes – an ointment that operates superficially on keratitis caused by the herpes virus. The standard treatment fails in being efficient as most of the drug is lost. However, the formulation has not yet been approved by the FDA due to its side effects and does not reach deep tissues.

Stromal keratitis is a severe infection, which develops with proliferation of the virus deep in the cornea. Repeated incidences of stromal keratitis could cause severe damage to the cornea causing complete vision loss. In the United States, 50,000 such cases have been reported every year. The popular prodrug choice for ocular infections is acyclovir (ACV), which is an antiviral drug with high specificity for herpes virus. It is mainly used in a form of ointment to treat the superficial HSV keratitis. This treatment is limited to the superficial level and is not as effective for stromal keratitis at deeper tissues. For macular disease, synthesizing a lipophilic medication instead of hydrophilic may optimize the drug delivery since a hydrophilic drug is unable to cross the blood-retinal barrier [3].

The compound should have two main properties to acquire the optimum hydrophilicity and lipophilicity:

1. Sufficient permeability across the corneal layers
2. Sufficient solubility to be favorable for eye drops

Contrary to these properties, ACV is hydrophobic in nature with a low solubility in water (0.2 mg/mL) and poor corneal permeability. Prodrug derivation is a trivial approach to raise the corneal permeability of ACV. In the dipeptide prodrug approach, amino acids, like glycine, tyrosine and valine, are attached to ACV in combination with valine and synthesized to target specific drug transporter, PEPT. From these designed drugs, Gly-Val-ACV and Val-Val-ACV were found to be the most permeable since they were able to successfully target the PEPT. Combinations of stereospecific dipeptide prodrugs (L- and D-isomers) based on the former designed prodrugs were synthesized to scrutinize their permeability across Caco-2 cell monolayer.

Treating Corneal Epithelial Keratitis with Ganciclovir

Corneal epithelial keratitis occurs in the outermost layer of the cornea, while stromal keratitis occurs in the middle layer of the cornea [4]. Another prodrug that has been used to formulate stereoisomeric dipeptides is ganciclovir (GVC). It is an antiviral drug with a guanosine nucleotide used as a treatment for herpes. Similar to acyclovir, GCV has a low lipophilicity that prevents the drug from reaching deeper tissues [5]. After examination, Val-Val-GCV was found to enhance the cellular uptake of the drug via a peptide transporter, PEPT-1, attributed to the increased aqueous solubility as well as stability [5]. Hydrolytic enzymes in the human body, like esterases and peptidases, are responsible for breaking down the prodrug into parent drug. There are also known for their stereospecificity as well as affinity for L-isomers. The conversion of prodrug to parent drug is a two-step mechanism:

(i) Peptidase hydrolyzes terminal peptide bond to form amino acid derivative GVC.
(ii) Esterases break the ester bonds in Val-GCV compound to give GCV.

Novel prodrugs synthesized with GCV as a parent drug, DLGCV, LLGCV and LDGCV, could be considered for ocular diseases depending on the location of the infection. For instance, LLGCV has the quickest bioreversion and would be beneficial for the treatment of acute or superficial keratitis. On the other hand, DLGCV would provide the most prolonged release for chronic ocular conditions, since it is most enzymatically stable.

Various Methods to Delivery Drugs

Nanoparticles

The term nanoparticle refers to a particle having a size of nanometers (1–1000 nm), and nanoparticles serve as drug delivery systems [3]. Nanoparticles are being substantially researched for their ability to infiltrate the physiological barriers [3]. They demonstrate this ability in mostly two different forms: nanospheres and nanocapsules. Both forms allow the parent drug to be dissolved, encapsulated, entrapped, absorbed on the surface, and also dispersed within particles [3]. A range of polymers with varying chemical and physiological properties comprising of metals, lipids, and phospholipids have been used to prepare nanoparticles. With the use of this delivery system, poorly soluble drugs could be encapsulated as the high dissolution rates improve their solubility [3]. These particles play an important role in carrying the drug intact to the targeted site and releasing the drug over a prolonged time period [3]. The following calculations were applied to determine the entrapment efficiency and prodrug loading:

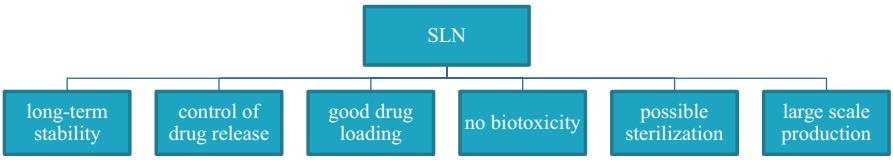

Fig. 20.3 Benefits of SLN (colloidal carriers)

$$\text{Entrapment efficiency}(\%) = \frac{\text{Amount of prodrug remained in nanoparticles}}{\text{Initial prodrug amount}} \times 100$$

$$\text{Drug loading}(\%) = \frac{\text{Weight of prodrug in nanoparticles}}{\text{Weight of nanoparticles}} \times 100$$

$$\text{Yield}\% = \frac{\text{Weight of dried nanoparticles recovered}}{\text{Sum of initial dry weight starting material}} \times 100$$

Solid lipid nanoparticles (SLN) are very reliable, nontoxic, and widely utilized particulates with a solid lipid core (Fig. 20.3). They are known to accommodate lipophilic drugs that are unable to solubilize in water by themselves. In the case of hydrophilic drugs, emulsions like microemulsions and water-in-oil emulsions have been examined to treat ocular disorders.

Conventional Dosage Versus Nanostructure

A myriad of issues have been tackled by nanotechnology in the various therapeutics regions; many of these were initially faced via the topical installation in the form of solutions, ointments, and suspensions. Even if the conventional method of treating the external has been prevalent, the tightness caused by corneal epithelium obstruction along with quick elimination of parent drug from the precorneal region and systemic absorption via conjunctiva has limited the amount of drug absorbed. This issue can be overcome by nanocarriers.

Challenges Associated with Drug Delivery

Although the stereoisomeric dipeptide prodrug approach significantly increases absorption efficiency, there are several issues in drug delivery of these prodrugs through oral administration. Biological barriers, like intestinal lumen as well as gastrointestinal mucosa, limit complete absorption since the peptides present have a broad range of substrate specificity to degrade the peptide-based prodrug [6].

Lipophilicity regulates passive diffusion of the intestinal lumen, and it has been found that an increase in lipophilicity causes a decrease in intestinal absorption [6].

At the nanoscale, the lipids of nanoparticles (SLN) are crystalline in nature and have complex properties [3]. This causes the overall temperature to decrease significantly compared to bulk lipids [3]. Frequently, the stored prodrug is ejected due to the lipid crystal transformation to a more preferred but space-consuming conformation [3]. Furthermore, nanoparticles could cause cell toxicity by the following:

- Attachment to the cell membrane because of the size
- Cellular internalization and disintegration
- Ionic charge (cationic surfactants are more damaging than anionic ones)
- Release of toxic by-products from disintegration

Future Applications: Described in Detail

The stereoisomeric prodrug approach has several useful applications in the field of target releasing [1]. These prodrugs possess a higher stability and have higher affinity toward peptide transporters at the corneal epithelium [5]. Delivering these drugs via nanoparticle formulation prevents degradation of the prodrug in precorneal and cellular enzymes allowing it to reach the targeted site [3]. The absorption of the parent drug in the systemic circulation and corneal tissues without much hindrance from any physiological barriers could be a great therapy for acute as well as chronic ocular diseases [5]. There are definitely some challenges to conquer including erroneous absorption rate, biotoxicity, early metabolic disintegration, and other side effects [1]. Such challenges encourage an extensive research to perfect the procedure that might result in even a wider range of application than the present ones.

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Part VI
Other Advances for Retina and Posterior Segment Diseases

Chapter 21
Receptor-Targeted Prodrug Approach for Retina and Posterior Segment Disease

Tejal Mehta, Viral Patel, and Om Prakash Sharma

Abstract Extensive research has been done in the field of ocular study, to develop and to enhance ocular bioavailability of drugs. Regardless it still faces challenges, as less than 5% of the dose administered reaches to the target site, which is insufficient to produce a pharmacological effect. The chemical methods including the development of prodrugs have proven to be a promising approach to improve ocular drug residence time and bioavailability.

Utilization of prodrugs for the treatment of posterior segment diseases was observed to be an innovative way to overcome barriers pertaining to drug delivery to the specific site. Prodrug effectively permeates the external ocular barriers, cornea and scleral tissues and has a greater partition coefficient. Prodrug approach offers a few points of interest like enhancement of drug solubility, stability, site-specific delivery, decreased toxicity and efflux pump evasion. This section stresses on hypothesis and uses of receptor-focused prodrug approach for ocular drug delivery systems.

Abbreviations

ACV	Acyclovir
ARMD/AMD	Age-related macular degeneration
AV	Arterial vein
BOB	Blood–ocular barrier
BRB	Blood–retinal barrier

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BRVO	Branched retinal vein occlusion
CRVO	Central retinal vein occlusion
EAAT	Excitatory amino acid receptors
GABA	Gamma amino butyric acid
GCV	Ganciclovir
HR	Hypertensive retinopathy
HSV	Herpes simplex virus
IV	Intravenous
MCTs	Monocarboxylic acid transporters
PCFT	Proton-coupled folate transporter
P-gp	P-glycoprotein
PHVP	Persistent hyperplastic primary vitreous
PVD	Posterior vitreous detachments
RBCs	Red blood cells
RFC	Reduced folate carrier
RVO	Retinal vein occlusion

Introduction

In the year 1958, the term prodrug was defined as a derivative of pharmacologically active compound which undergoes biological transformation either enzymatically or chemically to deliver the active parent drug, which is then capable of eliciting required pharmacological action. The journey of prodrug development began in 1899 with the development of methenamine, by Schering Corporation for the delivery of antibacterial formaldehyde to treat urinary tract infection, acetylsalicylic acid used as anti-inflammatory agent and which has been found less irritating than sodium salicylate, a blockbuster sulphanilamide which is a prodrug of Prontosil and the list continues [1].

In order to treat ocular disorders, the drug is delivered either locally or systemically. Local drug administration involves use of topically applicable products like eye drops, ointment, solutions, suspensions, etc. Figure 21.1 describes different approaches used for the drug delivery to different segments of the eye. Topical drug administration provides the advantage of preventing of systemic side effects. But it still faces challenges, as less than 5% of the dose administered reaches to the target site, which is insufficient to produce desirable pharmacological effect. With systemic drug administration, the blood–ocular barrier restricts the passage of the drug to intraocular tissues from systemic circulation. Hence, various approaches have been developed to enhance ocular bioavailability of drugs. Traditional methods used to increase ocular residence time of topically applied products involved increasing the viscosity of the formulations (solutions, suspensions, etc.), increased use of gels, polymeric inserts, micro- and nanoparticulate systems, etc., while the chemical methods included the development of prodrugs with an aim to improve ocular drug residence time and bioavailability.

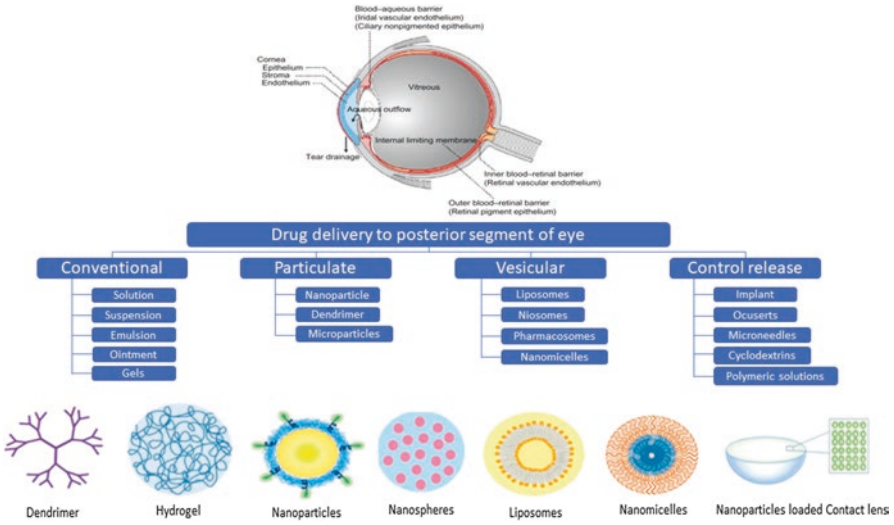

Fig. 21.1 Different approaches for drug targeting to posterior segment of the eye

Drug delivery targeting to posterior segment of the eye is a very a challenging task for formulations scientists. The use of prodrugs for the treatment of posterior segment diseases was found to be an innovative way to overcome barriers pertaining to drug delivery to the specific site. Prodrug effectively permeates the external ocular barriers, cornea and scleral tissues and has a greater partition coefficient [2]. Prodrug approach offers several advantages like enhancement of drug solubility, stability, site-specific delivery, decreased toxicity and efflux pump evasion. This chapter emphasizes on the theory and applications of receptor-targeted prodrug approach for ocular drug delivery systems.

Barriers to Posterior Segment

Due to rapid drainage of lacrimal fluid via nasolacrimal ducts, the therapeutic concentration of drugs could not be achieved in the posterior segment of the eye. This leads to low permeability and thereby poor bioavailability of drugs in posterior parts of the eye. The major reason behind limited permeation into the retina is due to blood–aqueous barrier (BAB) and blood–retinal barrier (BRB) which collectively contain blood–ocular barrier (BOB). This is a physical barrier between the local blood vessels, ocular tissues and fluids which restrict the passage of various solutes and fluids. These barriers can be static (e.g. sclera, Bruch's membrane-choroid (BC), retinal pigment epithelium (RPE) and conjunctiva) or dynamic type (e.g. drug clearance mechanism through blood and lymphatic vessels). This creates hurdle in absorption of systematically delivered drugs. Frequent administration of drugs leads to toxicities due to nonspecific absorption [3].

Bacterial and viral infections of the eyes are primarily treated by topical application of ophthalmic drugs. Other possible pathways for ocular delivery include transscleral and subconjunctival pathways, intraocular and periocular injections, etc.

Diseases of Posterior Segment/Retina of the Eye

The posterior segment of the eye comprises of everything that lies behind the lens. It consists of vitreous body, retina, sclera and choroid.

Diseases of Vitreous Body

The interior of the eyeball behind the lens is filled with jelly-like substance. This jelly-like substance consists majorly of protein, water and collagen. With the time, these components break down and form clumps that float within the vitreous body. As one moves their eyes, these clumps float as shadow and hinder the path of vision. These are commonly known as floaters.

Vitreous Liquefaction

It is a kind of degenerative change of vitreous. The cause of vitreous liquefaction is severe trauma to vitreous, thermal effects or radiation effects. Syneresis and opacities in the vitreous are usually associated with liquefaction. It is also called as black floaters in front of the eyes.

Vitreous Detachments

Posterior vitreous detachments

It is the detachment of cortical vitreous away from retina, in the posterior portion of the vitreous. It is a commonly occurring disorder above the age of 65 years of life. It is usually accompanied by the development of a hole in the posterior hyaloid membrane in the eyes with senile liquefaction. The space between the posterior hyaloid membrane and the internal membrane of retina is filled with synchytic fluid, resulting in PVD.

Detachment of the vitreous base and the anterior vitreous

The main cause of this is severe/blunt trauma causing medical emergency. Anterior retinal dialysis, dislocation of crystalline lens or vitreous haemorrhage may lead to detachment of the vitreous base and the anterior vitreous.

Vitreous Opacity

Vitreous body is composed of jelly-like transparent substance usually termed as vitreous humour. Any non-transparent material/structure falling in it will result in opacity. It will result in symptoms similar to that of floaters. Most common cause of such opacity includes muscae volitantes, persistent hyperplastic primary vitreous (PHPV), inflammatory vitreous opacities, vitreous aggregates and condensation with liquefaction, amyloid degeneration, asteroid hyalosis, synchysis scintillans, red cell opacities, tumour cells opacities, etc.

Vitreous Haemorrhage

Retinal vessel damage is the main cause of vitreous haemorrhage. It may be present as preretinal or intragel haemorrhage. The latter involves haemorrhage in anterior, middle, posterior or the whole vitreous body. There are many sources of vitreous haemorrhage like severe trauma to the eye, spontaneous retinal breakdown, inflammatory diseases involving erosion of vessel, vascular disorders (hypertensive retinopathy, retinal vein occlusion, etc.), metabolic diseases (diabetic retinopathy), blood dyscrasias, bleeding disorders (purpura, haemophilia and scurvy), neoplasms (retinoblastoma), etc.

Diseases of Sclera

Sclera consists of fibrous structures that cover five-sixth opaque part of the eyeball. It caters structural strength to the eye and protects the intraocular structures. The thickness of the sclera varies individually and with the age of the person. It is comparatively thick in posterior parts and gradually becomes thin in the interior parts. The inner surface of the sclera is separated from the choroid by suprachoroidal space. The canal of Schlemm is located in the anterior part of the sclera near the limbus. Various disorders of the sclera include:

Episcleritis

It is a form of recurrent inflammation involving the Tenon's capsule. Its exact etiology is not known. However, it is considered as a hypersensitivity reaction to endogenous tubercular or streptococcal toxins. It majorly affects adults having age above 50 years. It is twice more common in women than in men.

Scleritis

It is a protracted inflammation of the sclera. It is considered to be a sombre disease which, if not treated, may result in visual impairment and, in aggressive cases, loss of eyesight. However, occurrence of this disease is much less. It majorly affects elderly populations. It is usually associated with multiple conditions like metabolic disorders (gout, thyrotoxicosis, etc.), infections (chronic staphylococcal, streptococcal infection, herpes zoster ophthalmicus, etc.), granulomatous diseases (tuberculosis, leprosy, etc.), other conditions (irradiation, chemical burns, rosacea, etc.), surgery, etc.

Blue Sclera

It is portrayed by marked blue coloration of the sclera due to thinning of the sclera. It is an asymptomatic condition typically associated with osteogenesis imperfecta. Some other causes that may lead to blue sclera include high myopia, healed scleritis, Marfan's syndrome, buphthalmos and Ehlers-Danlos syndrome.

Diseases of the Retina

The retina is the most thin, delicate, transparent multilayered tissue of the eyeball. It is the most matured tissue of the eye that actually captures the light and transmits to the brain for interpretation. It has two types of light sensing cells: rods and cones. The rod cells are heavily concentrated in the peripheral parts. The main function of rod cells is sensing light and dark changes. The cone cells are present abundantly in central retina. Their main function is to identify different colors. The retina is the most critical part of the eye and is a home to various diseases. Some of the commonly occurring diseases involving the retina are discussed below:

1. Congenital and developmental disorders.
   (a) Coloboma of the optic disc: It results due to failure in closure of embryonic fissure. It arises in two forms: (i) minor defect (an inferior crescent is observed which is associated with hypermetropia or astigmatic refractive disorder) and (ii) fully developed (a very large whitish excavation is observed which is often associated with defective vision).
   (b) Drusen of the optic disc: They are characterized by papillary refractile bodies, which develop during childhood deep seated on the surface of the retina and emerge out during teen age. Ophthalmoscopically they are recognized as waxy pea-like irregular refractile bodies.
   (c) Hypoplasia of the optic disc: It is an anomaly associated with either the optic nerve or central nervous system. It is found to be due to the use of alcohol and certain drugs during maternity and diabetes. It has been found to be the reason of blindness at birth in many developed countries. The diagnosis is

difficult, but in typical cases, the disc size reduces and is encompassed by a yellow-colored ring.

(d) Medullated nerve fibres (opaque nerve fibres): This represents myelination of nerve fibres of the retina. In normal cases, the medullation of optic nerve begins from the brain and proceeds in descending manner towards the eyeball and the growth terminates at the lamina cribrosa. But in some cases, myelination of the optic nerve continues even after birth, and the growth increases beyond the optic disc. They then appear as a whitish patch present adjoining the optic disc.

2. Inflammatory disorders of the retina.

(a) Retinitis: It is again classified into two types – (i) nonspecific retinitis (it is occurring by invasion of pyrogenic organisms) and (ii) specific retinitis (it may be caused due to bacterial invasion (tuberculosis, leprosy, syphilis, etc.), viral infection (rubella, herpes zoster, etc.) or mycotic or parasitic origin).

(b) Retinal vasculitis: It is the inflammation of retinal blood vessels. It can be primary or secondary to uveitis. It involves inflammation of retinal veins which is characterized by recurrent vitreous haemorrhage.

3. Vascular disorders of the retina.

Different vascular disorders of the retina:

(a) Retinal artery occlusion: This disorder is more prevailing in patients suffering with hypertension and other cardiovascular diseases. Some commonly observed causes of occlusion are atherosclerosis, emboli, angiospasm, retinal arteritis, raised intraocular pressure, thrombophilic disorders, etc. the occlusion is usually unilateral and may be present in central artery or branched artery.

(b) Retinal vein occlusion: The occurrence of RVO is more common. It typically affects elderly population with age ranging from 60 to 70 years. Some of the causes of RVO include pressure on the retinal vein caused by sclerotic artery, increased viscosity of the blood (polycythemia), raised intraocular pressure, periphlebitis and some local causes (orbital cellulitis, sinus thrombosis, etc.). It may occur either in the central vein or in the branched vein. And hence, it is further classified as:

(i) Central retinal vein occlusion (CRVO) – Non-ischaemic CRVO: It is also called as venous stasis retinopathy. It is more common and is characterized by mild to moderate visual loss. Early examination reveals venous congestion and superficial haemorrhage. In later stages, it appears like a sheath around the vein.

(ii) Ischaemic CRVO: It attributes to acute and complete occlusion of central retinal vein. Early examination revels massive congestion and haemorrhage of the retinal vein. Neovascularization is observed near the disc and periphery. It is characterized by sudden visual loss.

(iii) Branched retinal vein occlusion (BRVO): It affects the main branch retinal vein causing hemispheric occlusion near the disc, the vein which is away from the disc, at the AV crossing leading to quadratic occlusion. Oedema and haemorrhage are restricted in the area weary by the damaged vein. Its occurrence is more as compared to CRVO.

4. Hypertensive retinopathy
   It occurs mainly in patients suffering from systemic hypertension. Increased vascular permeability, vasoconstriction and arteriosclerosis are the three main causes of hypertensive retinopathy. It is further classified based on the severity of occurrence, starting from grade I which is mild HR to grade IV which refers to severe HR.
5. Diabetic retinopathy
   In patients with diabetes mellitus, the retinal gradually undergoes several changes. It is associated with several risk factors like increase in duration of diabetes, being transmitted in heredity as a recessive trait, hypertension, smoking, obesity, hyperlipidaemia, pregnancy, etc. the disease progresses with occurrence of retinal ischemia followed by capillary leakage, retinal oedema, haemorrhage and neovascularization. In advanced cases it may lead to advanced diabetic eye disease which is characterized by uncontrolled proliferative retinopathy and may even end in causing blindness.
6. Sickle cell retinopathy.
   In patients suffering sickle cell anaemia, retinal hypoxia is the leading cause for retinopathy. It is caused by severe blockage in the blood vessels created by the abnormal shaped rigid RBCs. The disease slowly progresses following various stages – Peripheral arteriolar occlusion, peripheral arteriovenous anastomoses, neovascularization, vitreous haemorrhage and, lastly, vitreoretinal traction bands followed by retinal detachment.
7. Exudative retinopathy of coats.
   Idiopathic congenital vascular malformation of the retina (retinal telangiectasia) is called as coats' disease. It is characterized by yellowish exudates and haemorrhage of the retina which are associated with dilated retinal blood vessels. The disease progresses towards retinal detachment, and in late stages, cataract, uveitis and glaucoma may occur eventually causing phthisis bulbi.
8. Ocular ischaemic syndrome.
   It is a rare disorder caused due to chronic ocular hypoperfusion. It is a secondary disorder associated with carotid artery stenosis. Ulceration occurs in the carotid artery. Major factors leading to ischaemic syndrome include smoking, diabetes mellitus, hyperlipidaemia, hypertension, etc. various signs and symptoms are cornea oedema, dilated pupil, pain in ocular region, loss of vision, etc.
9. Photoretinitis.
   Also known as solar retinopathy or eclipse retinopathy, it is an injury caused to the retina by directly or indirectly viewing the sun. there are certain causes associated with solar retinopathy like solar eclipse observation with the naked eye, telescopic sungazing, religious sungazing, sun bathing, etc.; some of the clinical signs of the disease include the appearance of yellow spot with grey

margin in the foveolar and Para-foveolar region; lesions appear in pigment epithelium and in severe cases a macular hole may appear.

10. Age-related macular degeneration.
    It is a reciprocal disease and a leading cause of blindness in developed countries. Lack of proper nutrition, smoking, hypertension, exposure to sunlight heredity, etc. are some of the risk factors that may lead to ARMD. It is of two types:

    (i) Non-exudative: It is also called as dry geographic ARMD. Ninety per cent cases of ARMD are of non-exudative type. Patient suffers from distorted vision. It is characterized by development of pale region in the pigmented area of the retina. Small discrete yellowish elevated spots appear over the retina. In later stages, the atrophic areas enlarge, and the choroidal vessels become visible.

    Exudative: It is also called as wet or neovascular ARMD. The occurrence of this disease is rare, but compared to non-exudative ARMD, it rapidly progresses towards loss of vision. It passes through many stages – Drusen formation, REP detachment, choroidal neovascularization, haemorrhagic detachment of REP, neurosensory detachment and, lastly, disciform macular degeneration.

Criteria for Ideal Ophthalmic Prodrug

In order to overcome the physical, chemical or biopharmaceutical problems related with the parent drug molecule, a novel approach of designing prodrug for targeted drug delivery has emerged. Multiple issues associated with formulation development or drug delivery of parent drug molecule can be solved by designing prodrugs which would otherwise limit the clinical use of parent molecule. A schematic diagram of the prodrug transport is shown in Fig. 21.2. Below listed are some of the criteria for developing ophthalmic prodrug:

- Aqueous solubility: For water-insoluble or lipophilic drugs like steroids, aqueous solubility of the prodrug designed plays a crucial role, as most of the ophthalmic formulations are aqueous in nature.
- Partition coefficient: The prodrug must have sufficient lipophilicity in order to cross the cellular barrier and reach the target site. The ideal log P value range is 2–3.
- Chemical stability: The designed prodrug must be stable with sufficient shelf life to be formulated in the form of topical eye drop solution and suspension.
- Bioreversion: Upon reaching at the target site, the prodrug must cleave to parent drug. The active drug must be withheld at the target site for sufficient time to produce desired pharmacological action.
- Toxicity: The promoiety and the parent drug both should not have any toxicity of themselves. They should not cause cytotoxicity and irritability.

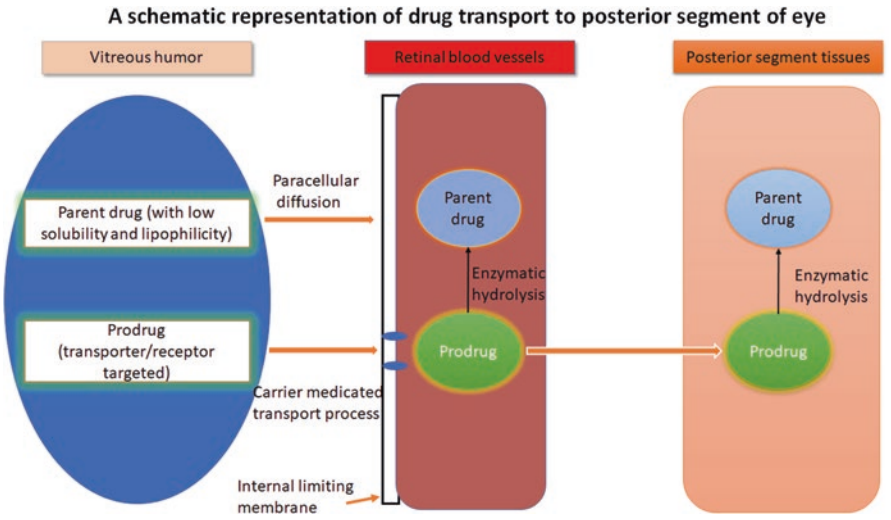

Fig. 21.2 A schematic representation of drug transport to posterior segment of the eye

Transporters/Receptors in Ocular Tissues

Delivery of drugs and prodrugs to ocular tissues involves different ocular routes like the cornea, conjunctiva, iris ciliary body and lens epithelium. Various transporters/receptors spotted on these tissues have been explored to revamp ocular drug delivery [4]. These transporters ameliorate site-specific drug delivery to posterior segment of the eye. Receptors are present in polarized manner in epithelium and endothelium for the purpose of transport of solutes. Receptors play a vital role in entry and exit of essential nutrients and ions across the cells. They can also act as efflux pump by driving out toxic metabolites of the cell. Various influx and efflux receptors are found in different parts of the eye. The influx receptors include amino acid, peptide, nucleoside, glucose, glutamate, organic cations, etc. [2]. The efflux receptors include (i) P-glycoproteins (P-gp) and (ii) multidrug resistance-associated proteins. A summary of various receptors present in different parts of the eye has been presented in Fig. 21.3.

Initially it was thought that membrane receptors were responsible for transferring nutrients across cell membranes, but later various studies have proved their role in the transportation of the drug molecules across various ocular tissues [5]. Targeting drug molecules towards these receptors will enhance their permeation across the membranes. The parent drug molecule can be translocated by this mechanism either by chemical modification or coupling to a ligand (which is a known substrate for the transporter). This prodrug, i.e. a drug with a promoeity, can be transported across the membranes, if it is recognized by the receptor. This prodrug upon reaching the targeted site is cleaved by the enzymes, thus, releasing the parent

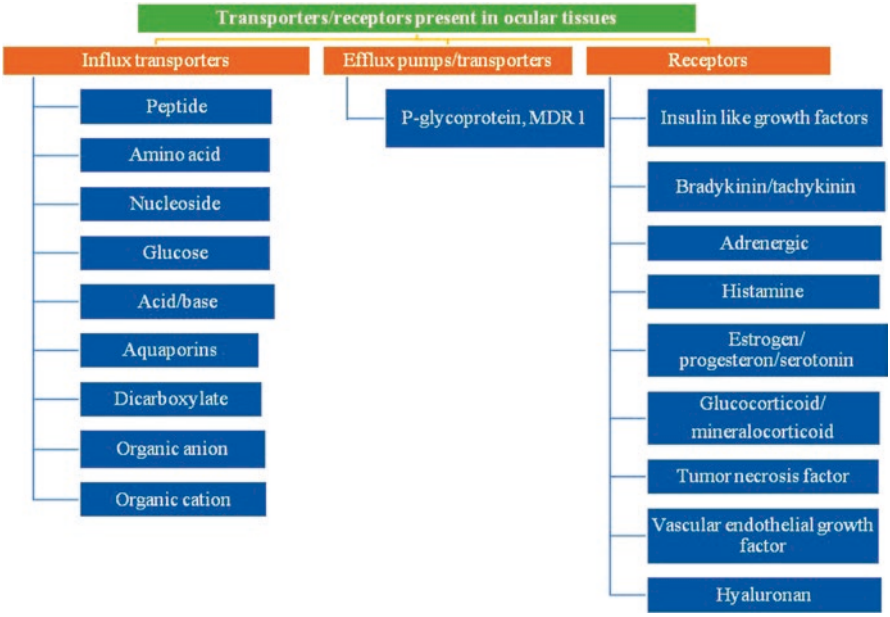

Fig. 21.3 Details of transporters/receptors present in different ocular tissues

drug molecule and the promoeity. The promoeity usually is a nutrient that doesn't cause toxicity concerns [4]. By appropriate selection of the promoeity, targeted drug delivery to posterior segments of the eye can be attained. A few notable transporters pertaining to posterior segment are discussed in the following section.

Peptide Transporters

Peptide transporters aid in translocation of dipeptides, tripeptides and peptidomimetics across the epithelial membranes. These membrane translocators are classified as PepT1, PepT2 and peptide/histidine transporters (PHT1 and PHT2). These transporters are proton coupled and are associated with transportation of a wide variety of substrates. Anand et al. reported that the dipeptide prodrug of acyclovir was less cytotoxic, highly water soluble and stable as compared to acyclovir, which showed excellent results in in vivo activity against herpes simplex virus 1(HSV-1). The permeability to the dipeptide prodrug of ACV was apparently the result of its identification by the oligopeptide transporters on the cornea, thereby showing increased amount in stromal tissues [6, 7]. Majumdar et al. conducted a study which reported that among different dipeptide monoester prodrugs of ganciclovir (GCV), val-GCV and gly-val-GCV serve as substrates for retinal peptide transport system. These prodrugs were stable in vitreous humour and were swiftly transformed into

GCV in retinal homogenates. Retinal GCV concentrations at the end of 5 h were almost identical, concluding these prodrugs to be potential candidates for treatment against human cytomegalovirus retinitis (HCMV) [8].

Amino Acid Transporter

Amino acid transporters are existing in BRB and intestinal mucosa and are found to be ubiquitous in nature, thus, leading to passage of only specific amino acids [9, 10]. Amino acid-based transporters can further be classified based on their sodium dependence, charge and substrate specificity. System B, $B^{0,+}$, IMINO, system X (anionic), ASC (cationic, anionic and neutral forms) and $ATB^{0,+}$ are the sodium-dependent transporters, and at the same time, system y^+ (cationic), $b^{0,+}$ and system L (large) are independent of sodium for transporting amino acids. LAT1 and LAT2 are the two isoforms, belonging to large amino acid transporter category. Gandhi et al. reported that the human cornea and rabbit corneal epithelium showed the presence of sodium-dependent, $B^{0,+}$ amino acid transporter and its interaction with the amino acid ester prodrugs [11]. Katragadda et al. investigated the in vivo corneal absorption of amino acid-based prodrugs of ACV using microdialysis technique in rabbits. They concluded that among L-alanine-ACV, L-serine-ACV, L-serine-succinate-ACV and L-cysteine-ACV prodrugs of ACV, L-serine-ACV was found to be most stable with high concentration till the last time point, thereby considering it to be a suitable prodrug for the management of HSV infection [12]. $ATB^{0,+}$is a substrate-specific transporter. Studies conducted with anionic amino acid-based prodrugs of ACV and ganciclovir showed the potential of $ATB^{0,+}$ for the delivery of antiviral drugs in the disease management of varicella zoster and herpes simplex virus.

Glutamate, GABA and glycine are the important amino acid-based neurotransmitters in the posterior segment of the eye [13]. In order to facilitate the neurotransmission, the extracellular glutamate concentrations within the retina need to be monitored [14]. Glutamate concentrations in the synaptic cleft govern the light stimulations with graded potential, which are responded by retinal neurons. Also, over-provocation of glutamate receptors causes neurotoxicity; thus, extracellular glutamate concentration needs to be regulated. Excitatory amino acid receptors (EAAT) located on the cell membranes maintain glutamate homeostasis within and outside the cell, thereby preventing its excitotoxicity. Overactivation of these glutamate receptors has been implicated in many retinal diseases [15, 16]. The presence of such transport system on the retina and the blood retina epithelium opens up opportunity for designing prodrugs targeting to posterior segment of the eye [17].

Vooturi et al. successfully synthesized prodrugs of gatifloxacin by modifying the amide bond. These modifications resulted in providing enzymatic stability to the prodrug until they reach the posterior segment [18]. The permeability study was carried out in the cornea, conjunctiva, sclera and retinal pigment epithelium using

chamber assembly and was compared with pure gatifloxacin. The formulated gatifloxacin prodrugs showed improved solubility and transporter-mediated permeability, thereby increasing its concentration in posterior segment of the eye [18].

Umapathy et al. concluded that amino acid transporters have potential for transporting α-carboxyl esters of neutral amino acids [19]. The α-carboxyl group of neutral amino acids can be altered by esterification to derivatize substrates that are transportable by $ATB^{0,+}$. If an antiviral drug, ganciclovir, is coupled with α-carboxyl group of valine by esterification, then it results in the formation of valganciclovir which becomes a substrate of $ATB^{0,+}$. Hatanaka et al. reported similar findings with valacyclovir, a valine-based prodrug of acyclovir [19, 20].

Yamamoto et al. reported that LAT1 and LAT2 are involved in L-leucine transport, thereby concluding that amino acid-based transport system can be investigated further for designing of different prodrugs that can improve ocular delivery and bioavailability via the retina into the posterior segments of the eye [21].

Monocarboxylic Acid Transport System

Proton-coupled MCTs transport lactate, pyruvate and other monocarboxylic acids across retinal pigment epithelium [22]. MCTs are abundantly found on the BRB, dwelling RPE and retinal micro-vessel endothelium. Among different types of MCTs, MCT1 and MCT2 have been explored in depth. MCT1 is enriched over RPE, muller cells, endothelium and photoreceptors, while MCT2 is expressed in Muller cell basal membrane. The location and distribution of these transporters propose that they play a key role in exchange of different monocarboxylic acid-based substrates [23, 24].

Glucose Transporter

Under normal conditions, the energy requirement of the retina is derived from glucose. Oxidative breakdown of glucose provides energy for various metabolic and electrochemical activities in the eyes. As reported by various scientists, the progression of ocular diseases is directly dependent on the metabolism and metabolic rate [25–28]. Researchers have identified seven isoforms of glucose transporters, GLUT1 to GLUT7 [28]. GLUT1 is a facilitative glucose transporter and is expressed in the cornea and retina [29, 30]. Apart from these, there are two isoforms of Na + −D-glucose transporters, SGLT1 and SGLT2, located in the conjunctiva and retinal cells [31–33].

Folate Transporters

Vitamin B9 is also named as folate. It is a water-soluble vitamin which binds to folate protein present in cell membranes. A synthetic analogue of folate and folic acid is crucial for the normal functioning of the visual system [34]. Its deficiency results in multiple abnormalities like optic neuropathy, retinal oedema and dysfunction and damage to photoreceptor cells, resulting in loss of visual function [35, 36]. Lipophilic nature of cell membranes prevents the transport of hydrophilic folic acid. The folate can cross cell membrane via three pathways – (i) folate receptors (FR), (ii) reduced folate carrier (RFC) and (iii) proton-coupled folate transporter (PCFT). FR-α and FR-β are two gene-coded FRs [37]. Folate-conjugated drug nanoparticles and micelles have been reported for targeting anticancer drugs in the form of prodrugs, since the tumour cells are found to overexpress FR [38, 39].

Drug Product Development Using Different Formulation Approaches to Target Posterior Segment of the Eye

Scientist all around the globe have worked for the development of different formulation strategies for efficient drug targeting to posterior segment of the eye [3]. Some of these approaches are discussed in Table 21.1.

In marketed formulations, numerous excipients are used in order to increase the drug penetration into ocular tissues. Commercially available controlled release ophthalmic drug delivery device “Ocusert®” was the first device containing pilocarpine and alginic acid. The invention of Ocusert was considered as one of the major innovations for topical delivery of ophthalmic drug delivery technology, but the complexity associated with the device insertion and removal resulted in poor patient compliance. The limitations of Ocusert lead to further invention of biodegradable polymers that made the use flexible. Various other nanotechnology-based products begin to be invented. Products that are approved by regulatory authorities across the globe in the past 5 years are discussed in Table 21.2 [69, 70].

Table 21.1 Approaches to target posterior segment of the eye

Route of administration	Formulation approach	Drugs	Treatment target	References
Intravitreal	Solution	Pegaptanib sodium	Wet AMD	[40]
		Fomivirsen	CMV retinitis	[41]
		Oligonucleotide		
	Implant (biodegradable)	Dexamethasone	Diabetic macular oedema, Uveitis, post-cataract surgery	[42]
		Ciliary neurotrophic factor	Retinitis pigmentosa and AMD	[43]
	Implant (non-biodegradable)	Fluocinolone acetonide	Chronic non-infectious uveitis, diabetic macular oedema	[44, 45]
		Triamcinolone acetonide	Wet AMD	[46]
		Ganciclovir	CMV retinitis	[47]
	Nanoparticles	Plasmid DNA	N/A	[48]
		Nile red, Rh-6G	N/A	[49]
	Vectosomes (light-sensitive)	Oligonucleotides	N/A	[50]
Transscleral	Suspension	Triamcinolone acetonide	Wet AMD	[51]
	Microspheres	RNA aptamer	Choroidal neovascularization	[52]
	Microneedle	Pilocarpine and sulphorhodamine	Various	[53]
	Episcleral implants	Betamethasone		[54]
	Osmotic pump	Immunoglobulin G	N/A	[55]
	Iontophoresis	Antibiotics, steroids and antiviral	Various	[56]
Subconjunctival	Microparticles	Celecoxib	Diabetic macular oedema	[57]
	Microspheres	Antisense TGF-b2 phosphorothioate Oligonucleotides	Post-glaucoma filtering surgery	[58]
	Gels	Dexamethasone and 5-fluorouracil	Post-glaucoma filtering surgery	[59]
		Cisplatin	Retinoblastoma	[60]
	Nanoparticles	Budesonide	Diabetic macular oedema	[61]
Periocular	Microspheres	PKC412	Choroidal neovascularization	[62]
Suprachoroidal	Microcannulation	Triamcinolone acetonide	Wet AMD	[63]
Intrascleral	Implant	Betamethasone	Uveitis	[64]
	Film (PLGA)	Ethacrynic acid	Glaucoma	[65]
Intravenous	Micelles	Dendrimer porphyrin	Choroidal neovascularization	[66]
	Liposomes	Verteporfin	Wet AMD	[67, 68]

Products Approved for Ophthalmic Drug Delivery in Last 5 Years

Table 21.2 List of products approved for ophthalmic drugs

Brand	Manufacturer	Drug	Approval year	Clinical use
Zerviate	Nicox	Cetirizine	2017, May	For the treatment of ocular itching associated with allergic conjunctivitis
Xiidra	Shire Development, LLC	Lifitegrast	2016, July	To treat the signs and symptoms of dry eye disease
Oralair™	Greer labs	Sweet Vernal, Orchard, Perennial Rye, Timothy and Kentucky Blue Grass Mixed Pollens Allergen Extract	2014, April	Grass pollen-induced allergic rhinitis with or without conjunctivitis
Omidria™	Omeros	Phenylephrine and ketorolac	2014, June	To prevent intraoperative miosis and reduce post-operative pain during and after eye surgery
Hetlioz ™	Vanda Pharmaceuticals	Tasimelteon	2014, January	Treatment of non-24-hour sleep–wake disorder in the totally blind
Cystaran™	Sigma Tau Pharmaceuticals	Cysteamine hydrochloride	2012, October	Management of corneal cystine crystal accumulation due to cystinosis
Jetrea™	Thrombogenics	Ocriplasmin	2012, October	Treatment of symptomatic vitreomacular adhesion
Lucentis™	Genentech	Ranibizumab	2012, August	Treatment of diabetic macular oedema
Zioptan™	Merck	Tafluprost ophthalmic solution	2012, February	Treatment of elevated intraocular pressure
Eylea™	Regeneron Pharmaceuticals	Aflibercept	2011, November	Treatment of neovascular (wet) age-related macular degeneration
Zymaxid™	Allergan	Gatifloxacin ophthalmic solution	2010, May	Treatment of bacterial conjunctivitis

Conclusion

The research on ocular diseases that results in visual or ocular impairment is ongoing and has shown promising outcomes in this era. This has become possible due to the novel strategies and newer targets identified which delivered the drug effectively to different segments of the eye. The prodrug approach is advantageous for the treatment of ocular diseases and at the same time challenging due to requirements of certain criteria like solubility, stability, safety. etc. Receptor-targeted drug delivery is an emerging area, which involves drug targeting to specific receptors that will enhance its absorption at targeted site. Research in this area suggests that the folate receptor is found ideal for tumour targeting compared to transferrin and vitamin B12. Some of the researches towards synthesizing receptor-targeted prodrugs have been commenced. However, the work in this area is not fully explored and has shown an urgent need to move faster in this direction to grab the opportunities for effective delivery of prodrugs for the treatment of ocular diseases of the retina and posterior segment of the eye.

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Chapter 22
Intravitreal Injection Drug Delivery for Retina and Posterior Segment Disease: Challenges and the Future Ahead

Tejal Mehta and Munira Momin

Abstract Intravitreal drug delivery system has been found to successfully cross the blood-ocular barrier and providing an immediate healing effect. Although these advantages help in overcoming the challenges faced by conventional treatment methods, intravitreal injections have to be given by a skilled physician with precaution. Currently, the novel implants could replace injections, which prevent the side effects. The implants or injections can contain formulations involving liposomes, nanoparticles, and hydrogels to administer sustained drug release to the ocular tissues. Sterilization is ensured via the use of membrane filters for aqueous and oil-based solutions. Meanwhile, stability of active ingredients is preserved by controlling its storage environment. The hydrogel contact lens drug delivery system and suprachoroidal space (SCS) system have displayed great improvement in targeting precision when tested on animals. With recent studies involving in vivo and ex vivo models, the chapter briefly talks about the advances as well as drawbacks in terms of tackling ocular diseases.

Introduction to Intravitreal Drug Delivery Systems

The increasing ocular diseases day by day demand newer approaches to be used for posterior ocular drug delivery systems. Moreover, the complex anatomical and physiological constraints of the eye are the hurdles in developing effective products for the posterior segment of the eye. An intravitreal injection is one of such kind. It is a shot of medicine which is applied into the vitreous, near the retina at the back of the eye. The inside of the eye is filled with a jelly-like fluid. The triamcinolone acetonide (Kenalog) was the first invented intravitreal injection for the treatment of macular edema. It has been explored in the treatment of AMD (neovascular age-related macular degeneration), CSME/PDR (clinically significant macular edema/

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proliferative diabetic retinopathy), endophthalmitis, uveitis, retinal vein occlusions, CNVM (choroidal neovascular membrane) secondary to multiple retinal diseases, etc [1, 2].

The advantages of intravitreal injections are as follows:

- Provides immediate and increased therapeutic effect in the intended retinal tissue.
- It bypasses the blood-ocular barrier to achieve constant therapeutic levels in the eye while minimizing systemic side effects.

The major limitations are as follows [3]:

- Intravitreal injections require experienced personnels for its administration. Also, appropriate size of syringeplay a significant role in accuracy, precision and reproducibility of the delivered volume.
- Frequent administration of drugs through this route can lead to retinal detachment, endophthalmitis, and increased intraocular pressure.
- Most of the drugs are rapidly cleared from the vitreous humor, and thus to achieve required therapeutic effect, multiple injections are given.

This strongly indicates huge need to carry out research in this newer area of injectable drug delivery.

Major advantages of intravitreal injection drug delivery over other ocular drug delivery systems:

Intravitreal drug administration is employed to overcome the inefficiency of topical ocular drug delivery to deliver therapeutic drug concentrations to the posterior segments of the eye. Systemic route of ocular drug delivery which may lead to side effects makes them less desirable as compared to intravitreal drug delivery. Intravitreal route of drug delivery comparatively is more invasive than periocular routes like subconjunctival, sub-Tenon, retrobulbar, and peribulbar administration. Intravitreal route enables direct delivery of drug in desired concentration to the retina and vitreous segment of the eye [4, 5]. Also, it aids in providing sustained drug delivery with the use of nanocarriers. Drugs delivered as solution and suspension disappear rapidly after topical administration, while with intravitreal drug delivery, it seems to be the most efficacious method for targeting molecules to the tissues in the posterior segment of the eye. Drug suspensions delivered by intravitreal route show prolonged drug residence by allowing slow and steady drug dissolution based on concentration gradient, thereby providing effective drug therapy and better patient compliance. With topical ocular drug delivery, there is higher tear dilution; hence, frequent drug administration is required for effective drug therapy, which can be avoided by intravitreal drug delivery. Therefore, in nutshell, intravitreal drug delivery has a winning edge over other ocular drug delivery systems when the purpose is to deliver targeted drug to the posterior segment of the eye. The general way of intravitreal injection administration and its distribution in the eye is shown in Fig. 22.1. As shown in the figure, upon intravitreal injection of the delivery system in the form of solution or suspension, the delivery system would behave depending on the composition of the delivery system. If the delivery is of simple solution or suspension of drug, it would be dispersed/distributed

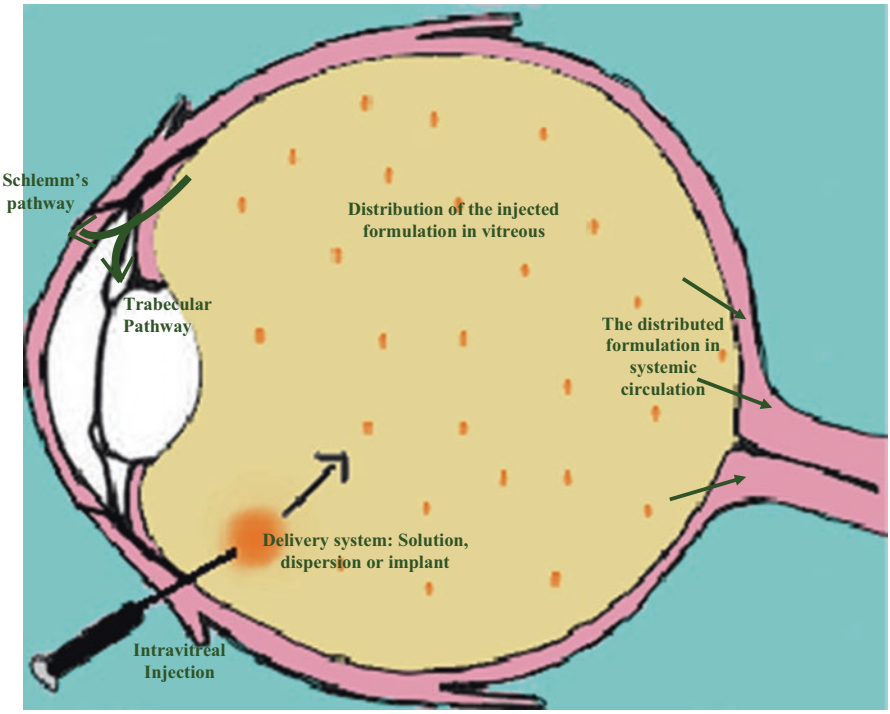

Fig. 22.1 The general way of intravitreal injection administration and its distribution in the eye

in the vitreous. If the delivery system is planned to be converted into an implant, it would behave accordingly. Upon distribution, the drug would solubilize and be available locally for its action. Some part of the drug will also enter systemic circulation as shown in the figure. Simultaneous to the distribution of the drug, the clearance also takes place either through trabecular meshwork pathway or Schlemm's pathway [6–8].

Types of Drug Delivery Systems

Various approaches are tried by researchers for delivering drug through intravitreal route. Conventionally direct intravitreal injections were used. However, the introduction of intravitreous implants has replaced them due to enormous advantages. Implantable devices like solid biocompatible implantable devices for sustained or controlled intravitreal osmotic mini-pumps, non-bioerodible and bio-erodible drug-loaded pellets, configured capillary fibers, biodegradable scleral plugs, scleral discs, polymeric matrices, and scaffolds of various geometries are tried by researchers. Literature proved high patient compliance of this drug delivery in terms of achieving sustained release, ease in removal in case of allergy, good targeting, higher drug level at site,

minimizing lower dose and side effects, etc. Biodegradable and non-biodegradable devices are widely used in intravitreal implants [9, 10]. Some of the new developments and work done toward implantable technology in this area are mentioned in Table 22.1.

Table 22.1 New developments and work done in intravitreal implants

Name of implantable technology	Description	Ref.
OphthaCoil	It is a drug-loaded mucoadhesive hydrogel on a thin-coiled metallic wire which ends are capped using an adhesive. It was tried using ciprofloxacin and showed promising results in bacterial infections of the posterior segment of the eye. Here, upon contact with tear fluid, the hydrogel coating swells, and drug is released into the tear film	Pitts et al.
Imprinted hydrogel contact lens device	This is a molecularly imprinted hydrogel device which controls drug release kinetics the results of using the same for ketotifen fumarate showed zero-order kinetics. As this device delivers drug in days, it is not suitable for chronic suppressive maintenance therapy which requires several weeks or months for treatment	Ali et al.
Cyclosporine-loaded discoid device	This device is developed for the constant release of cyclosporine (CsA) in inflammatory episodes of uveitis in horses. The result showed that CsA devices did not completely eliminated the recurrence of disease. However, the duration and severity of inflammation, cellular filtration, and pro-inflammatory cytokines RNA transcript levels were reduced in eyes which were implanted with cyclosporine loaded discoid device	Gilger et al.
Gelfoam-based device for the delivery of insulin	It is a soft and acidified absorbable gelatin sponge-based device (6 mm diameter) which, without presence of absorption enhancer, helps in the efficient absorption of drug into the systemic circulation. This is due to the change in Gelfoam due to reaction with acid which helps in the absorption of the drug from the device	Lee et al.
Retisert and Medidur devices	Retisert is a reservoir-based fluocinolone-loaded implant designed to deliver drugs for 1000 days. However, this device showed some risks and complications. Medidur is similar but small device. This is a reservoir-type non-biodegradable implant which is not sutured to the eyeball and floats freely in the vitreous space. This device release the drug for 3–4 months	Kuppermann et al.
Microelectromechanical device	It is an intraocular device governed by the principle of electronic control of drug delivery and provides targeted drug delivery. Electrolysis-actuated pumping is used to deliver drug to the posterior segment of the eye	Li et al.

Intravitreal injection drug delivery is administered in different forms which are discussed over here.

Liposomes

Conventional ocular intravitreal drug delivery requires repeated injections as many drugs are rapidly cleared from the vitreous humor. Liposomes, for ocular delivery, have been widely explored with formulation available on marketed product for treating macular degeneration to be administered intravenously. Liposomes, if administered intravitreally, may offer several advantages primarily of reducing the dose of drug required. They are being developed to incorporate a wide variety of drug molecules, proteins, nucleotides, and even plasmids giving them great potential for use in ophthalmology. But it has remained under critical discussion considering the limitations pertaining to delivery intravitreally and to formulation. Apart from expected limitations, toxicity of drug-associated lipidic vesicles intraocularly has been reported. Such toxicity is in the form of blurring effect or sparkling opacities in the lower part of the eye were addressed by PEGylating the vesicles, thereby, preventing ocular inflammation. With this concept, drugs like anti-infectious, anti-inflammatory, immunosuppressives, or antimetabolites have been reported to be less toxic when administered as liposomes. Keeping the observed toxicity apart and antivitreal delivery of liposomes, reports are published which exhibit increase in half-life of drug and residence time of formulation in ocular tissues and hence reduced dosing frequency of the drug. The metabolism or removal of injected liposomes can follow two pathways, i.e., through trabecular meshwork or through Schlemm's canal [11, 12].

Collectively, liposomes, so far explored for other route, have potential for intravitreal delivery by overcoming the toxicities and improving the formulation properties.

Nanoparticles

The conventional treatment to the eyes has restricted efficacy owing to certain formulation and physiological factors. Thus, having a delivery system and a route that can effectively deliver drugs into the eye without altering ocular physiology has been encouraged. Intravitreal route has opened passage for competent delivery of drugs within the eye. Nanosize-based formulation strategies can improve the efficacy of drugs to the eye. Such formulations are in a size range between 100 and 400 nm or up to 500 nm [13]. For such delivery, nanoparticles can modulate the release of drug from the nanoparticulate formulation by modifying the composition. The mechanism of penetration of nanoparticles within the ocular tissues, when studied through labelling, depicted multiple pathways. In order to enhance

penetration intravitreally, the surface of nanoparticles can be coated with specific polymer which induces interaction of nanoparticles with the ocular tissues and hence, allow permeation. Further, to reduce the clearance of drugs from the vitreous, polymeric nanoparticles are prepared which allow the drug to be released gradually. In spite of nanoparticles being a promising approach for intravitreal drug delivery, the effect of drugs administered via nanoparticles is a subject of the vitreous physiology and other factors like tear drainage, blinking of eye, etc. which has put boundaries around nanoparticles. Apart from these, accumulation in the eye, variable clearance of formulation, and drug from ocular tissues depending on ocular vasculature also determine the rate of efficacy of a formulation. Thus, studying and controlling these parameters can stimulate the applicability of nanoparticles in the intravitreal delivery. Moreover, the type of polymer used also influences this drug delivery [14–17].

Other Drug Delivery Systems

Apart from the direct nanoparticulate delivery to the vitreous, various other modifications to such systems have been reported. Pachis et al. have reported sustained release of flurbiprofen in the form of liposomal hydrogel. After conducting in vitro and in vivo studies, it was found that bioavailability of flurbiprofen was increased nearly twofold from the liposomal hydrogel as compared to other formulations. In another study conducted by Feiyan et al., micellar delivery of the prodrug delivery of cidofovir was done. The micelles of size nearly 250 micron were formed using lipid derivatized nucleoside analog, hexadecyloxypropyl-cidofovir. This formulation demonstrated a sustained release profile of cidofovir in vitro and good safety profile in rabbits. Injectable biodegradable thermogel delivery of dexamethasone has been researched by Zhang et al [18, 19]. The poly(lactic acid-*co*-glycolic acid)–poly(ethylene glycol)–poly(lactic acid-*co*-glycolic acid) (PLGA–PEG–PLGA) triblock copolymer formed the thermogelling system which behaved as a low-viscous liquid at low temperature and formed a non-flowing gel at body temperature. The release of dexamethasone could be modulated by varying ratios of triblock copolymers. The thermogel provided long-lasting retention intraocularly from 12 h to nearly 1 week as compared to drug suspension. The thermogel also demonstrated the safety in terms of retaining the morphology of the retina and cornea. Puras et al. explored cationic niosomes for intravitreal gene delivery [20]. After ocular administration, the expression of EGFP was detected in different cells of the retina depending on the administration route. This novel niosome formulation represents a promising approach to deliver genetic material into the retina to treat inherited retinal diseases. This indicates that variety of formulations can be used for intravitreal injection drug delivery. Controlling the formulation and process parameters is very essential for desired ocular drug release [4].

Characterization of Intravitreal Injection Drug Delivery

The characterization of this drug delivery is very complex and requires suitable methods to be utilized to thoroughly evaluate the same.

In Vitro Characterization

The nano-based formulations are widely applicable for this drug delivery. Thus, the evaluation of developed formulations for intravitreal drug delivery involves measurement of particle size, zeta potential, solubility, pH, surface morphology, clarity, sterility, and stability. Particle size and zeta potential measurements play a vital role in the development of formulations suitable for intravitreal drug delivery. For the measurement of particle size, various methods are employed – optical microscopy, laser diffraction particle analyzer, dynamic light scattering (DLS), electron microscopy (SEM, TEM, AFM), dynamic imaging analysis, Coulter counter test, light obscuration particle count test, and nanoparticle tracking analysis (NTA). Pharmacopeia specifications recommend that not more than 50 particles per ml may be of size >10 μm, not more than 5 particles per ml should be of size >25 μm, and not more than 2 particles per ml may be of size >50 μm. Among all the methods for particle size measurement, NTA is a new technique. Particles ranging between 30 and 1000 nm can be measured with the aid of NTA. This technique enables visualization and recording of the particles in a solution using laser light scattering microscope attached with the instrument. High rate of precision is obtained in the size measurements in comparison to other methods. However, the only limitation with this technique is requirement of large volume of sample.

Potentiometric methods are usually employed for the pH determination of solutions, drops, suspensions, and in situ gels. pH range between 7 and 7.4 is considered ideal for the formulations that are to be instilled in the eye.

The general tests for sterile products as mentioned in pharmacopeia are also very essential. The formulations that are developed for the ophthalmic drug delivery should be free from particulate matter. The clarity and sterility of these formulations play a key role during developmental stage. A novel method for determining clarity of the formulations involves the use of transmittance measurement using UV-visible spectrophotometer. This method is specially employed for the medicated contact lenses. The medicated lenses are hydrated with saline and are then placed on the quartz cuvette. The transmittance is measured at wavelengths between 200 and 1000 nm.

Sterility is the basic requirement for the drug forms developed for intravitreal administration. The sterility of the formulations is examined by direct inoculation of samples for aerobic and anaerobic microorganisms, on two different microbiological media: thioglycolate media and soyaneam casein digest media for aerobic bacteria and fungi. Membrane filtration method (indirect method) is now widely employed for water- and oil-based solutions. Filters usually consist of cellulose nitrate with

pore size of 0.45 μm. Products containing fatty bases, such as ointment, are sterilized by indirect method. Apart from these characterizations, examination of preservative content is conducted using relevant analytical technique. Also, the multidose ocular products are challenged for preservative content.

The purpose of stability study is to ensure that the potency of the active ingredient is retained during the process of formulation development. The stability requirements for ophthalmic formulations must comply with International Conference on Harmonization's criteria. The storage conditions for active ingredients should enable thermal stability and prevent degradation caused due to humidity and light. Stability examination should be carried out in different conditions which must be in correlation with the warehousing, transport, and usage conditions that are in compliance with ICH guidlines.

Other in vitro characterization includes viscosity measurement using viscometers, osmolarity examination, and refractive index measurements with the aid of refractometers or ellipsometers.

Ex Vivo Characterization

In literature, various methods are reported for the ex vivo examination. They include bottle method, modified rotating basket method, modified flow-through cell, and Franz diffusion cell. Among all, Franz diffusion cell is widely used. The apparatus is designed to have two compartments: donor and receptor. The receptor compartment is thermoregulated at 37 °C ± 0.5 °C and is filled with artificial tear fluid or phosphate buffer pH 7.4, which is subjected to continuous stirring with aid of magnetic stirrer, usually at 50 rpm. The donor compartment resides above the receptor compartment. At the junction of two compartments, diffusion cell membrane or eye membrane is uniformly placed. The formulations containing the drug are placed over the membrane. At different specified time points, required aliquots are withdrawn and analyzed for drug release. This method gives precise estimate of drug release and so it is the official method of estimation of drug release.

In Vivo Characterization

The animal models are developed for various researchers for studying permeability and release of drug in the eye. Some of the in vivo models are discussed here [21].

Microdialysis Rabbit Model

The exceptionally poor delivery of drug from the blood to vitreous urges the administration of large systemic dose of medications to achieve intravitreal therapeutic level. Administration of such large doses systemically may result in the emergence

of adverse effects of the drugs. In lieu of this, microdialysis method, initially developed for examination of neurotransmitters in the cerebrum, was modified for intraocular utilization.

Microdialysis with Mountable Probe

The assembly for microdialysis consisted of a probe, bent at 60–90°, with both inlet and outlet tubes mounted on a solitary, stiff plastic tube, with a membrane attached at its end. Probe is inserted in the vitreous through an opening in the sclera. This model eliminated risk of bacterial contamination; the probes are well tolerated in rabbits and functions for about 21 days. This model has advantage over the sustained release system that it can be used both for administration and collection of compounds from the vitreous around the dialysis membrane. Administration can be continuously modified or even interrupted, and there is no volume expansion in the eye because the molecules enter by diffusion, obviating the need for vitrectomy.

Microdialysis with permanently implanted probes

In this method, a tube of outer diameter of about 0.6 mm with a dialysis membrane mounted on one end is used for dialysis. The open end of the tube is turned toward the site of origin where dialysis takes place, i.e., mainly the retina. The mounted membrane is composed of polycarbonate-polyether copolymer, with an optical density of 0.52 mm and I.D. of 0.4 mm.

Hydrogel Contact Lens Drug Delivery: Rabbit Model

The anaesthetized healthy New Zealand white rabbits (using isoflurane gas) received contact lenses, over the surface of both eyes, treated with beclomethasone, prednisolone, ranibizumab, or saline for injection (control). Ensuring that the lenses were placed properly, the fur surrounding the eyelids was closed with standard surgical tape without undue stress on the eyelids. Anaesthesia was discontinued after 4 hr. of inoculation period followed by removal of tape and then contact lenses. The removed lenses were examined to ensure that they remained flat on the surface of the eye. Unless otherwise specified, this method was practiced on days 1, 2, 5, 8, and 10. On day 11, animals were anaesthetized as described above, and blood was collected prior to euthanasia. After plasma collection from sampled blood, ocular tissues, i.e., cornea, conjunctiva and limbal areas (anterior segment), the vitreous humor, retina, macula, and sclera (posterior segment), were excised and snap-frozen in a liquid nitrogen bath until evaluation.

Suprachoroidal Space (SCS): Pig Model

Various studies conducted for supracolloidal space delivery include use of different animal models to study for efficacy of formulations, one of the studies reported used the pig model in which the animals were sedated using ketamine (10 mg/kg) and maintained with an intravenous propofol drip, titrated to effect, followed by conjunctival peritomy from 7.00 am up to 10.00 am exposing clear access to the 9:30 region of the equatorial sclera. Two parallel 3 mm radial scleral incisions were made to expose the bare choroid. Here, the second sclerotomy accommodated the solid rod GRIN endoscope. Viscoelastic was used to facilitate the posterior drug delivery system (PDS) in the SCS at the entry site. After the PDS was inserted, a light pipe was introduced through a separate 2 o'clock pars plana sclerotomy to illuminate the beacon probe tip in the perimacular region. This is followed by cannulation; the illuminated endoscope was used to image the PDS in the SCS. Last, the scleral and conjunctival incisions were closed using 7–0 Vicryl suture. The animal was maintained under sedation and returned to the imaging suite. The fluorescein angiography, indocyanine green (ICG), red-free, and infrared images were again observed. The study showed good targeting of drug through this drug delivery (Fig. 22.2).

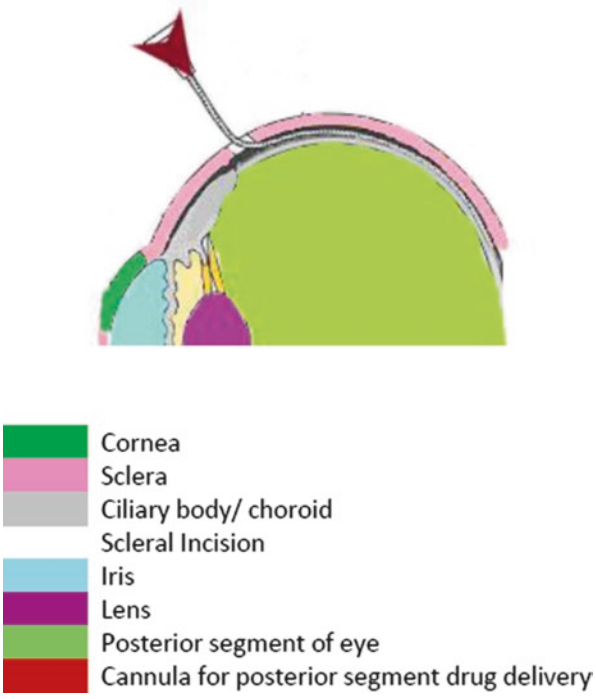

Fig. 22.2 Posterior delivery system cannula in suprachoroidal space (SCS). (Modified from Timothy et al. [22])

Advances and Limitations of Current In Vitro, Ex Vivo, and In Vivo Retinal Models

The research in this area led many new developments and improved therapy. Some of them are discussed in brief over here [3].

(i) *Advances and Limitations in* in vitro *Models*: Developing the in vitro models that mimic and correlate to in vivo fluid dynamics and drug clearance is really challenging. However, several groups have successfully developed compartmental models to accelerate the drug development process in IVT administration. For instance, Awwad et al. developed a pharmacokinetic model in administration of ranibizumab that displayed a clearance time of 8.1 ± 3.1 days comparable to humans (Awwad et al., 2015) [23]. The group also tested clearance of bevacizumab in the presence or absence of albumin and drug release of Kenalog®. The model could significantly mimic minimum and maximum vitreous clearance, effect of protein on fluid dynamics, protein function, and stability and can be used as an IVIVC tool. Haghjou et al. developed finite volume model for quantitative assessment of commonly used ophthalmic drugs. The group investigated the permeability of 32 drugs through intravitreal injection and found correlation between the physiochemical properties of drug and permeability. The model can be used for the prediction of colloidal systems without the use of experimental data [3].

The major limitation is that these in vitro models cannot be perfectly correlated with the diseased conditions, and the drug clearance and pharmacokinetics differ widely in conditions like chronic inflammation.

(ii) *Advances and Limitations in* ex vivo *Models*: Ex vivo models for intravitreal drug delivery serve advantageous as it does not involve the approval of ethics committee, can contain a large sample for study and thus yield statistically significant results, and is comparatively cheaper as compared to in vivo models since it does not include the cost of maintenance. Ex vivo models in this delivery has been used to investigate the effect of physiochemical properties of drug on distribution to different regional areas. For instance, Kadam et al. investigated the influence of the drug property on the pharmacokinetics and targeted delivery of drugs through different routes using ex vivo white rabbit eyes and concluded that suprachoroidal route serves targeted delivery for the retina and choroid [24].

The major limitations in ex vivo models are that these models do not directly correlate to the pharmacodynamics, biodistribution, and diseased conditions

like macular degeneration, chronic inflammation, changes in volume, and viscosity of vitreous humor in diseased conditions.

(iii) *Advances in* in vivo *Models*: Fernández et al. examined the pharmacokinetic profile through intravitreal injections through radiolabeling PET studies. The studies found that the chronic inflammation increased the retention of molecules in the eye as compared to the normal eye. Moreover, studies reflected that the volume of injection did not affect the overall clearance.

The main limitations in in vivo models include-, rats are difficult to handle and have comparatively small volume of vitreous humor volume as compared to humans.

The Future Ahead

The current intravitreal injection products in the market include bevacizumab (Avastin), ranibizumab (Lucentis), triamcinolone acetonide (Kenalog), ganciclovir, foscarnet, cidofovir, fomivirsen, methotrexate, vancomycin, ceftazidime, amikacin, and many more. The in vitro models for intravitreal drug delivery are new and need to be investigated to warrant their use for effective IVIVC. Nevertheless, several investigations have proven the IVIVC using appropriate models. However, during the establishment of such correlation, physiochemical properties of drugs have to be considered as their interaction with proteins, clearance rate, and distribution varies greatly according to the diseased conditions. The most promising new treatments in development are anti-PDGF therapies and smaller anti-VEGF products for treating AMD in the future. The reason for the same is their stronger binding capacity which improves its efficacy and provides sustained drug release. Other developments include RTH258, a small, humanized anti-VEGF antibody fragment which is in development phase by Alcon (Fort Worth, Texas) and Novartis (Basel, Switzerland) that inhibits all isoforms of VEGF-A. Allergan is developing an anti-VEGF DARPin® (abicipar pegol), a small, engineered protein that mimics the action of an antibody with high specificity and binding affinity. The anti-PDGF agent Fovista (Ophthotech, New York) is being developed and is currently in phase 3 clinical trials. Corticosteroids like Alimera (Alpharetta, Ga.) and pSivida (Watertown, Mass.) have partnered to manufacture ILUVIEN, a non-biodegradable implant indicated for DME that elutes the steroid fluocinolone acetonide to provide zero-order kinetics that allows it for release up to 3 years. ILUVIEN received FDA approval for the treatment of DME in the USA in September 2014, and the drug is expected to be commercially available in the country soon. It is commercially available in the UK and Germany, while for other countries the approval process are in pipeline.

Conclusion

Intravitreal injections are an upfront technique that address the challenges of delivering drugs into the vitreous body. The drugs given through this route have long retentions in ocular segment owing to restricted exposure to the vasculature and the viscous vitreous which allows gradual distribution of the drugs. Though this route has manifested many advantages to vitreous delivery as compared to the other routes, certain limitations have been reported like blurred vision, the variable retention of drugs in vitreous body, and accumulation of formulation components in lower part of eye. In addition to this, the pharmacokinetics of the drugs within ocular segment is varied as it depends on the type of formulation and its composition, physicochemical properties of drugs and anoumt of lacriamal fluid. Various formulations can be administered through this route in the form of solution, suspension, implants and nanoparticulate systems like nanosuspensions, liposomes, etc. Nanosystem based formulations can be embedded or dispersed in a suitable vehicle for drug delivery. These formulation strategies predict promising future for treatment of ocular disease, but research is required for developing formulations devoid of toxicity. Thus, considering the pros and cons of the route and the delivery systems, intravitreal injections can be considered as a milestone in ocular drug delivery with close monitoring of the adverse effects expected to be observed. New therapies have appeared, but there is still a lot of room for improvement as several unmet medical needs prevail and need to be addressed in order to ameliorate efficacy, safety, and applicability of this drug delivery system.

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Chapter 23
Thermoresponsive Gel Drug Delivery for Retina and Posterior Segment Disease

Komal Parmar, Jayvadan K. Patel, Deepak Bhatia, and Yashwant V. Pathak

Abstract Significant melioration has been done in the optimization of drug delivery to target the tissues in the eye and gain maximum therapeutic efficacy with the drug doses within the organ. Thermoresponsive drug delivery systems propose immense prospective among their equivalents due to their flexibility in design, targeting ability and in situ temperature sensible phase transitions. The approach can be found advantageous for specific applications as it does not require organic solvents in formulation, copolymerization agents for gel formation or any externally applied stimuli for gelation. Thus, this chapter focuses on the emerging thermoresponsive gel technology for drug delivery to anterior and posterior segment of eye disease. This review examines the characteristics of this system including design, assessment and optimization and merits and limitations of thermoresponsive gels for ocular therapy. Future potential of the system for targeted and sustained drug delivery of drugs to ocular part is also presented.

Keywords Thermoresponsive gels · Retinal delivery · Target delivery · Posterior segment

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Introduction

Eye as the most important organ of human body senses is mentioned first. The posterior segment of the eye which consists of retina, choroid and optic nerves serves the most in maintaining a good visual sense. Disease prevailing in this important division may lead to impairment or total loss of vision. Irreversible vision impairment could result from the disorders like age-related macular degeneration, diabetic retinopathy, glaucoma, retinitis pigmentosa, cytomegalovirus retinitis, retinal detachment, ocular melanoma and retinoblastoma [24, 69, 84]. Modern era of ophthalmic pharmacology has been started for which new drugs are being developed for the treatment of different segments of eye disease. The route of drug delivery made to reach the back of the eye included topical, systemic, intraocular, periocular and intravitreal [63]. Major limitation of the conventional drug delivery for the anterior and posterior segment of eye disease includes extensive precorneal loss resulting from drainage and tear fluid turnover which in turn lead to the need of developing novel targeted drug delivery systems for eye disease. Thus, recent advances are made in delivering the drug molecules to the posterior pole of the eye and thereby enhancing the contact time of the drug and therapeutic efficacy [14, 31, 81, 82, 93]. Despite the development of new ocular drug delivery systems, targeting the drug to the posterior portion of the eye remains the noteworthy challenging assignment. The purpose of this review is to illustrate the thermoresponsive gel technology development with its evaluation for drug delivery to the retina and posterior segment of eye disease.

Thermoresponsive Gel Technology

Thermoresponsive/thermosensitive gel technology is a class of ‘smart’ polymer technology that have the ability to respond to the external stimuli: change of temperature. These smart polymers imbibe water and convert into gel form instantaneously once the gelation temperature is achieved. This characteristic of the gelling materials exhibits a wide range of pharmaceutical applications and thus draws the scientific attention. Thermoresponsive gel forms three-dimensional highly interconnected polymeric matrices consisting of water within the entanglement. After absorbing water, these polymers form micelle-like aggregates and rise to highly viscous fluids.

Owing to their excellent responsive characteristics, water intake capacity and compatibility with the biological tissues, thermoresponsive gel technology is extensively investigated for effective drug delivery, biomedical applications and in tissue engineering [7, 8, 33, 70, 87, 95, 102]. Thermoresponsive gel technology can be utilized for a wide variety of drug molecules, viz. hydrophilic, hydrophobic and macromolecules like proteins and peptides [9, 38, 61, 95]. Drug release kinetics follows a mechanism like drug diffusion, swelling of gels, mechanical squeezing of

gel and degradation of gel. The inter-polymer repulsive and attractive forces, chemical composition of hydrophilic and hydrophobic macromers, hydrophilic-hydrophobic interactions, polymer structure, molecular mass, degradation rate and affinity between drug and polymer play an important role in determining the swelling of polymeric gel and release kinetics of drug from the thermoresponsive gels [5, 12, 22, 29, 32, 33, 45, 52, 64]. Thus, factors mentioned above can be tuned to manipulate the swelling and thereby release kinetics of drug from the gel network. Recently the thermoresponsive gel system is investigated much for the treatment of posterior segment of eye disease. Phase behaviour, design, development, challenges and applications of the thermoresponsive gel technology for delivering drug molecules to the posterior portion of eye are reviewed further.

Phase Behaviour of Thermoresponsive Polymers

Volume phase transition is observed by the thermoresponsive polymers at certain temperature which changes their solubility properties. The ideal system is a solution which acts as a free flowing fluid at normal room temperature and converts into gel at body temperature [73]. The common characteristics of these polymers are critical solution temperature which means the temperature at which the polymer solution will undergo phase separation. Having lower critical solution temperature (LCST), thermoresponsive polymers remain in soluble form below LCST and show precipitation with rise in temperature. This is the crucial and most important process for the application of thermoresponsive polymeric gel into technology for drug delivery [9]. Below the LCST, hydrogen bonding is the driving force for the solubility of polymeric material in water, whereas above the LCST, volume phase transition occurs where the attractive forces of the hydrogen bonding are favoured between the macromers as equated to the water molecules. This leads to coil-to-globule transition (Fig. 23.1). The phenomenon is reversible; with lowering of temperature, the system again favours hydrogen bonding leading to solubilization of polymer into the solvent [51, 60, 80, 91].

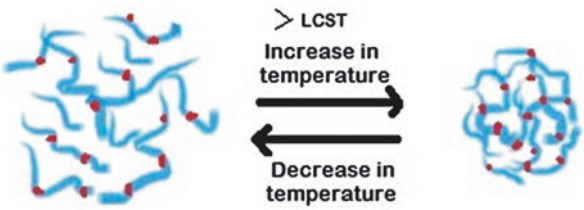

Fig. 23.1 Reversible coil-to-globule transition of thermoresponsive polymers

Design, Characterization and Optimization of Thermoresponsive Gels

Chemical and physical properties of the components of thermoresponsive gels should be taken into consideration for design and formulation for ophthalmic use. Compatibility of polymeric components and ocular portion and release kinetics from the gel formulation are influenced by hydrophilic-hydrophobic interactions of polymeric chains, degree of crosslinking and interaction between drug and polymer. Additionally the presence of additives such as salt, surfactant and co-solvent affect the volume phase transition and thereby transition temperature. Generally synthetic polymers are used to formulate thermoresponsive gels. However, many natural polymers have been revealed to show gelation upon change in temperature. Thus, investigators have exploited natural polymers to fabricate thermally responsive polymers with desired characteristics.

Biodegradable semisynthetic polymers included cellulose derivatives, viz. methylcellulose. It shows thermoreversible gelation in aqueous solution, with sol-gel transition in the temperature range of 60–80 °C and gel-sol transition upon cooling [40, 50, 86], whereas hydroxypropyl methylcellulose (HPMC) exhibits phase transition between 75 °C and 90 °C [79]. However, it has been found that the LCST of the HPMC was observed to be reduced drastically to 38 °C in the presence of acrylic acid (AA). This was attributed to hydrogen bonding and hydrophobic interaction of the two different polymeric molecules leading to shifting of LCST [99]. Thermosensitive chitosan-polyol salt gels have been developed until 2004 which forms monolithic gels at body temperature [72, 73]. More recent advancement includes formation of thermosensitive gel by incorporation of poly(ethylene glycol) (PEG) into chitosan with no additional crosslinking agent [6]. An approach based on a dextran polysaccharide is reported. A dextran macromer containing oligolactate and 2-hydroxyethyl methacrylate units (Dex-lactate-HEMA) showed LCST below 32 °C [33]. Gelatin also exhibits thermoreversible properties. Researchers have successfully examined its gelling ability in response to thermal transitions. It is observed that upon increasing the temperature, gelatin shows a random coil conformation in the solution which upon cooling exhibits a continuous network [25, 30, 67, 98].

Thermoresponsive hydrogel based on poly(*N*-isopropylacrylamide) (pNiPAAm) and its copolymers is the most investigated thermoreversible system [13, 43, 56, 65, 66, 101]. It belongs to non-biodegradable polymer and shows sharp phase transition with an LCST at about 32 °C in water. Addition of hydrophilic monomers increases the LCST, while incorporation of hydrophobic monomers shows decline in LCST [19]. Jeong and co-researchers described thermosensitive and biodegradable hydrogel based on poly(lactic acid). Block copolymer with polyethylene oxide (PEO) and poly(lactic acid) showed sol state at 45 °C and gel state at body temperature [41]. A thermosensitive gel consisting of poly(lactic acid-co-glycolic acid)-poly(ethylene glycol)-poly(lactic acid-co-glycolic acid) (PLGA-PEG-PLGA) has been demonstrated. The solution is transparent solution at 4 °C which converts into opalescent gel at body temperature [97].

Triblock copolymers poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide) (PEO-PPO-PEO), known also as Pluronics® or poloxamers, are another group of polymers utilized for the formulation of thermoreversible systems. The poloxamer series covers a range of liquids, pastes and solids, with molecular weights and ethylene oxide-propylene oxide weight ratios varying from 1100 to 14,000 gram/mole and 1:9 to 8:2, respectively. By optimization in composition, molecular weight and concentration of the polymer, gelation can be achieved at physiological temperature and pH. Their amphiphilic structure is due to hydrophilic ethylene oxide and the hydrophobic propylene oxide, which can self-assemble to form micelle in aqueous solution and is dependent on the temperature changes [10, 94, 103]. Many researchers have successfully investigated poloxamers for thermoresponsive gel formulations for ocular drug delivery [17, 53, 57, 68, 90, 92].

Characterization of prepared thermoresponsive gels is an important parameter after its formulation, before the gel system can be employed for controlled drug delivery system via ocular route. Characterization study includes ascertainment of physical properties, confirmation of chemical structure and evaluation of biological properties of the formulation. Confirmation of chemical structure can be determined by attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), nuclear magnetic resonance (NMR) spectroscopy and mass spectroscopy [33, 34]. Surface chemical composition can be assessed by using X-ray photoelectron spectroscopy [76, 89]. Crystallinity and glass transition temperature of thermoresponsive gels can be evaluated by X-ray diffractometer [26, 55] and differential scanning calorimeter [36, 37, 49]. Thermoresponsive gelling properties can be ascertained by measuring swelling ratios as a function of temperature [33, 34]. Dynamic viscosity of the gel is measured by rheometer [68]. Porous morphology of thermoresponsive gel can be determined by scanning electron microscopy [12, 58, 85]. Mechanical properties of thermosensitive gel formulations can be assessed by using a texture analyser [23]. Cytotoxicity studies of thermoresponsive gels can be carried out by cell viability assays with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) [11, 62] and 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) [43]. The abovementioned characterization studies give an important information regarding physical, chemical and biological status of the thermoresponsive gels which is further required for the optimization purpose for desired ocular drug delivery demand.

Optimization of thermoresponsive gels provides an accurate composition of additives for its successful formulation. Optimization of thermoresponsive gels depends on various factors affecting the process of gelation and release of drugs from the gel. Concentration of the thermoresponsive polymers utilized determines the diffusion-based release of drug from the gel matrix, and it affects the breakdown of the gel network as well. However, the concentration of polymers depends on the aqueous solubility of gelling polymers and/or the viscosity of the polymeric solutions. Thus, high concentrations of polymers can be utilized by selecting low molecular weight gel precursors. Different rates of drug release may be desirable

for different applications and can be achieved by varying the concentration and polymers utilized for gelation. Fast-release thermoresponsive gels can be prepared by using surfactant-based formulation which may facilitate dissolution of drug in the physiological fluid and help in further diffusion process. Slower gelling formulations with high-viscosity polymers may give bioadhesion and help in sustaining the drug release for prolonged period of time [32].

Application of thermoresponsive gel is sometimes inapt clinically as interacting polymeric solution may have to be kept either in extra-protective storage condition or form a reconstituted preparation. During application via injection, the needle may be clogged because of pre-conversion of solution into gel form which might be due to change in temperature during handling.

Intravitreal Administration for Ocular Drug Delivery

Administration of pharmacotherapies via intravitreal route dates to the mid-1940s and, in the last decade, had a major contribution in ophthalmic practice. Indications of intravitreal pharmacotherapies include choroidal neovascular membranes, diabetic macular oedema, ischaemic neovascularization, inflammatory and infectious processes and neoplasia. Drug delivery by intravitreal route has been used in frequent manner in the treatment of retinal disorder. Various approaches for drug delivery have been studied for vitreoretinal disease [100]. Sustained drug delivery for the treatment of posterior segment of the eye is now feasible with ocular implants given via intravitreal route, such as Vitrasert™, Retisert™, Iluvien™, MicroPump, Ozurdex™ and Verisome™. Vitrasert™ is a reservoir implant device consisting of a drug pellet of ganciclovir coated with polyvinyl alcohol (PVA), a permeable polymer that regulates drug diffusion and ethylene vinyl acetate (EVA), an impermeable polymer that controls the area through which a drug is released [48, 75]. Retisert™ is an insert which exhibits constant release of fluocinolone acetonide for the treatment of noninfectious uveitis for 2.5 years [3, 39]. Iluvein™, an intravitreal implant (Alimera Sciences, Inc.), contains fluocinolone acetonide indicated for the treatment of impairment of vision associated with chronic diabetic macular oedema. The recommended dosage is one implant containing fluocinolone acetonide 190 μg (0.2 μ/ day) for which drug is released for at least 3 years [42, 77]. A novel microdrug pump, MicroPump, for intravitreal drug delivery for up to 1 year, was studied, and results suggested biocompatibility of the device with eye [28, 35, 78]. Ozurdex ™ is a slow-release dexamethasone containing intravitreal implant. It shows duration of efficacy for at least 3 months [4, 20]. Verisome™ is a biodegradable formulation containing triamcinolone acetonide to deliver the drug for up to 1 year for the treatment of macular oedema [54].

Intravitreally injectable thermoresponsive gels have been investigated recently, which contains several microliters of volume of formulation injected via fine needle. These polymeric solutions are liquid at ambient temperature and get converted into gel form as it comes in contact with physiological fluid having certain

temperature. Thermoresponsive gels consisting of poly(ethylene glycol)-poly-(serinol hexamethylene urethane), a thermoresponsive polymer, have been investigated for intravitreal administration of bevacizumab, a monoclonal antibody for its cytotoxicity efficacy [71]. Poly(ethylene glycol)-poly-(serinol hexamethylene urethane)-based thermoresponsive gel is biocompatible in vitro and in vivo and can maintain concentrations of bevacizumab five times higher than the controls. Self-assembled thermoresponsive nanogels have been studied for the ophthalmic delivery of Muscone, a poorly soluble drug. A dispersion of Muscone-loaded micelle nanogel of poloxamer 407 solution has been used as an intravitreal injectable thermoresponsive gel-forming solution [92]. Poloxamer 407, a triblock polymer consisting of a central hydrophobic block of polypropylene glycol edged by two hydrophilic blocks of polyethylene glycol, is in solution form at ambient temperature and undergoes temperature-induced phase transition around body temperature. Nanogels produced a 3.4-fold increase in apparent permeability coefficients. Thermoresponsive gel using poly(*N*-isopropylacrylamide) (PNIPAAm), cross-linked with poly(ethylene glycol) diacrylate (PEG-DA) has been investigated [88]. Proteins such as bovine serum albumin and immunoglobulin and drugs such as bevacizumab and ranibizumab were encapsulated into the gel. PNIPAAm with lower critical solution temperature at ~32 °C is a non-biodegradable hydrophobic polymer which is cross-linked with PEG that induces hydrophilic property into thermoresponsive gels [43]. Poly(d,L-lactide-co-glycolide) (PLGA) microspheres in thermogelling PLGA-PEG-PLGA gel consisting ganciclovir has been studied. The formulation exhibited mean vitreal concentrations of the drug at approximately 0.8 μg/mL for 14 days as compared to only 54 h with direct injections [16].

However, intravitreal thermoresponsive gel implants/injections can efficiently deliver the drug to the posterior segment of the eye; the process is invasive and can cause complications such as cataract formation, glaucoma, vitreous haemorrhage, choroidal detachment, retinal detachment, hypotony, vitreous loss, intraocular inflammation and endophthalmitis [18, 74, 83].

Conjunctival Administration for Retinal Therapy

Conjunctival retinal drug delivery is considered as better option as compared to invasive intravitreal therapy. The subconjunctival sac provides enough space for the administration of modified drug release formulations to the posterior segment of the eye [27, 47, 59]. However, conjunctival tissue constitutes about 80% of the total ocular space in humans but is less efficient than the intravitreal route of drug delivery. On the other hand, blood retinal barrier and retinal pigment epithelial cells act as a barrier for the absorption of drug via subconjunctival route; it is reported that molecules of the size up to 70 KDa can cross the sac and reach the retina [1, 2, 46, 59]. Various approaches in novel drug delivery systems have been applied in order to achieve prolonged drug release in the posterior segment of the eye, viz. polymer implants, nanoparticles and microparticles. Implants were fabricated using

polyvinyl alcohol, a non-reactive biocompatible polymer with 2 mm diameter. Sustained release of cytochalasin E, an epoxide containing a fungal-derived metabolite, was achieved from the implant with initial release of 9.8 ± 3.0 μg/day over the initial 4 days followed by constant release of 4.7 ± 0.8 μg/day between days 5 and 28 [46]. Subconjunctival injection of carboplatin-loaded dendrimer nanoparticle was developed for the treatment of transgenic murine retinoblastoma. Poly(amidoamine) (PAMAM) dendrimers were fabricated with an objective of improving the solubility properties of carboplatin. Results implicated significant reduction in mean tumour burden compared with the conventional formulation [44]. Fu et al. developed subconjunctival injection loaded with dorzolamide microparticles for lowering intraocular pressure in glaucoma. Dorzolamide was encapsulated with poly(ethylene glycol)-co-poly(sebacic acid) (PEG3-PSA) microparticles which released the drug over 12 days in vitro. However, in vivo fluorescently labelled PEG3-PSA microparticles were detected for at least 42 days [21].

In situ gel of curcumin-loaded nanoparticles was formulated using thermoresponsive polymers such as poloxamer 188 and poloxamer 407. Polymeric solution was liquid at 30.9 °C that transformed into gel form at 34.2 °C in the presence of simulated tear fluid [57]. Conjunctival vaccine delivery was fabricated using a thermoresponsive in situ gel of poloxamer 407 loaded with polymeric antigen BLSOmp31. Mucoadhesive in situ gel of chitosan was also prepared as comparative drug delivery system. Results depicted better performance of thermoresponsive in situ gel than mucoadhesive gel [15]. Ocular delivery of cyclosporine A was developed using thermoresponsive hyaluronic acid-based in situ forming microgels. Hyaluronic acid-g-poly(N-isopropylacrylamide) (HA-g-PNIPAAm) was the thermoresponsive polymer synthesized by the researchers and investigated for the drug release. The LCST of HA-g-PNIPAAm solution was found to be 32.7 °C. HA-g-PNIPAAm microgels achieved significantly higher drug concentration levels (1455.8 ng/g of tissue) as compared to commercial eye drops [96].

Future Directions

In succinct, thermoresponsive gels have potential to modify the release of different types of drug, such as anti-vascular endothelial growth factor, anticancer agents, anti-inflammatory agents, antibiotic agents and antigens via ocular route. The formulation facilitates sustained release of drug in the posterior segment of the eye. Apart from prolonged release of therapeutic agents, thermoresponsive gels are also responsible for improving the release properties of poorly soluble drugs. Likewise it has been proposed that thermoresponsive hydrogels might prove an efficient drug delivery system to deliver large macromolecules to the posterior segment with sustained release over time. Presently, intravitreal route of administration is the most efficient way for the posterior segment drug delivery. However, it is a highly invasive method with some complications. Thus, another subconjunctival route of administration can be chosen as an alternative.

Future directions may include formulation and design of smart thermoresponsive gels with an objective of controlled and sustained drug delivery to the retina. Various factors pertaining to drug-polymer interactions, physicochemical properties of therapeutic agents and interactive characteristics of smart polymers are needed to be considered while formulating thermoresponsive gel. Nanoscale particulate system encapsulated in thermoresponsive gel formulation directs promising future of targeted and controlled drug delivery in the treatment of disease of posterior segment of the eye.

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Chapter 24
Ocular Delivery of Peptides and Proteins

Rajiv Dahiya and Sunita Dahiya

Abstract The delivery of protein and peptide therapeutics through ocular route requires considerable knowledge and understanding about eye's anatomy and physiology. Although this delivery route has high potency and specificity, it exhibits difficulty in absorption through barriers resulting in lower bioavailability as well as crucial stability issues. Due to the complications associated with the most common injectable route for the peptide and protein delivery, there is a surge for the noninvasive route such as ocular which include intravitreal and periocular route. Newer techniques for delivery of these macromolecules involve targeting transporters or receptors to enhance specificity, while approaches such as nanocarriers, prodrug, mucoadhesion, and permeation enhancers have been employed to attain enhanced bioavailability. This chapter addresses pros and cons of ocular delivery of peptides and proteins, significant features of their chemistry, potential and challenges associated with their local and systemic delivery, as well as different ways to attain better protein bioavailability and stability.

Background and Introduction

Although extensive research efforts have been carried out for modifying the drug release of small molecules for the conventional oral delivery [1–8], peptide and protein therapeutics are the macromolecules whose delivery requires special approaches due to associated inherent problems. Recent biotechnological advancements have resulted in the development of many novel therapeutically active proteins and

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peptides. These biomacromolecules are potent therapeutics and are indicated for several chronic conditions such as cancer, hepatitis, diabetes, rheumatoid arthritis, and leukemia [9]. However, several pharmaceutical and biopharmaceutical challenges limit their clinical application. It is a well-known fact that the route of administration has a significant impact on the therapeutic outcome of a drug. Parenteral administration with the needle and syringe is a well-established choice for the therapeutic protein and peptide delivery till now, but it has some drawbacks related to patients such as requirement of frequent injections due to short in vivo half-life of proteins and peptides, associated pain, and poor patient compliance if it is continued for longer time. Also, from the formulation point of view, cost, sterility, etc. are major concern with parenteral protein and peptide delivery. Thus, the noninvasive routes are assumed to have added importance in protein and peptide drug delivery, and these include nasal, ocular, buccal, vaginal, transdermal, and pulmonary routes.

The unique and peculiar anatomy of the eye poses several barriers to ocular drug delivery. These barriers may be static/tissue barriers, circulatory and clearance systems, as well as metabolic barriers. The main routes of ocular drug delivery are topical, systemic, intravitreal, and periocular. For anterior segment disorders, topical route is the most common and least invasive. Systemic administration has limited efficacy due to the presence of blood-ocular barriers. Intravitreal injection can deliver therapeutic amounts to posterior ocular tissues but is invasive. Periocular route is less invasive and proven successful in anterior segment diseases but yet to be proven for posterior segment conditions.

Protein and peptide delivery via ocular route is an interesting but delicate approach, and a good understanding of the physiological parameters of the eye is necessary before designing an ocular product. For example, low drug-contact time and poor ocular bioavailability due to the drainage of solution/suspensions, tear production (lacrimation) and turnover, and consequent dilution are key problems [10]. Moreover, the main challenge in ocular peptide and protein delivery is to circumvent the protective barriers of the eye so that the therapeutic molecule can penetrate into the bio-milieu quantities sufficient to treat ophthalmic diseases or to exert its pharmacological action [11]. Although conventional drug delivery systems such as solutions, suspensions, gels, ointments, and inserts have been investigated for controlled ocular delivery, they suffer from problems such as poor drainage of instilled solutions, tear turnover, poor corneal permeability, nasolacrimal drainage, systemic absorption, and blurred vision. Advanced drug delivery systems have been developed with the intention of optimizing and controlling the delivery of ocular therapeutics to the target sites. This can be achieved either by increasing its penetration across the mucosa or by prolonging the contact time of the carrier with the ocular surface and have shown promising results [11, 12].

To date, most of the proteins and peptides that have been delivered to the eye have been for the treatment of local ocular disorders. Although ocular route for systemic delivery of peptides and proteins suffers from limitations such as the poor permeability through a membrane, enzymatic degradation, and low capacity for transport, some significant efforts have been made in this area. These include the ocular delivery of insulin and also the use of various nanocarriers for controlled and/

or targeted delivery [13–15]. The prodrug approach has also been developed to overcome the poor membrane permeability of peptides. The present chapter deals with different aspects related to peptide and protein delivery via ocular route for treatment of local and systemic disorders.

Significant Pathways of Ocular Administration

For most diseases of the anterior segment, topical instillation of eye drops is the preferred and conventional route of treatment. However, it is associated with limitations such as limited penetration to tissue of interest, the need for repeated dosing, rapid washout by tear and lachrymal drainage systems, and poor patient compliance. These factors limit the tissue bioavailability of topical route to less than 5%. Although there are some reports of attempts to treat posterior segment diseases by topical delivery [16, 17], it is not a viable route due to the presence of multiple tissue barriers to drug delivery and huge wastage of the drug due to the low bioavailability of such treatment methods, in addition to the possibility of systemic side effects. Systemic route of administration is used for treatment of certain conditions of the anterior and posterior segments, such as antibiotics and medications for severely raised intraocular pressure (IOP) or for pain relief. This route of administration, however, has limited success in treatment of posterior segment disorders due to the presence of blood-ocular barriers, particularly the blood-retinal barrier.

For posterior segment disorders, the intravitreal delivery method is the most preferred route. This is due to the ability of intravitreal route to deliver the therapeutic agents in close proximity to the target tissue. However, certain chronic conditions affecting the posterior segment such as age-related macular degeneration (AMD) and diabetic macular edema (DME) require repeated intravitreal injections. These repeated injections may lead to several complications such as retinal detachment, cataract, and endophthalmitis. The viscosity of the vitreous humor hinders diffusion of drugs and macromolecules and leads to nonuniform diffusion kinetics and distribution profiles of drugs delivered intravitreally. Small molecules can distribute rapidly and in more quantity through the vitreous, compared with large molecules [18].

Periocular routes of drug delivery are gaining popularity in recent times as a noninvasive alternative to intravitreal delivery. In this, the drug molecules (with or without entrapment in carriers) are introduced in the subconjunctival, retrobulbar, posterior juxtascleral, or sub-Tenon spaces, without affecting the integrity of the eyeball, from where it reaches the target tissues (the retina or retinal pigment epithelium [RPE] usually) [19]. Of the periocular routes, the subconjunctival route is considered less invasive as compared to subretinal, suprachoroidal, or sub-Tenon routes of delivery. The drug administered by the periocular route may reach the posterior segment by any of the three different pathways: transscleral diffusion, systemic circulation through the choroid, or the anterior pathway through the tear film, cornea, aqueous humor, and vitreous humor. Although this method has its own limitation, as the drug washout is high and the drug needs to pass through static, dynamic, and

metabolic barriers to achieve therapeutic levels at the site of action [18], it can be made viable if the drugs are protected from washout and clearance. This can be achieved by entrapment of the therapeutic agent in carriers such as microparticles or nanoparticles, or polymer films or implants, to provide protection from degradation/clearance as well as sustained delivery. The chronic nature of several ocular disorders also benefits from such a sustained delivery approach, which can help to deliver the therapeutic dose over a longer duration.

Major Considerations in Ocular Delivery of Peptides and Proteins

Ocular Delivery of Peptides and Proteins: Pros and Cons

Therapeutic proteins and peptides, due to their high potency and selectivity, are very attractive from the pharmaceutical point of view. But their instability and low bioavailability make their administration through non-parenteral routes very difficult. This is a fact that hampers efficient exploitation of peptides and proteins in spite of their great potential in therapeutics. Since about last five decades, significant amount of research in the area of drug delivery and nanotechnology has been done in order to overcome those hurdles. In particular, biodegradable and biocompatible lipid and polymer-based nanocarriers have emerged as promising delivery platforms to enable the administration of proteins and peptides. Proteins are vital constituent of the body as they perform many of its major physiological and biological processes. Recently, proteins and peptides have attracted much attention as potential treatments for various dangerous and traditionally incurable diseases such as cancer, AIDS, dwarfism, and autoimmune disorders. Furthermore, proteins could be used for diagnostics. At present, most therapeutic proteins are administered via parenteral routes that have many drawbacks, for example, they are painful and expensive and may cause toxicity. Finding more effective, easier, and safer alternative routes for administering proteins and peptides is the key to therapeutic and commercial success. In this context, much research has been focused on noninvasive routes such as nasal, pulmonary, oral, ocular, and rectal routes for administering proteins and peptides. Unfortunately, the widespread use of proteins and peptides as drugs still faces many obstacles such as low bioavailability, short half-life in the bloodstream, in vivo instability, and numerous other problems. In order to overcome these hurdles and improve protein/peptide drug efficacy, various strategies have been developed such as permeability enhancement, enzyme inhibition, protein structure modification, and protection by encapsulation. These strategies are discussed in subsequent sections of this chapter. Although ocular route of protein delivery presents many advantages as it is easier and faster than traditional injection routes, ocular protein drug administration protects proteins by avoiding gastrointestinal and hepatic first-pass metabolism which leads to the low bioavailability of proteins and peptides administered through the oral route [20, 21]. However, again many drawbacks limit

the widespread use of ocular protein delivery route, such as low bioavailability due to poor eye membrane permeability for hydrophilic macromolecules like proteins. In addition, the ocular tissue contains many enzymes such as protease and aminopeptidase that can degrade the proteins and peptides administered [22].

Challenges in Ocular Delivery of Peptides and Proteins

Unique properties such as high hydrophilicity, large size, structural fragility and complexity, as well as substantial physical and chemical lability strongly influence the pharmacokinetic and pharmacodynamic behavior of the peptide and protein drugs in vivo. They also limit the reactions, solvents, and environmental conditions that can be used in the preparation and application of protein- or peptide-based pharmaceuticals. At the same time, drug delivery to the eye is also a challenging task due to peculiar anatomical features of the eye. That is why the technique to formulate peptide or protein ocular drug delivery system is very different from formulating conventional small molecule drugs. Additionally, the delivery of a peptide or protein-based drugs is particularly challenging due to the protein instability issues. For all kinds of systemic delivery except intravenous, one of the first barriers for absorption is the permeation across a cell layer. Being highly vulnerable molecules, proteins usually present short in vivo half-lives, due to degradation by enzymes, either at the site of administration or in every anatomical location, on their way to the site of pharmacological action. Moreover, being charged, large, and hydrophilic, proteins are notoriously poor permeators giving rise to poor bioavailability. Therefore, it is often necessary to add enhancers to the protein formulation. In formulating ocular dosage forms of protein and peptides drugs, an intelligent selection of additives which enhance their absorption across membranes and their stability is very significant. The physical size of the protein drugs and their susceptibility to degradation are key determinants of their delivery route. Noninvasive delivery of proteins would be very desirable [23, 24], and there have been interesting efforts to develop oral protein formulation similar to the ones continuously going on with regard to insulin. Unfortunately, these efforts are being hampered by the low bioavailability. The clinical approaches for successful delivery of peptides and proteins are continuously going on for the most common eye disorders including age-related macular degeneration, diabetic macular edema, cataract, proliferative vitreoretinopathy, uveitis, cytomegalovirus, and glaucoma.

Low Absorption

The specific challenge in designing a therapeutic system is to achieve an optimal concentration of a drug at the active site for an appropriate duration in order to provide ocular delivery systems with high therapeutic efficacy. The anatomy, physiology, and barrier functions of the cornea compromise the rapid absorption of drugs which necessitates frequent instillations of eye drops to maintain a therapeutic drug level in

the tear film or at the site of action. But at the same time, it must not be neglected that the frequent use of highly concentrated solutions may induce toxic side effects and cellular damage at the ocular surface. Precorneal loss factors including solution drainage, lacrimation, tear dynamics, tear dilution, tear turnover, conjunctival absorption, nonproductive absorption, transient residence time in the cul-de-sac, and the relative impermeability of the corneal epithelial membrane are responsible for poor bioavailability of drugs from ocular dosage forms for anterior segment drug delivery following topical administration. Due to these physiological and anatomical constraints, only a small fraction of the drug, effectively 1% or even less of the instilled dose, is ocularly absorbed. To be clinically effective, topical formulation has to possess balance between lipophilicity and hydrophilicity with higher contact time [25].

Barriers to Transport

The barriers encountered by protein molecules before reaching the back of the eye vary depending on the mode and/or site of administration. The outermost ocular barrier is the conjunctival epithelium (CE) or the cornea, depending on the site of topical application. The epithelial pore sizes of palpebral and bulbar conjunctiva are 4.9 ± 2.5 nm and 3.0 ± 1.6 nm in diameter, respectively [26]. CE limits the penetration of molecules between 20 and 40 kDa and often acts as a mechanical barrier to the transport of larger protein molecules. Upon crossing the CE, there are additional barriers apart from molecular weight, that are associated with enzymatic degradation and vascular drainage. During penetration after subconjunctival administration, one of the major barriers is the blood flow of episclera and conjunctiva along with the lymphatic flow. The next major barrier for protein transport is the sclera, which comprises an interconnected network of collagen fibrils that combine to form interlacing fiber bundles or defined lamellae [27]. In addition to molecular weight, the shape and charge of the proteins are also important criteria for scleral transport. Positively charged proteins could bind to negatively charged proteoglycans in the sclera, which would result in reduced permeability across sclera compared with that achieved with negatively charged proteins. Also, it was observed that proteins that are globular in shape have better permeability than those that are linear. Furthermore, protein transport into the retina is restricted by both the outer limiting membrane (OLM) and the inner limiting membrane (ILM) of the retina. The endothelial tight junctions of retinal vessels comprise the zonulae occludentes, which are known to limit the entry of proteins. It is generally considered that large proteins with molecular weight of 50–75 kDa cannot penetrate into the retina. However, some studies indicate that proteins up to 150 kDa can diffuse across ILM, which has a pore size of 10–25 nm. In the case of intravitreal administration, reported studies suggested that proteins such as bevacizumab could penetrate well into the retina, RPE, choroid, and outer segments of the photoreceptors. This suggests that an active transport mechanism is involved in the transport of protein drugs from vitreous humor to blood [9, 28].

Peptide and Protein Chemistry

Fundamental Structural Features of Peptides and Proteins

Proteins are the workhorses of our bodies which make up about 42% of the dry weight of our bodies. Proteins are made up of natural polypeptide chains having 50–2000 amino acid residues. Insulin is a polypeptide hormone consisting of two linear chains A and B of 21 and 30 amino acids, linked by disulfide sulfur-sulfur bridges between cysteine residues. The human insulin protein is made up of 51 amino acids and has a molecular mass of 5808 Da [29]. It is a dimer of an A-chain and a B-chain. Insulin from animal sources differs in carbohydrate metabolism effects from human insulin because of the variations in insulin structures. Insulin molecules have a tendency to form dimers in solution due to hydrogen-bonding. Moreover, in the presence of zinc ions, insulin dimers associate into hexamers. These interactions have vital clinical consequences. Monomers and dimers readily diffuse into blood, whereas hexamers diffuse poorly. So, absorption of insulin preparations containing a high proportion of hexamers is delayed and somewhat slow. This phenomenon has stimulated the development of a number of recombinant insulin analogs.

Cyclosporine is a lipophilic cyclic undecapeptide with a selective immunosuppressive action. It has 11 amino acids, including a novel amino acid, (4*R*)-4-((*E*)-2-butenyl-4,N-dimethyl-L-threonine [30]. Immunoglobulin G (IgG) antibodies are large molecules of about 150 kDa made of four peptide chains. IgG contains two identical class γ heavy chains of about 50 kDa and two identical light chains of about 25 kDa, thus a tetrameric quaternary structure [31]. The two heavy chains are linked to each other and to a light chain each by disulfide bonds. The resulting tetramer has two identical halves, which together form the Y-like shape. Vancomycin is a branched tricyclic glycosylated peptide with bactericidal activity, whereas ganciclovir is a synthetic analog of 2′-deoxy-guanosine which is used to treat cytomegalovirus (CMV) infections [32]. Vasoactive intestinal peptide (VIP) is a peptide hormone 28 amino acid residues that belongs to a glucagon/secretin superfamily, the ligand of class II G protein-coupled receptors. VIP is present in the peripheral and the central nervous systems where it functions as a nonadrenergic, noncholinergic neurotransmitter or neuromodulator [33, 34]. Figure 24.1 shows structures of some therapeutic proteins/peptides (a) cyclosporine, (b) insulin, (c) immunoglobulin G, (d) vancomycin, and (e) vasoactive intestinal peptide.

Comparison of Linear and Cyclic Peptides

Although linear peptides are associated with pharmacological activities, cyclic peptides dominate over them due to the fact that inherent flexibility of linear peptides leads to different conformations which can bind to more than one receptor molecules, resulting in undesirable adverse effects. Further, cyclization of peptides

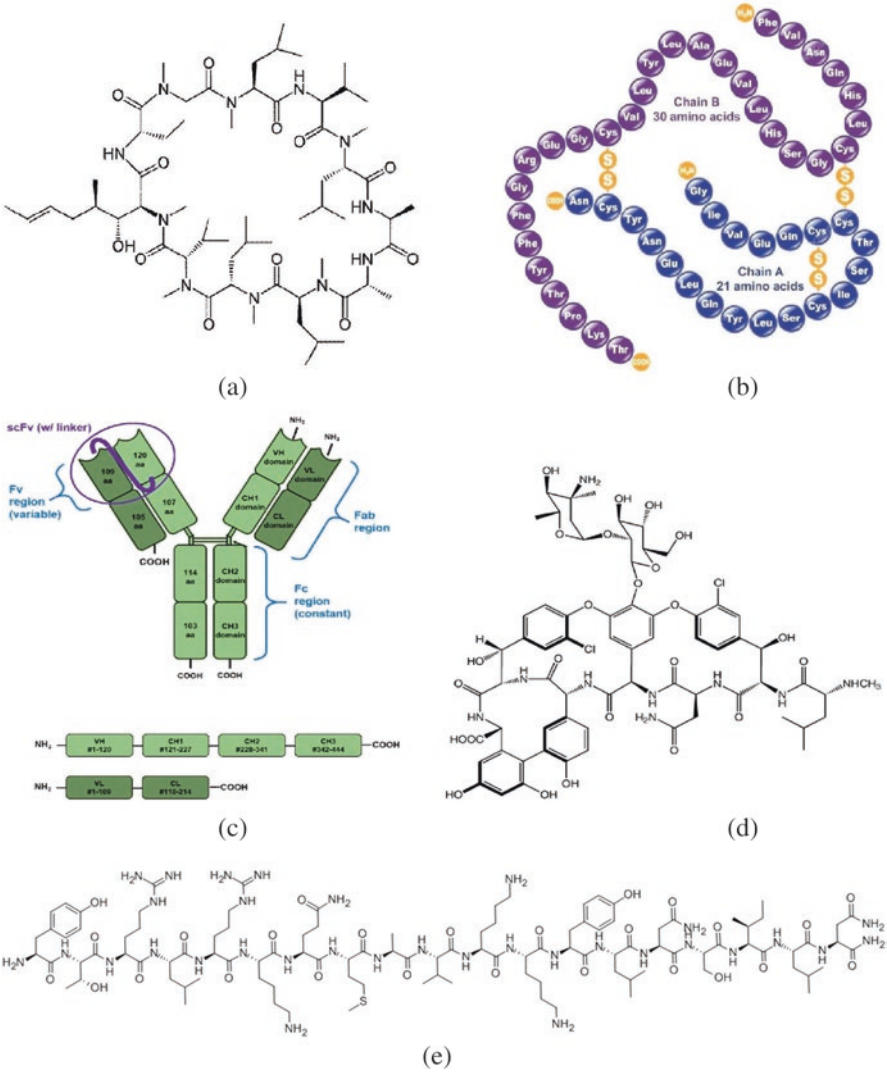

Fig. 24.1 Structures of some therapeutic proteins/peptides (**a**) cyclosporine, (**b**) insulin, (**c**) immunoglobulin G, (**d**) vancomycin, (**e**) vasoactive intestinal peptide

reduces the degree of freedom for each constituent within the ring and thus substantially leads to reduced flexibility, increased potency, and selectivity of cyclic peptides [35–37].

Synthesis of Bioactive Peptides

Solution-phase peptide synthesis is a classical approach for peptide synthesis but is replaced widely by solid-phase synthesis. However, it retains usefulness in large-scale production of peptides for industrial purposes [38]. Solid-phase peptide synthesis is the most common technique for peptide synthesis and is much faster than classical solution process. Solid-phase synthesis allows a formation of 20 amino acids peptide in 24 h and longer ones in less than a week. Further, literature is enriched with several reports involving use of solution-phase and solid-phase techniques to carry out the synthesis of natural peptides [39–65].

Synthesis of cyclosporine involves the utility of microwave-mediated carboxylic acid isonitrile couplings, thioacid isonitrile couplings at ambient temperature, and isonitrile-mediated couplings of carboxylic acids and thioacids with amines to form challenging amide bonds [66]. The synthesis of vancomycin starts from simple chemical building blocks and opens the way for the design and synthesis of peptide libraries for biological studies. The synthesis involves sequential glycosidations of a suitably protected derivative of the previously synthesized vancomycin aglycon and delivers the target molecule in a highly efficient and stereoselective manner [67]. Synthesis of vasoactive intestinal peptide (VIP) involves coupling of pentadecapeptide with a heptapeptide derivative followed by deprotection and acylation [68]. Insulin is synthesized in significant quantities only in beta cells in the pancreas. Within the endoplasmic reticulum, proinsulin is exposed to several specific endopeptidases which excise the C peptide, thereby generating the mature form of insulin [69].

Peptide Transport Systems and Ocular Bioavailability

Transporters are important to the cell because they aid in the entry and exit of essential nutrients and ions. Transporters can also act as an efflux pump by expelling toxic compounds out of the cell. All transmembrane transport processes are mediated by integral membrane proteins, sometimes functioning in conjunction with other receptors or protein domains. Usually transporters are thought to be localized in a polarized manner in the epithelium or endothelium for the purpose of transport of solutes. Membrane transporters/receptors are involved in drug transport processes and play a key role in absorption, tissue distribution, and elimination. An increasing number of drugs are currently being targeted to transporters and receptors to aid in site-specific carrier-mediated absorption. Drug targeting to specific transporters and receptors using carrier-mediated absorption is emerging as a novel and clinically significant approach. Transport processes in the eye have been targeted in an effort to increase ocular bioavailability of drugs administered to anterior and posterior chamber of the eye [70]. Various nutrient transporters and receptors do exist on membrane surfaces of the corneal epithelial cells which aid in the movement of different vitamins and amino acids across the cell membrane [71]. Peptide

transporter systems in the eye have gained attention in current time for the targeted ocular drug delivery. These proton-coupled transporters help in the translocation of di- and tripeptides across the epithelium [72]. The transporters are mainly classified into PepT1, PepT2, and peptide/histidine transporters (PHT1 and PHT2), and many drug molecules are known to be substrates for these transporters.

The presence of an oligopeptide transporter on rabbit cornea has been confirmed. Other peptide-derived drugs including the β-lactam antibiotics, renin inhibitors, and angiotensin-converting enzyme (ACE) inhibitors are known to be substrates for PepT1 and PepT2. The expression of PHT1 in bovine and human retinal pigment epithelium cells (BRPE and HRPE cells, respectively), ARPE-19 cells (a human RPE cell line), and bovine and human neural retina cells has been demonstrated [73]. However, PepT2 and PHT2 expressions were reported in bovine and human retina, whereas PepT1 was not detected. It was also concluded that glycylsarcosine uptake studies did not demonstrate any significant functional activity of PHT1 on plasma membranes of RPE. The mechanism of model dipeptide (glycylsarcosine) transport across the blood-ocular barriers following systemic administration has been investigated. This included carrier-mediated uptake of glycylsarcosine across the blood-ocular barrier, and a dependence on time and concentration was also discovered. It has been shown that as compared to the parent drug, peptide prodrugs such as valine-ACV (where ACV = 6-(L-alpha- aminoadipy1)-L-cysteinyl-D-valine) and valine-valine-ACV exhibited higher concentrations of ACV in the aqueous humor following systemic administration. This confirmed two facts: firstly, that the peptide prodrugs can be taken up from the systemic circulation into the eye via carrier-mediated transport mechanisms and, secondly, that the drugs with poor ocular bioavailability can be suitably modified by rational design to be recognized and taken up by peptide transporters for enhanced ocular bioavailability. These findings revealed that the drugs with poor ocular bioavailability could be suitably modified by design to facilitate recognition and uptake by peptide transporters.

Peptide and Protein Delivery Approaches to the Eye

Prodrugs

Prodrug approach consists of a transient and reversible modification of the physicochemical properties of a given compound through chemical derivatization in order to improve drug permeation across the cornea and thus enhance the bioavailability of peptide or protein drugs. The first prodrug for ocular delivery was Dipivefrin, prodrug of epinephrine used to treat glaucoma [74]. After, many other prodrugs have been designed to improve the ophthalmic bioavailability of various drug molecules, prolong their duration of action, improve their formulation properties, or reduce systemic side effects. Figure 24.2 shows the chemical structures of peptide prodrugs developed for ocular delivery. Further, the prodrugs developed for ophthalmic use

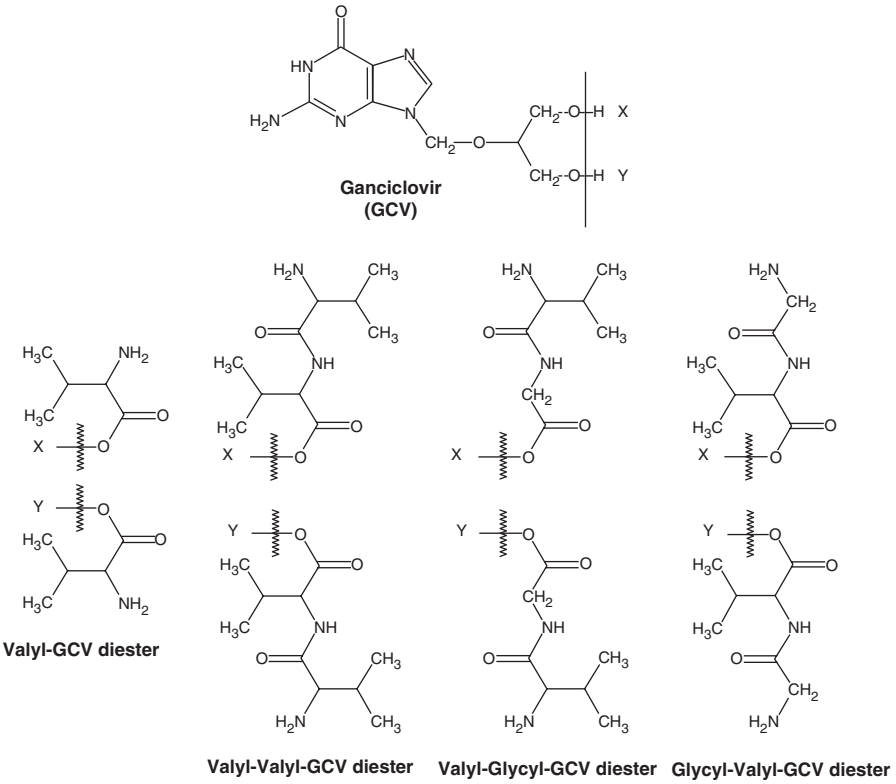

Fig. 24.2 Chemical structure of ganciclovir and its chemical modifications (peptide diesterification) that have been used for the treatment of ocular disorders

are expected to possess good chemical stability and high enzyme lability that is often a challenge for the development of ophthalmic prodrugs which are intended to be rapidly converted to the active drug after absorption [75]. Therefore, only those prodrugs that show good chemical stability combined with a sufficiently high enzymatic lability can be easily developed without resorting to multi-vial reconstitutable products. Prodrugs are designed with major goal to overcome various physicochemical, biopharmaceutical, and/or pharmacokinetic problems that may be associated with the parent drug molecules, which would otherwise limit their clinical use.

Ophthalmic peptide and protein prodrug approach can also be utilized for preventing a low aqueous solubility (which prevents the development of aqueous eye drops), a low lipid solubility (which results in low corneal permeation and low ophthalmic bioavailability), a short duration of action [28, 76] due to rapid drug elimination from site of action, and systemic side effects due to low corneal and high systemic absorption, which necessitates frequent administration resulting in poor patient compliance.

Mucoadhesion

Mucoadhesive systems utilize the property of bioadhesion of certain polymers that can be used for targeting a drug to a particular region of the body for extended period of time. The mucus membrane lining the ocular region acts as strong site for attachment of bioadhesive system. Thus, mucoadhesion may solve bioavailability problems resulting from a too short stay of the dosage form at the absorption site. The cornea and conjunctiva are extraocular structures possessing a net negative charge due to which mucoadhesive cationic polymers might interact intimately with these structures, increasing the concentration and residence time of polymer-associated drug. Chitosan has attracted a great deal of attention as a mucoadhesive polymer because of its unique properties such as acceptable biocompatibility, a biodegradable backbone, and an ability to enhance the paracellular transport of drugs possibly through a transient loosening of the tight junctions [77, 78].

Nanosized Carriers

Nanocarriers offer selective targeting of peptide and protein drugs along with sustained release of molecules at the desired site. Liposomes, niosomes, biodegradable nanoparticles, solid lipid nanoparticles, dendrimers, etc. are some of the examples [79] of nanosized carriers which provide protection to encapsulated peptide drugs from enzymatic degradation and also from loss due to tear turnover, thereby maintaining a sustained drug release over longer periods of time. Also, if the mucoadhesive polymers are incorporated with the drug in the nanocarriers complex, it allows adherence of nanocarriers to the corneal epithelium. The potential of chitosan nanoparticles as a new vehicle for the improvement of the delivery of the hydrophobic, cyclic peptide cyclosporine A (CsA) to the ocular mucosa has been investigated, and it was concluded that, following topical instillation of CsA-loaded chitosan nanoparticles to rabbits, it was possible to achieve therapeutic concentrations in external ocular tissues including the cornea and conjunctiva for at least 48 h while maintaining negligible or undetectable CsA levels in the inner ocular structures, i.e., the iris, ciliary body, and aqueous humor, blood, and plasma [80, 81].

The effectiveness of liposomes in aiding the ocular absorption of entrapped insulin in normal rabbits has been reported [13]. Administration of insulin entrapped in positively charged liposomes to normal rabbits produced a substantial reduction in blood glucose concentration 90–120 min after the administration of the formulation. The ability of liposomes to deliver the immunosuppressive agent cyclosporine A (CsA) to the cornea, anterior sclera, and aqueous and vitreous humor in rabbit eyes is investigated [82]. Reports include testing of liposome-encapsulated CsA (CsA-LIP) or olive oil drops containing an equivalent concentration of CsA (CsA-DR) against “collagen shields” soaked for 30 min in the liposome preparation (CsA-LIP-CS), both in vitro and in vivo. CsA-CS-LIP yielded significantly higher levels of CsA in the aqueous and

vitreous humor and in sclera compared to CsA-DR, evincing the requirement for the nanocarrier system. CsA-loaded, solid lipid nanoparticles (SLNs) for topical ophthalmic applications have also been investigated [83]. SLNs were prepared by using a high shear homogenization and ultrasound method, with Compritol 888 ATO (a wax for hot-melt coating and prolonged-release), Poloxamer 188, and Tween 80 (polyethylene glycol sorbitan monooleate). These SLNs were then investigated for cellular uptake into rabbit corneal epithelial (RCE) cells and evaluated for potential cytotoxicity.

CsA release from the SLNs was found to be enzyme (lipase/co-lipase complex) dependent. In the subsequent studies, it was observed that the topical ophthalmic efficacy of CsA was enhanced remarkably via administration of SLNs with a particle size of 225.9 ± 5.5 nm and a negative surface charge [84]. An aqueous humor drug level of up to 50.53 ng/ml was achieved without any serious irritation in the rabbit eye. Similarly, CsA levels in ocular tissues and fluids after topical administration of poly-epsilon-caprolactone (PCL)/benzalkonium chloride (BKC) nanospheres and hyaluronic acid (HA)-coated PCL/BKC nanospheres into healthy rabbit corneas have been reported [85]. The CsA-loaded PCL/BKC and HA-coated PCL/BKC nanospheres were found to achieve high levels of CsA in the cornea, 10- to 15-fold higher than could be achieved with CsA that had been solubilized in castor oil. It can be concluded that the nanospheres formulation and HA coating both played an important role in delivering high levels of CsA into the cornea. A novel formulation of vasoactive intestinal peptide (VIP) based on the incorporation of VIP-loaded rhodamine-conjugated liposomes (VIP-Rh-Lip) within an HA gel, for the treatment of endotoxin-induced uveitis (EIU), has been reported [86]. It was observed that interactions between the HA chains and liposomes resulted in an increased viscosity and reinforced elasticity of the gel. Retention of the liposomes by the HA gel was confirmed by in vitro and in vivo studies. It was further noted that the severity of the inflammatory response profoundly influenced the stability of the liposomal system, thereby resulting in the delayed release of VIP, which is desired for the treatment of uveitis. Hence, it was concluded that the HA-gel-containing VIP-Rh-Lip served as an efficient strategy for the sustained delivery of VIP in both the ocular and local lymph node tissues for better immunosupressor activity of VIP.

Permeation Enhancers

Permeation enhancers increase the penetration of drugs through the corneal barrier thereby altering the integrity of the epithelial cell layer. The commonly used penetration enhancers in ocular formulations include cyclodextrin, dimethyl sulfoxide (DMSO), ethylenediaminetetraacetic acid (EDTA), sodium glycocholate and related cholates, Tween 20 (a nonionic polysorbate surfactant), Brij 35 (polyoxyethylene lauryl ether), saponins, and bile salts [87]. Generally, penetration enhancers such as EDTA and cholates transiently loosen the tight junctions between adjacent cells of the corneal epithelium. Thus, penetration enhancers, when applied topically to the eye, have been successfully applied to the delivery of protein and peptides through

the corneal epithelium. Several studies have reported the improved delivery of peptides and proteins into the systemic circulation via the ocular route [88, 89]. Brij 78 and BL-9 were found to be effective for the systemic delivery of somatostatin, vasoactive intestinal peptide (VIP), α-melanocyte-stimulating hormone (α-MSH), and adrenocorticotropic hormone (ACTH) via ocular route [90]. The effect of several penetration enhancers on the bioavailability of insulin administered via the ocular route has been studied, and it was concluded that improved insulin delivery was achieved by enhancers, in the order of polyoxyethylene-9-lauryl ether > sodium deoxycholate > sodium glycocholate ~ sodium taurocholate [91]. When cyclosporine was topically applied in conjunction with the novel cutaneous penetration enhancer, Azone® (1-dodecylazacycloheptan-2-one), to allografted rabbit eyes, a clinically significant concentration of cyclosporine could be measured in the treated corneas, but little or no cyclosporine could be found in the aqueous humor or blood revealing that cyclosporine delivered in conjunction with Azone may result in suppression in the severity and incidence of graft rejection. While using the penetration enhancers for the eye, the risk of toxicity is of utmost importance to be taken care of. In this respect, toxicological parameters must be thoroughly addressed prior to choosing a penetration enhancer for ophthalmological products.

Miscellaneous

The literature on ocular delivery of peptides and proteins has been reported with some other approaches discussed above, including other vehicle or delivery platforms. The efficacy of transactivator of transcription protein transduction domain (TAT-PTD, $TAT_{49–57}$) as a vehicle was evaluated to deliver acidic FGF (aFGF) to retina in rats. TAT-conjugated aFGF-His (TAT-aFGF-His) exhibited efficient penetration into the retina following topical administration to the ocular surface. Immunochemical staining with anti-His revealed that TAT-aFGF-His proteins were readily found in the retina (mainly in the ganglion cell layer) at 30 min. and remained detectable for at least 8 h after administration. In contrast, His^+ proteins were undetectable in the retina after topical administration of aFGF-His, indicating that aFGF-His cannot penetrate the ocular barrier. Furthermore, TAT-aFGF-His, but not aFGF-His, mediated significant protection against retinal ischemia-reperfusion (IR) injury. After IR injury, the retina from TAT-aFGF-His-treated rats showed better-maintained inner retinal layer structure, reduced apoptosis of retinal ganglion cells, and improved retinal function compared to those treated with aFGF-His or PBS. These results indicate that conjugation of TAT to aFGF-His can markedly improve the ability of aFGF-His to penetrate the ocular barrier without impairing its biological function. Thus, $TAT_{49–57}$ provides a potential vehicle for efficient drug delivery in the treatment of retinal disease [92]. Such one more example is about the efficacy of the TAT (the trans-activating transcription factor from the human immunodeficiency virus) protein transduction domain as a carrier to deliver acidic fibroblast growth factor (aFGF) to the retina of the rat eye. The TAT-conjugated aFGF-His peptide

(TAT-aFGF-His) exhibited efficient penetration to the retina after topical administration. Immunohistochemical staining with anti-His antibody revealed that TAT-aFGF-His proteins were readily found in the retina, mainly in the ganglion cell layer (GCL), after 30 min, and remained detectable for at least 8 h after administration. In contrast, His-positive proteins were undetectable in the retina after topical administration of aFGF-His, indicating that aFGF-His cannot penetrate the ocular barrier alone. This fact suggested that conjugation of TAT to aFGF-His can remarkably improve the ability of aFGF-His to penetrate the ocular barrier without impairing its biological function for combating retinal disease. Intravitreal injections can cause several ocular complications, including vitreous hemorrhage, endophthalmitis, retinal detachment, and cataract, while repeated injections can multiply the risk of these complications [93]. A recombinant antibody, bevacizumab, is used for the treatment of several different ocular diseases but is delivered by intravitreal injection. In order to improve and prolong its ocular bioavailability after intravitreal administration, liposomal bevacizumab, as a novel drug delivery system, has been described and compared with conventional formulations on the market. The mean concentration of free bevacizumab in the vitreous humor of eyes that received liposomal bevacizumab was compared with eyes injected with soluble bevacizumab and shown to be nearly twofold (48 versus 28 mg/ml) and fivefold (16versus 3.3 mg/ml) higher at days 28 and 42, respectively. In contrast, the mean concentration of free bevacizumab in the aqueous humor of both liposomal and soluble formulations was almost equivalent at the same time intervals. It concluded that the liposomal formulation provided a beneficial effect in prolonging the residency of bevacizumab in the vitreous humor [94].

Local Ocular Delivery of Peptides and Proteins

Controlled local delivery of proteins and peptides to ocular sites can be achieved by suitable manipulation of their physicochemical properties without affecting their biological activity. Several peptides have been demonstrated as therapeutic agents in a number of ocular disorders including dry eye disease, age-related macular degeneration or proliferative diabetic retinopathy, etc. Some of the therapeutic peptides indicated in certain disorders through ocular route [11] include ACTH as anti-allergic and anti-inflammatory, beta-endorphin and leu-enkephalin as analgesic, integrin-binding peptide as anti-scarring agent in glaucoma filtration surgery, insulin for diabetes mellitus, vasopressin for diabetes insipidus, TSH for diagnosis of thyroid cancer, cyclosporine A for dry eye disease, VIP for secretion of insulin, calcitonin for induction of vitreous detachment in Paget's disease, oxytocin for induction of uterine contractions, glucagon for hypoglycemic crisis, etc. However, adverse physicochemical properties and enzymatic degradation of these peptides within the ocular environment, as discussed above, may render them less effective. Sustained release of peptides, after loading them on a carrier system such as a liposome or biodegradable nanoparticle, may limit some of these problems.

Systemic Ocular Delivery of Peptides and Proteins

Benefits of the ocular route include the delivery of precise doses of peptide or protein; the relative ease and low cost of formulating and administering eye drops compared to injection; the relatively rapid rate of systemic absorption compared to oral delivery; the relative insensitivity of the eye tissues toward immunological reactions compared to other tissues such as the lung and gut; the absence of first-pass metabolism through the hepatic circulation as occurs for oral delivery platforms; and an apparently good tolerance without ocular side effects. Systemic delivery of insulin via the ocular route is especially challenging with regard to the requirement for reproducible delivery.

Ocular administration of free insulin (400 U/mL) to normal rabbits produced no change in blood glucose level unless permeation enhancer was included in the dosing solution. A concentration of insulin (10 U/kg dose) is the optimum for ocular instillation. Among various penetration enhancers, polyoxyethylene-9-lauryl ether (POE) in 0.8% w/w concentration was found to exhibit better ocular compatibility and penetration-enhancing effect. Further, it seems that administration of insulin in liposomes (egg phosphatidylcholine with cholesterol and stearylamine) not only promotes the ocular absorption but also controls and prolongs the drug action [13].

Polylactide-co-glycolic acid (PLGA) microspheres are proved carriers for the topical ocular delivery of a peptide drug – vancomycin (VA). So, PLGA microspheres can be proposed as a system for ocular delivery of peptide drugs. The microspheres were able to modulate the in vitro drug release of VA with a behavior dependent on their composition, i.e., the highest drug content corresponded to the highest release rate. High and prolonged VA concentrations and increased AUC values (twofold) with respect to an aqueous solution of the drug were observed. Increasing the viscosity of the microsphere suspension by addition of a suspending-viscosizing agent (hydroxypropyl cellulose) did not produce an increase of the ocular bioavailability [95]. Further, vancomycin microemulsion (VM-ME) is promising for ocular drug delivery for the treatment of topical eye diseases as the sol to gel transition increases viscosity which enhances the ocular retention while retaining the therapeutic efficiency [96]. VM incorporation into SLNs, i.e., VM-loaded solid lipid nanoparticles, proved as effective tool for enhanced ophthalmic delivery of vancomycin [97]. Moreover, a 0.3% w/v chitosan solution appears to be a highly promising, cost-effective candidate for biomedical use as a vehicle for VM ocular drug delivery [98].

An osmotic pump was used to deliver IgG across the sclera of pigmented rabbits, and levels were measured in the choroid, retina, vitreous humor, aqueous humor, orbit, and plasma. This method which involves use of minimally invasive transscleral delivery of ocular drug delivery to deliver therapeutic levels of bioactive drugs to the choroid and retina with negligible systemic absorption, may be used in the treatment of a variety of chorioretinal disorders [99, 100].

An intravitreal (i.v.t.) injection of vasoactive intestinal peptide (VIP) loaded in rhodamine-conjugated liposomes (VIP-Rh-Lip) reduced experimental autoimmune uveoretinitis (EAU) pathology through the immunomodulation of intraocular macrophages and deviant stimulation of T-cells in ILN. Thus, the encapsulation of VIP

Table 24.1 Significant strategies in systemic delivery of peptides and proteins through ocular route

Peptide/protein	Delivery strategy	Peptide/protein	Delivery strategy
Insulin (polypeptide hormone)	Penetration enhancers (polyoxyethylene-9-lauryl ether, sodium taurocholate, sodium deoxycholate)	Vancomycin (amphoteric glycopeptide antibiotic)	PLGA microspheres, nanoparticles, microemulsions
Cyclosporine A (cyclic undecapeptide immunosuppressant)	Azone penetration enhancer	Ganciclovir (anti-CMV)	Dipeptide ester prodrugs
IgG protein (large molecules of 150 kDa made of four peptide chains)	Transscleral delivery	Vasoactive intestinal peptide (VIP, potent vasodilatory neuropeptide)	Conjugated liposome

within liposomes appears as an effective strategy to deliver VIP into the eye and is an efficient means of the prevention of EAU severity [101]. Table 24.1 summarizes significant strategies in systemic delivery of peptides and proteins.

Peptide and Protein Delivery for Back of the Eye

Topical ocular medications do not reach the posterior segment drug targets. Posterior segment (retina, vitreous, choroid) can be treated by high drug doses given intravenously or by intravitreal administration. Currently, there is rapidly growing interest in the posterior segment drug delivery. Ever since the emergence of the first USFDA (US Food and Drug Administration)-approved recombinant protein, human insulin, in 1982, there has been a tremendous surge in the development of commercial protein therapeutics for applications in various fields of medicine. The role of therapeutic proteins used in the eye ranges from the neutralization of biomolecules, such as cytokines and growth factors, to protection of photoreceptors and prevention of angiogenesis. Among these, many proteins are used for the treatment of diseases affecting the back of the eye, collectively called "posterior segment diseases of the eye" [102]. These diseases include age-related macular degeneration (AMD), retinal vein occlusion with cystoid macular edema (CME), posterior uveitis, glaucoma, diabetic retinopathy (DR), cytomegalovirus (CMV) retinitis, and retinitis pigmentosa (RP), many of which can lead to visual impairment and sometimes blindness. Hence, a huge amount of research time and money has been spent on the development of drugs and treatment modalities in this area [103]. The disease progression in DR involves neovascularization of the retina and choroidal neovascularization (CNV) in the case of AMD, which is responsible for the loss of vision. The gold-standard treatment for retinal neovascularization involves ablation of CNV or laser-assisted thermal photocoagulation to make the retina anoxic. However, these

approaches are gradually being replaced by intravitreal injections of anti-vascular endothelial growth factor (anti-VEGF) agents. An inhibitory peptide that specifically acts against mitochondrial μ-calpain (Tat-μCL, 23 amino acid, 2857.37 Da) and protects photoreceptors in retinal dystrophic rats was developed. Tat-μCL was topically administered to the eyes of Sprague-Dawley rats for 7 days to determine both the delivery route of the peptide to the posterior segment of the eye and the kinetics after topical application in adult rats. The results suggest that while the topically applied Tat-μCL peptide reaches the posterior segment of the retina and the optic nerve, the sufficient concentration (>IC50) is maintained for at least 6 h in the rat retina. Research findings revealed that the delivery of topically applied peptide to the posterior segment and optic nerve occurred through the conjunctiva, periocular connective tissue, sclera, and optic nerve sheath [104].

Anti-VEGF Proteins

Protein drugs that neutralize vascular endothelial growth factor (VEGF), such as aflibercept or ranibizumab, rescue vision in patients with retinal vascular diseases. Nonetheless, optimal visual outcomes require intraocular injections as frequently as every month [105]. Some of the major protein drugs for AMD and DR that are currently being used clinically or are in the development pipeline include aflibercept (glycosylated recombinant fusion protein), AGN-150998 (recombinant ankyrin repeat protein), ALG-1001 (integrin peptide), bevacizumab (monoclonal IgG), conbercept, VGX-300 (recombinant fusion proteins) [106–109], etc. Crucial aspects of anti-VEGF proteins include their binding affinity and ability to bind to different isoforms of VEGF. Biological advantages of aflibercept include its greater binding affinity for VEGF, a longer intravitreal half-life relative to other anti-VEGF agents, and the capacity to antagonize growth factors other than VEGF. Similarly, newer anti-VEGF proteins are being developed, such as conbercept, an antibody fragment comprising the Fc portion of immunoglobulin 1 (Ig1) and extracellular domain 2 of VEGF receptor 1 (VEGFR-1) and extracellular domains 3 and 4 of VEGFR-2. Newer anti-VEGF proteins block all isoforms of VEGF-A, VEGF-B, VEGF-C, and placental growth factor (PGF) and has a high binding affinity for VEGF and a long half-life in the vitreous [110].

Protein Stability during Drug Development

Protein stability is a crucial factor at various stages of drug development and controlled-release device fabrication [111, 112]. The functionality of proteins is heavily dependent on their complex secondary, tertiary, and quaternary structures, which are susceptible to modifications in the presence of many physical, chemical, as well as biological stimuli. The physical and chemical stabilities of proteins are determined by the intra- and intermolecular chemical modifications that they can

undergo, such as oxidation, deamidation, beta-elimination, disulfide scrambling, hydrolysis, and aggregation. Surrounding conditions, such as pH, temperature, and the presence of salts or surfactants, interfere with the structural and functional stability of protein entities [113]. Pharmaceutical companies enhance protein stability not only by molecular engineering but also by using excipients, such as protein stabilizers. Protein stabilization works by reducing protein dynamics and motion, eliminating conformational transitions that can disrupt the native state of the protein. Protein degradation follows a sequence of stages that can be prevented by careful interventions at various phases. The first step in protein degradation involves the exposition of buried hydrophobic amino acids to the aqueous solvent, leading to protein unfolding. However, the presence of sugars, such as trehalose, sucrose, lactose, maltose, and dextran, and polyols, such as sorbitol, mannitol, and glycerol, promote the strengthening of interactions between these hydrophobic amino acids, which predominantly comprise nonpolar amino acids, making the protein more thermostable and rigid. For example, the commercial formulations of ranibizumab and aflibercept contain trehalose and sucrose, respectively, which form hydrogen bonds on the protein surface, replacing water molecules and thereby enhancing stability. Furthermore, an additional freeze-drying step is often used to stabilize the protein after the addition of these sugars or polyols and is known to improve the shelf life of these formulations. In addition to lyophilization and additives, encapsulation within controlled release systems for enhancing protein stability has been investigated by some research groups. For example, a promising long-term nanostructured polymer device for sustained stable protein release comprised a nanoporous surface layer and a nonporous film, which are thermosealed with each other to prevent nonspecific protein leakage. The protein used is lyophilized and placed as a pellet between these two membranes, wherein it diffuses through the nanoporous surface with zero-order release kinetics. Proteins can also undergo degradation and aggregation cause of factors that range from the chemicals used during system fabrication to degradation products used during drug release. For example, proteins can interact with polymers used in the fabrication of controlled-release systems, such as PLGA particles, by binding to the polymers or interacting with the organic solvents, resulting in aggregation, degradation, activity loss, and, in some cases, immunogenicity. This has been previously observed for recombinant human interleukin-1a as well as for porcine insulin, where the incorporation of a basic excipient improved the protein-release profile significantly. Recently, a study investigating the stability of ranibizumab and aflibercept in different acid conditions showed that these proteins aggregate at pH 4.5 based on a lowering of denaturation temperature, as observed using DSC, which indicates a potential formation of protein aggregates at this pH [114]. The environment in which the proteins are stored or embedded has an important role in determining the tertiary and quaternary architecture and molecular motion of proteins during controlled-release device fabrication. Hence, careful selection and control of these parameters are essential to maintain the functional efficiency of the final product.

Emerging Technologies for Ocular Peptides and Protein Delivery

Currently, ocular route is mainly used for the delivery of proteins and peptides for the treatment of local ocular disorders such as age-related macular degeneration, dry eye disease, or proliferative diabetic retinopathy. Lucentis® and Eylea® are the recently marketed proteins intended for the treatment of ocular diseases. The physiological and anatomical barriers and enzymatic degradation within the ocular environment limit the efficacy of proteins and peptides administered by ocular route. Polymeric NPs have been successfully designed to overcome these barriers and improve ocular bioavailability of proteins and peptides. Polymeric NPs can improve localized ocular delivery by providing sustain release, protection from enzymatic degradation and enhancing precorneal residence time compared to aqueous eye drops [9].

To reduce the number of barriers encountered, anti-VEGF delivery to the posterior segment of the eye is typically carried out by intravitreal injections that make use of a needle to penetrate the globe and release the drug into the vitreous. These injections are often administered repeatedly and have increased propensity for complications, such as endophthalmitis, cataracts, retinal tears, and retinal detachment [115]. Moreover, presence of proteolytic enzymes, such as trypsin, in the vitreous, which can increase with aging, result in the degradation of free protein drugs injected therein. Controlled protein delivery improves the efficacy of treatment by increasing the residence time of active drugs at the target site, thereby reducing the frequency of drug administration. Moreover, some of these carriers are designed to enhance protein transport across various ocular barriers following less invasive topical or periocular administration. Although drug delivery systems for prolonging the release of small Mw drugs were developed early on, it was not until the late 1970s that the idea of using biocompatible materials for controlled delivery of macromolecular drugs was investigated. Recent years have seen the emergence of protein delivery systems specifically developed for applications to the back of the eye. These systems can be broadly classified as injectable colloidal particles, injectable hydrogels, implants, and cell-based systems. Although most of these systems have been developed for the sustained release of bevacizumab, recent studies that demonstrate the controlled release of ranibizumab have also been reported. Injectable colloidal particles comprise protein-loaded nano- or micron-sized particles loaded with drug molecules that can act as depots for prolonged sustained release or enhanced protein transport across ocular barriers. Polylactic-co-glycolic acid (PLGA) polymer-based systems constitute a major class of colloidal carriers that have been explored for use as bevacizumab depots for controlled release.

In a recent study, coating of cyclosporine A (CsA)-loaded poly-epsilone-caprolactone (PCL)/benzalkonium chloride (BKC) nanospheres with hyaluronic acid resulted in high concentrations of cyclosporine A into the cornea compared with non-coated NPs [116]. Cationic polymers such as Eudragit® have also been employed to increase precorneal residence time. It was observed that Eudragit® can prolong the residence time of NPs by interacting with the anionic mucins present in the mucus layer at the eye

surface. Evaluation of efficiency of Eudragit® in improving effectiveness of CsA NPs formulations against inflammation of the eye surface is reported in literature [117]. The NPs were prepared using either PLGA alone or a mixture of Eudragit® RL with PLGA or were coated with Carbopol®. Tear kinetic parameters were determined following topical application of CsA-loaded NPs suspension and RestasisA® (drug-loaded emulsion) in rabbit eye. Cellular uptake, tear film concentration of the drug, and $AUC_{0 \rightarrow 24h}$ were significantly higher for PLGA: Eudragit® RL (75:25)-CsA NP (cationic NPs) and Carbopol®-coated PLGA-CsA NP (adhesive) formulations compared to RestasisA®. These results clearly demonstrate the effect of surface characteristics of NPs in the improvement of ocular retention and bioavailability.

Concluding Remarks and Future Perspective

Emerging trends in the development of ocular biotechnology products, especially for proteins and peptides, include the design of more specific delivery strategies intended to achieve therapeutic responses with minimal doses and controlled ocular pharmacokinetics. The literature supports the fact that significant efforts have been done in the disease management involving the posterior segment of the eye, which ranges from intravitreal protein therapy to the destructive laser therapy. Also, several research collaborations with pharmaceutical industry have generated several promising protein molecules that are efficacious in arresting the progression in many of the back-of-the-eye diseases. In spite of the fact that such studies have been faced with the challenges involved in pharmacokinetics and pharmacodynamics of the anti-VEGF agents, for most of protein molecules, intravitreal injections remain the mainstay of administration. Usually, intravitreal injections are done on a monthly basis, leading to possible adverse effects, including retinal detachment. In this context, the current challenge is to devise a minimally invasive and long-lasting mode of administration for protein drugs. Although research activity is intense in this area, no system has advanced beyond the preclinical stage. This is true even for sustained-release systems injected into the posterior segment of the eye, primarily because of the agglomeration of the injected particles, leading to partially obscured vision. Thus, a delivery system containing encapsulated proteins that can be administered topically, subconjuctivally, or periocularly remains a goal for researchers worldwide. Although cellular delivery systems that generate the protein drug in situ have been investigated, they remain an even more distant goal because of regulatory issues. More recently, major protein delivery approaches such as prodrug and use of permeation enhancers and nanosized carrier systems have been studied with several peptide molecules. Despite the fact that these ocular protein delivery approaches theoretically address solutions to many associated complications and seem to exhibit promising therapeutic outcomes, a successful commercial product based on any one of these approach is still awaited. This is probably because they require a thorough assessment of the concerned potential toxicology issues of the delivery system. All efforts oriented toward delivery strategies of peptides and protein

molecules revealed that wider use of ocular route for protein and peptide delivery needs much more research and improvement. Considering potential results as well as associated challenges, the oclar route could be used in order to deliver protein drugs for local and systemic treatment, but may not be suggested as very promising and preferred route for systemic protein administration.

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Chapter 25
Corneal Haze, Refractive Surgery, and Implications for Choroidal Neovascularization

Amitoj Singh, Afrah Jalil Abd, Aseel Al-Mashahedah, and Jagat Rakesh Kanwar

Abstract Visual impairment is a multifactorial issue affecting 285 million people world-wide and influencing their quality of life. Although surgical procedures such as photorefractive keratectomy (PRK), laser in situ keratomileusis (LASIK), laser sub-epithelial keratomileusis (LASEK), and implantation of synthetic intra-ocular lenses have shown promise in correcting refractive errors, post-operative complications represented by blurry vision (corneal haze) are widely recorded, with the duration of the symptom varying with each case from a few days to months. In addition to surgical procedures, corneal injuries such as alkali burns, infections, etc. cause keratocyte apoptosis, which triggers a wound-healing cascade leading to corneal haze. Corneal haze is the result of aggressive wound healing and the formation of scar tissue post-surgery, which involves the differentiation of keratocytes to myofibroblasts causing fibrosis, and unorganized deposition of collagen types IV and VII leading to reduced ocular clarity. Therefore, the goal of future research is to promote wound healing through regeneration without fibrosis, and reducing the oxidative damage caused by reactive oxygen species. Consequently, significant research is being undertaken in reducing these complications, in addition to increasing the efficacy of the existing drug formulations to reduce ocular toxicity, corneal haze, and reduce the rate of wound healing. This chapter presents a comprehensive review of the current treatments available, and new prospects for therapy.

Keywords Haze · PRK · LASIK · LASEK · Myopia · Choroidal neovascularization

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Introduction

Vision loss poses a major concern for individuals, with refractive errors accounting for low vision in more than 50% of the population world-wide. This incidence proportionally increases with age and subsequently affects the patient's quality of life [1]. Corneal haze is one of the major complications of refractive error surgery and leads to diminished corneal clarity. It occurs as a result of aggressive wound healing and initiation of keratocyte apoptosis at the site of injury followed by migration of myofibroblasts which reduce transparency [2, 3]. Corneal haze also leads to the generation of free radicals [4], and a reduction of naturally present antioxidants [5, 6]. Corneal haze is an outcome of corneal epithelium damage originating from surgical operation, infection, alkali burns, or other forms of trauma that lead to corneal opacification with varying degrees of severity, normally graded between 0 and 4; grade 0 represents no haze, whereas 4 represents complete opacification [7]. Surgical procedures such as photorefractive keratectomy (PRK), laser in situ keratomileusis (LASIK), and LASEK commonly involve partial excision of corneal tissue using an ionizing laser to reshape the central optical zone of the underlying stroma in order to treat refractive errors [8]. Similarly, laser is also used in cataract surgery that delivers ultra-short pulses to create a small incision in the cornea, followed by removal and replacement of the lens [9]. This therapeutic strategy is accompanied by traumatic injuries and an inflammatory response, and leads to formation of scar tissue, which is part of the immune system's natural response to injury [2, 10]. Although some of these symptoms are treatable and might persist for a few days [11], others such as corneal haze could take several weeks or months to disappear [2]. In severe cases, corneal haze could lead to refractive regression, irregular astigmatism, and corneal surface irregularity [9, 12]. This chapter focuses on corneal haze as the key complication after refractive surgery and deals with prevailing and prospective strategies to manage the severity of this condition.

Refractive Surgeries

A variety of techniques has been used to manage refractive errors through use of excimer lasers in order to reshape the stroma after complete or temporary removal of the corneal epithelium (Fig. 25.1). Nevertheless, the choice of effective therapy should be determined by the physical condition of the eye.

Photorefractive Keratectomy

PRK was introduced to reshape corneal tissue without creating a flap, and this was done by using laser rather than a blade. The process involves the complete removal of a thin layer of corneal epithelium through the use of an alcoholic solution,

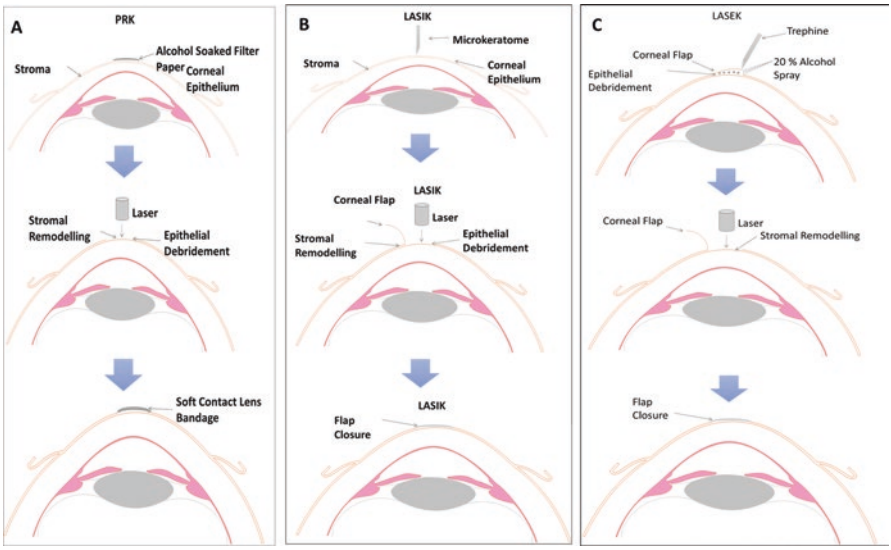

Fig. 25.1 Diagrammatic representation of PRK, LASIK, and LASEK. (**A**) PRK involves the excision of the central cornea to access the stroma for reshaping, the excised cornea is discarded; (**B**) LASIK involves the creation of a corneal flap using a microkeratome, the cornea is repositioned carefully after stromal reshaping; (**C**) LASEK involves the creation of a flap using a finer trephine and loosening the epithelium with 20% alcohol to access the stromal tissue. The cornea is carefully repositioned after reshaping of the stromal tissue. This image was made using the PowerPoint Drawing Toolkit from MOTIFOLIO

followed by reshaping the stroma. In general, PRK is not recommended for patients suffering from high myopia and with extremely thin corneas. PRK was reported to cause several undesirable effects such as severe dry eye, corneal haze, and moderate post-operative pain [13]. Furthermore, myopic regression was frequently noticed following PRK in subjects with high diopter (5–6 D) [14, 15]. Pathological changes are represented by a pronouncedly higher rate of keratocyte apoptosis and myofibroblast density in patients affected by high myopia than in others with low myopia [16]. Keratocyte apoptosis is initiated immediately after PRK; in addition, the myofibroblasts formed as a result of the wound-healing cascade inhibit the proliferation and differentiation of epithelial cells, leading to epithelial hyperplasia, which in turn causes myopic regression [17, 18]. Corneal haze is another issue that is experienced in all patients after PRK surgery. The haze disappears between 1 and 2 years following surgery. Patients occasionally develop a severe bilateral haze with myopic regression accompanied by best-corrected vision loss [3, 19, 20]. Moreover, the risk of developing late haze rises when the attempted level of correction increases above 5 D. Further exacerbation was also observed during exposure to ultraviolet light [21], which negatively influences visual acuity [22]. Coincidental factors that could determine the severity of a corneal haze are the depth of ablation, recovery time required for wound closure, stromal surface irregularities, and removal of the

epithelial basement membrane and Bowman's layer ablation [22–29]. Despite the limitations of PRK, this approach is only suitable for correcting vision with low diopters (< 6 D) because the degree of developed corneal haze is insignificant with PRK when compared to other techniques [22].

LASIK

LASIK is a technique commonly used to correct vision. With this approach, the shape of the cornea is changed completely by using a microkeratome to create a hinged flap, then reshaping the stroma with an excimer laser, and replacing the hinged flap to cover the exposed stroma [30]. Although this approach is the optimal choice for corneal corrections, major complications can arise from corneal flap irregularities, such as visual aberrations, dry eye, and infectious keratitis. Moreover, repositioning of the flap is irritating and painful for the patient [31]. Nevertheless, the corneal wound-healing cascade after LASIK is localized at the interface of the ablated area, which is distant from the epithelium (the epithelium is responsible for cytokine production). Such a functional separation is likely to be the reason for the minor intensity of epithelial hyperplasia following LASIK in contrast to PRK [17]. A further advantage of LASIK is the minor level of ablation required to produce the same level of correction in identical myopias, and the inferior rate of myopic regression recorded after surgery [19, 20]. The use of sharp blades in LASIK minimizes the introduction of the epithelial tissue onto the laser-ablated area, and consequently lower levels of cytokines occur. Cytokines are the key initiators of keratocyte apoptosis. Additionally, the presence of epithelial debris is also minimized by proper irrigation of the injured area with a balanced salt solution and controlled aspiration of the residual solution [19, 20, 32, 33]. The alternative technique involves using a femtosecond laser for creating the epithelial flap to access the underlying stroma with minimal debris production [19, 20, 34–36]. Femtosecond lasers can augment visual acuity toward 20/40 or better, and can be considered a safer alternative to microkeratomes [34], which is more likely to boost the release of cytokines [20]. Although LASIK is significantly preferred in comparison to PRK, handling errors are frequently seen since complicated devices are involved in this technique that lead to significant variation [37]. Microkeratome on the other hand can be problematic [38], and is affiliated with unexpected complications, such as free flaps, buttonhole flaps, flaps with a centralized hole, improper flap alignment or decentration, epithelial defects, flap striae or folds in the epithelial flap, flap dislocation, recurrent corneal erosion, and dry eye [37]. In this sense, careful planning and precise skills are required when performing LASIK.

LASEK

Laser-associated subepithelial keratomileusis (LASEK) is a new approach for vision correction. This technique is relatively recent and combines the advantages of PRK and LASIK to cause minimum irritation. In this technique, trephine is used to create an epithelial flap and this flap remains attached to the remaining epithelium at a single point; this is followed by repositioning of the loosened flap to expose the underlying stroma and reshaping with the excimer laser [13, 39]. LASEK is used for correcting vision in patients with high myopia and thin corneas [13, 40]. However, in some cases it is followed by undesirable effects, such as prolonged visual recovery and persistent dry eye, which can last up to 12 months [13]. LASEK was designed to overcome the limitations of other techniques; for instance, the use of alcohol to loosen the corneal epithelial flap causes minimal complications and enhanced visual recovery when compared to PRK [41, 42]. The process of wound healing is affected by other circumstances like viability of the corneal epithelium, concentration of alcohol used, epithelial layer debris, dead cells, and temperature of the balanced salt solution used [8]. Significant corneal epithelial damage and increased healing time as a result of alcohol has been reported in several investigations. Recent data confirm the reduction in epithelial viability with elevated concentrations and exposure time of ethanol, with typical results obtained with 20% ethanol for 30 s [43–45]. These effects can contribute to the formation of minor levels of TGF-β [46], which initiate a mild healing cascade in contrast to LASEK and PRK. However, the underlying mechanism causing these events is not fully understood [46]. Since the success of LASEK surgery depends upon the creation of an efficient epithelial flap, it is important to accurately predict the extent of basal epithelial cell adhesion to the anterior stromal cells [8], which could be assumed to be the major limitation with this technique due to the absence of effective clinical tests to evaluate the degree of adhesion. Subsequently, epithelial flap damage can develop when the epithelial layer is loosened from the stromal tissue with improper alcohol exposure [47–51]. Recently, gel-assisted LASEK has been introduced as a sophisticated technique to overcome the limitations of LASEK. The approach involves insertion of a methylcellulose gel using a specialized cannula between the interface of the corneal epithelial layer and the stroma, followed by the creation of a flap in a butterfly fashion, which initiates a minor degree of corneal haze and reduces the immunological response as the epithelium remains separated from the stroma at all times [52, 53]. Gel-assisted LASEK has also been followed by adverse effects, such as slower visual recovery and high post-operative discomfort [54]. LASEK is a relatively recent technique and further studies involving a large number of patients are necessary to evaluate the efficacy of LASEK to manage visual aberrations [8]. In comparison to PRK and LASEK, LASIK is still the most satisfactory technique for correcting visual perturbations.

Pathophysiology of Corneal Wound Healing Cascade and Haze Initiation

The corneal wound-healing cascade is a complicated mechanism that involves the mediated interaction of cytokines with various structures of the ocular environment, namely, stromal keratocytes, stromal tissue, corneal nerves, tear film, lacrimal glands, corneal epithelial cells, and Bowman's membrane [6, 19, 20]. The cascade develops rapidly following epithelial insult arising from surgical and non-surgical traumatic injury, ultraviolet radiation, [19, 20, 55] and post-operative inflammatory response [6]. Interleukin 1 (IL-1) acts as a master regulator for these events. Following epithelial injury, IL-1 crosses the epithelium and reaches the underlying stromal tissue, binds to its receptors on the keratocytes [19, 20], and modulates the process of keratocyte apoptosis [56]; furthermore, it stimulates the production of hepatocyte growth factor (HGF) and keratinocyte growth factor (KGF) [57], and promotes the production of collagenases and metalloproteinases [55, 58–60].

Keratocyte apoptosis is induced by the role of IL-1 through synthesizing Fas ligand, which binds to the keratocyte Fas receptor. Further increases in the production of growth factors such as HGF and epidermal growth factor (EGF) in the lacrimal gland stimulates the proliferation, migration, and differentiation of keratocytes at the injured site, and recruitment of activated keratocytes, fibroblasts, and myofibroblasts to the depleted stroma and the epithelial wound during the 24-h period after injury. After this, macrophages/monocytes, T-cells, and polymorphonuclear cells (PMN) travel via the limbal blood supply and the tear film to the injured site to phagocytose apoptotic and necrotic debris from the wound. Following the initial wound-healing phase, stromal remodeling is initiated to replace the early haphazard arrangement of collagen fibrils and proteoglycans, so that the reorganized corneal tissue closely resembles the uninjured transparent corneal epithelium. This process occurs over a period of months to years.

However, the key mediators in stromal remodeling are myofibroblasts that originated from keratocyte differentiation from the action of transforming growth factor beta (TGF-β). Myofibroblasts remodel stroma through the production of collagen, glycosaminoglycans, collagenases, gelatinases, and matrix metalloproteinases (MMPs). Normal corneas have a repeating-orthogonal structure arrangement of collagen types I, III, V, and VI; however, laminin, fibronectin, tenascin, types IV and VII fibrillar collagen are deposited as a modified extracellular matrix following injury, and this encourages fibroblast migration [61, 62] and attendance of types I and III fibrillar collagen in a non-orthogonal arrangement [2]. Together with decreased crystallin production, faulty collagen deposition, and random scattering of light by myofibroblasts, this results in decreased corneal transparency and corneal haze (see Fig. 25.2).

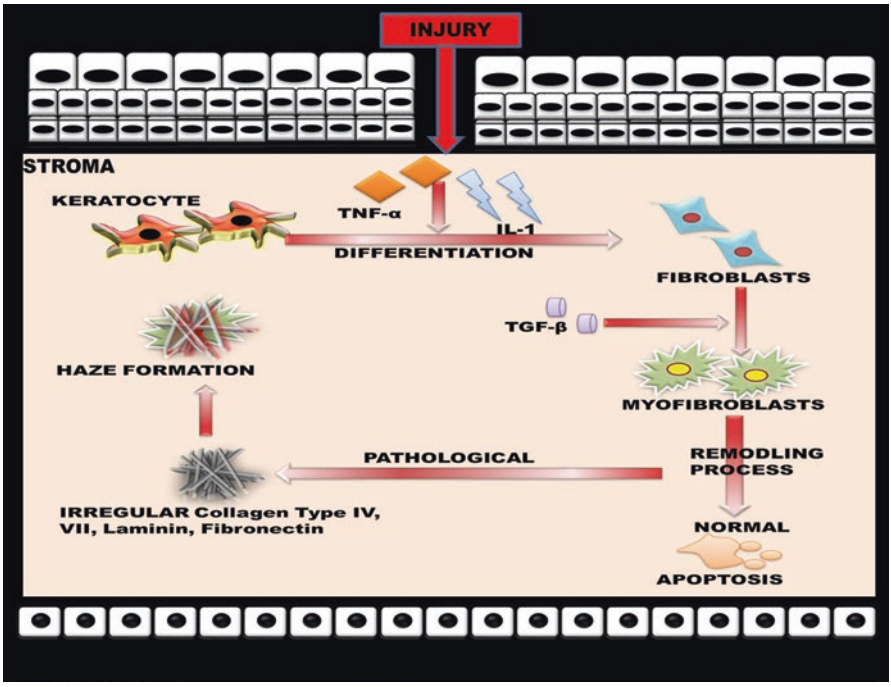

Fig. 25.2 The Corneal Wound Healing Cascade. The cascade starts with the release of cytokines from the wounded area and keratocyte apoptosis. Keratocyte proliferation and migration marks the second stage, which ultimately leads to the formation of fibroblasts that are transdifferentiated into myofibroblasts. Cytokine upregulation causes myofibroblast differentiation. Secretion of irregular collagen types IV and VII, laminin and fibronectin by the myofibroblasts causes visual opacities or corneal haze on account of random light scattering by the irregular collagen deposition

Oxidative Stress in Keratocyte Apoptosis

Excimer laser interaction (193 nm) with corneal tissue is often used to correct refractive errors of the cornea; however, this procedure is associated with adverse effects such as the formation of oxygen free radicals that produce oxidative stress and cause tissue damage by lipid peroxidation of the cell membranes, impairment of nucleic acids and sulfur containing enzymes, and ultimate initiation of keratocyte apoptosis [5, 63–66]. The deleterious effects of oxidative stress are illustrated in Table 25.1.

ROS is normally generated due to the cleavage of peptide and adjacent carbon-carbon bonds by high photon energy (6.4 eV), hydrogen radical production by water photo dissociation [4], increased surface temperature [89], or PMN invasion [65, 90, 91] to the ablated area. Recent investigation has shown a double accumulation of superoxide anion production in basal 6.3 ± 0.7 nmol/10^6 cells/h–15.6 ± 5.4 nmol/10^6 cells/h following corneal excimer laser irradiation in New Zealand white rabbits

Table 25.1 Deleterious effects of oxidative stress on corneal tissue

Factor	Effect	References
Lipid peroxidation and apoptosis	Induction of corneal wound-healing cascade, aggressive wound healing, cellular toxicity	[67–71]
Detachment of epithelial cells from basement membrane	Release of cytokines and inflammatory cell infiltration	[72–75]
Nuclear fragmentation and mitochondrial DNA	Decreased cell viability	[75–77]
Reduced oxidative phosphorylation and intense reactive oxygen species formation	Reduced levels of antioxidants, increased cellular toxicity leading to necrosis and apoptosis, initiation of wound-healing cascade	[75–77]
Aggregation and disorganization of collagen fibrils	Corneal surface irregularities leading to corneal opacities	[68, 78–80]
Altered collagen solubility, mechanical strength, and collagen-fibroblast interaction	Disorganized synthesis of collagen type IV and VII, leading to differential light scattering and ultimately visual aberrations	[81–83]
Corneal edema	Inflammation, trauma, infection, blurry vision	[84]
Corneal clouding	Loss of corneal transparency, increased IOP	[85]
Infiltration of PMN	Corneal ulceration, corneal perforation, destruction of corneal collagen	[68]
Nuclear DNA damage	Decreased cellular proliferation	[86]
Lowered Gpx, SOD, catalase and aldehyde dehydrogenase activity	Lowered antioxidant activity thereby affecting cell viability, initiation of apoptosis and the wound-healing cascade	[87, 88]

using a dose of 200 mJ cm^{-2} [72]. Moreover, extremely toxic aldehyde formation contributes to the biomolecule modifications of mainly lipids, DNA, and proteins [92]. Additional burden is produced by environmental factors since the cornea is constantly exposed to environmental UV radiation, which leads to ROS formation, induction of pro-inflammatory cytokines, and subsequent corneal cell death by apoptosis [92–94]. Both UV-A (320–400 nm) and UV-B (290–320 nm) have been demonstrated to induce cellular damage, reduce cell viability, increase lipid peroxidation, and suppress the activity of catalase in cultured rabbit lenses and corneal epithelium by approximately 50% [95]. The severity and extent of the contribution of ROS to corneal haze has not been completely investigated.

The cornea possesses the capability to synthesize a higher level of antioxidants in response to oxidative stress, mainly superoxide dismutase (SOD), catalase, glutathione peroxidase (Gpx), nicotinamide adenine dinucleotide phosphate (NADPH) cytochrome p450 reductase, and catalase to protect the cornea from radical injury [92, 96–98]. Both SOD and Gpx work together to maintain corneal integrity; SOD acts to catalyze the conversion of superoxide radicals to hydrogen peroxide and oxygen, while hydrogen peroxide is reduced to water and oxygen by the action of Gpx [67, 79].

Despite the higher levels of antioxidants [96, 97], IL-1 upregulation could initiate keratocyte apoptosis [67]. IL-1 has been reported to suppress the SOD-mediated antioxidant activity in keratoconus [99], leaving the stromal matrix predisposed to ROS-induced damage [92].

Effect of Oxidative Stress on Cellular Communication

The eye is continuously exposed to oxidative stress from environmental factors and especially following ocular surgery. Several mechanisms exist to protect the eye from oxidative stress, and facilitate the transport of antioxidants across cells to maintain eye integrity. However, transport systems, specifically gap junctions, are subject to the detrimental effects of oxidative stress [100]. Connexin 43 (Cx43) plays a major role in corneal wound healing [101]. Both Cx43 and connexin 26 (Cx26) expression upregulate within 24 h after photorefractive keratectomy [35, 36]. Following corneal injury, keratocytes rapidly proliferate to plug the corneal wound against the outside environment [102, 103]. Concurrently, Cx rearrangement occurs around the wound edges and the limbal area [104]. Hence, the modulation of the gap junction expression appears to be induced according to the requirement of Cx's half-life, which ranges between 1 and 3 h [105] in variation with the gap junction conformity, following increased levels of oxidative stress. The role of connexin upregulation still requires further investigation [35, 36]. Modulation of gap junction communication using Cx 43 antisense oligodeoxynucleotides (AsODN) is showing promising results in promoting corneal wound healing [106]. Cx 43 generally presents in the basal layers of the rat corneal epithelium to maintain stromal homeostasis by coordinating nutrient and waste exchange [101, 107]. Cx 43 AsODN-mediated Cx 43 downregulation was effective in stimulating wound closure following corneal injury as well as reduced myofibroblast differentiation following Cx 43 AsODN treatment. This can be attributed to the fact that Cx 43 downregulation stimulates cells to migrate at a greater rate to plug the wound. In addition, there was reduced edema in the stromal layer, and evidence of pro-inflammatory signals between keratocytes that limit myofibroblast proliferation and differentiation [106]. Targeted deletion of Cx 46 and Cx 50 has been implicated in causing lens opacities in mice [108]. However, the current knowledge regarding corneal gap junctions is only limited to Cx 43 and 26. The involvement of other connexins needs to be investigated.

Heat Shock Proteins in Ocular Disorders

Heat shock proteins are principally considered to preserve and maintain cellular integrity under stressful conditions by reflecting proteins. The potential effects of these proteins were studied during ocular disorders, UV exposure, and temperature elevation (REF). A study on Statens Seruminstitut Rabbit Cornea (SIRC) cells using a mild heat shock of 39 °C reported an increase in the level of IL-1β, IL-6, and HSP 90 after 30 min of heat shock. Further, mild heat shock also elevated the levels of AKT, IκBα phosphorylation, NF-κB nuclear translocation, IL-1β, and IL-6, followed by SIRC cell apoptosis [109].

Similarly, another study was performed with an *ex vivo* model involving canine spontaneous chronic corneal epithelial defects (SCCEDs); here, expression of HSP 47 and 70 was found to be reduced after corneal injury, highlighting the suppressed ability of corneal epithelial cells (CECs) to induce cytokines and growth factors to promote cell proliferation, migration, and adhesion to the basement membrane during the wound-healing cascade. HSP 70 is a relatively crucial protein involved in the wound-healing process and its inhibition might significantly delay the wound-healing cycle, while its exogenous supply restores the formation of the corneal fibroblast monolayer [110]. Further research was performed to demonstrate the effect of phosphorylated HSP 27 expression. It was found to be upregulated in a scratch wound-healing model in a time-dependent manner over 60 min, while these events did not exist with the expression of non-phosphorylated HSP 27 [111]. HSP 27 protects cells by upregulating enzymes involved in maintaining cellular functions such as chaperoning ability, glutathione activity, and actin filament stabilization [112, 113]. HSP 27 knockdown robustly limited the cell's capability to proliferate, migrate, and stimulate Bax expression when compared to the control group [111].

Thymosin β_4 Effect on Corneal Wound Healing

Thymosin β_4 ($T\beta_4$), a 4964 Da, 43 amino acid acidic polypeptide [114, 115], is a potent regulator of actin polymerization, and plays a key role in dermal and corneal wound healing [115]. $T\beta_4$ downregulates the expression of membrane-type matrix metalloproteinases such as MMP 2, 9 and MT6-MMP and ultimately preserves the basement membrane integrity, which is essential for tissue remodeling and reducing the inflammatory response modulated by MMP-9 expression [116]. The $T\beta_4$ splice variant has vigorous anti-inflammatory, wound healing, angiogenic, and tissue remodeling properties [117].

Focal Adhesion Kinases in Corneal Wound Healing

Epithelial migration is the first phase of the corneal wound-healing process. Basically, the adhesion of epithelial cells to the extracellular matrix is an important process, since the tight adhesion limits the migration of epithelial cells to the damaged area. Corneal cells were found to be subjected to repeated cycles of adhesion and de-adhesion in order to repopulate the damaged area [118]. Such adhesion is promoted by integrin $\alpha5\beta1$ and fibronectin upregulation through structures known as focal contacts, which cause aggregation of actin stress fibers and other intracellular proteins to enhance epithelial cell migration to the wounded

area [119]. Similarly, downregulation of these proteins is considered to be a feature of the healing process [120–122].

Several observations were noticed regarding the underlying mechanism of corneal wound healing. Focal adhesion kinase (FAK), integrins α5 and β1 were reported to stimulate the formation of focal contacts between corneal epithelial cells and extracellular matrix (ECM) in SV40-transformed human corneal epithelial cells [118]. Similarly, enhanced tyrosine phosphorylation of FAK occurs as a result of integrin-mediated cell attachment by the synergistic effect of substance P and insulin-like growth factor 1 (IGF-1), which in turn upregulates mitogen-activated protein (MAP) kinase in order to regulate cell proliferation and differentiation [123]. TGF-β administration promotes the formation of mature and 'supermature' focal adhesions that upregulate tensin, FAK, vinculin, and paxillin. These events in turn enhance the expression of α-smooth muscle actin fibers, the characteristic feature of myofibroblast differentiation. Hence, differentiation of myofibroblasts involves a cascade through the cornea that is more likely to be reliant on TGF-β stimulation and subsequent effects on FAK and other related intercellular proteins [124, 125].

Role of Tryptophan Against Oxidative Stress

Tryptophan (Trp), an essential amino acid, is a natural UV filter present in the cornea [126, 127]. Trp levels have been reported to reduce with age in men [128, 129]. Tryptophan is degraded through the kynurenine metabolic pathway in the lens [130–133], and together with the major kynurenine metabolites [134], 3-hydroxykynurenine [133, 135], and L-3-hydroxykynurenine O-D-glucoside [132, 136], act as primary UV filters in the lens and yield other UV filters through their interaction with lens crystallins such as 4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid O-β-D-glucoside (AHBG) [137] and glutathione [138]. These compounds absorb more than 90% of the UV radiation in the lens. It has been reported that the human corneal endothelium is reduced naturally with age; furthermore, cellular apoptosis also occurs after corneal transplantation [139]. Thus, it is reasonable to assume that Trp levels also reduce as a result of depleted cell numbers and therefore might increase the risk of oxidative damage following surgery and with age. Additionally, Trp metabolism activates indoleamine 2, 3-diooxygenase (IDO), which utilizes two free oxygen radicals to form another UV filter, kynurenine [135]. IDO has also been shown to protect corneal endothelial cells from UV-mediated damage by upregulation in a dose-dependent manner and reduce apoptosis and lipid peroxidation [140]. Accordingly, supplementation with Trp or its metabolites could support corneal tissue integrity after stressful conditions that are responsible for keratocyte apoptosis and ultimately corneal haze initiation.

Role of Corneal Crystallins Against Oxidative Stress

The cornea possesses a significant accumulation of proteins analogous to lens crystallins, called corneal crystallins. Corneal crystallins function as structural elements to maintain corneal clarity by scavenging free radicals, producing NADPH, absorbing UV radiation, and detoxifying toxic aldehyde products of reactive oxygen species (ROS) [79]. Table 25.2 lists the various human crystallins and their known functions. Owing to the high levels of crystallins, the cornea seems to be well equipped to deal with various stressors introduced either surgically or environmentally. Moreover, the immense quantity of crystallins also suggests other functions related to maintenance of corneal clarity, light-scattering properties, and antioxidant properties that remain to be identified [79]. It could be argued that both the cornea and lens are working together to maintain ocular clarity [148], and, hence, both the corneal and lens crystallins might operate by sharing genes [79]. Understanding the underlying genetic basis that governs crystallin function might assist in the design of appropriate strategies to minimize corneal haze and improve surgical outcomes after refractive surgery.

In vertebrate lenses, αA and αB crystallins resist stress-induced apoptosis. α Crystallin in general prevents the translocation of Bac and Bcl-Xs, thereby inhibiting the release of cytochrome c, and subsequent activation of the caspase 3 pathway [149], and stimulates the AKT survival pathway [150]. However, the extent of the involvement of crystallins in corneal mitigation of apoptosis has not yet been fully elucidated.

Table 25.2 Human crystallins and their known functions

Corneal crystallin	Function	Reference
ALDH3A1	Metabolizes 4-hydroxyl-2-noneal (4-HNE) Production of NADPH Scavenging ROS Chaperone like activity	[88, 141, 142]
ALDH1A1	Metabolizes 4-HNE and malondialdehyde (MDA)	[143]
Transketolase (TKT)	Continuous production of NADPH	[79]
Serum Albumin	Binding toxic metabolites, maintenance of osmotic balance, transport of hormones and fatty acids UV light filtration Protection of stroma from oxidative stress by chaperone like activity	[79, 144–146]
α-Enolase	Epithelial cell differentiation	[147]

Models for Corneal Wound Healing

Alkali Burn Models

Controlled alkali burns in animal models are generally performed to study corneal opacification [151, 152], corneal transplantation [153, 154], and other corneal pathologies [155–158]. The process involves inducing an alkali insult with a 1N NaOH-soaked filter paper applied to the cornea of one eye for a varying time, followed by rinsing the area with a balanced salt solution [154, 159]. Following insult, mesenchymal stem cells aid in the re-establishment of the cornea and improved ocular clarity [154]. Similarly, therapeutic agents like bevacizumab and recombinant adeno-associated virus (rAAV)-angiostatin can accelerate wound healing, suppress neovascularization, and increase corneal clarity [151, 160].

Variations in the cultured bovine cornea have been assessed for mimicking alkali burns *in vitro* after insulting the cornea with a 2 M NaOH solution for 60 s. This results in disruption of corneal, limbal, and stromal tissues, followed by fragmentation of collagen fibrils after 7 days [161]. In a further study, cultured bovine corneas were subjected to alkali burns using 0.5 N NaOH, while the original ocular opacity was reversed using a combination of nano-formulated bovine lactoferrin and a dominant negative form of Survivin (SuR9-C84A) proteins [162].

Radiation-Induced Models

Since corneal haze is a complication dependent partly on the deleterious effects of excimer laser radiation, a model of excimer radiation was employed to induce corneal haze. Mohan et al. [163] developed a novel model for corneal haze by subjecting black C57BL/6 mice to excimer laser radiation (193 nm) following irregular phototherapeutic keratectomy (PTK). Significant corneal haze was detected in the anterior stroma after 4 weeks of insult [163]. Plasminogen deficiency was also investigated as a possible agent for corneal haze subsequent to refractive surgery; the authors induced corneal opacity using a Summit Apex Laser (Summit, Waltham, MA, USA) after PRK in plasminogen-deficient ($Plg^{-/-}$) and control mice. Results illustrated a persistent fibrinogen deposition in $Plg^{-/-}$ but not in control mice, which is strongly correlated with the development of corneal haze [164]. Similar wound-healing mechanisms after PRK have been studied in rhesus monkey corneas using 193 nm argon fluoride laser, with corneal haze reported in 93% of the cases after 6 months [165].

Scrape Injury Model

Keratocyte apoptosis is the key initiating factor in the wound-healing cascade. To assess the role of mechanical injuries in inducing keratocyte apoptosis, a scrape injury model was developed by using a Paton spatula for removing the corneal epithelium. The IL-1 system was reported to regulate corneal tissue remodeling and wound healing in a scrape injury model using adult BALB/c mice, and therefore the administration of apoptosis inhibitors following PRK can reduce the incidence of corneal haze [56].

In a further study, cultured bovine and human corneas were damaged by removing epithelial and superficial stromal tissue. The authors found that the rate of healing was similar to that *in vivo*, and this confirms the role of TGF-β in suppressing corneal re-epithelialization [166].

Haze Treatment

Mitomycin C

Mitomycin C (MMC) was discovered and first isolated in 1964 by Japanese scientists from fermentation cultures of *Streptomyces caespitosus* [167], and was known as a bioreductive alkylating agent [168]. Since that time, MMC usage has expanded from anti-tumor therapeutics to preventing haze formation after refractive surgeries [169]. MMC cross-links the complementary base strands of DNA, causes mutations, inhibits DNA synthesis, induces apoptosis, and ultimately causes cell death [168, 170].

MMC (0.02%) has shown promising results in preventing haze formation by curbing the proliferation of overactive keratocytes following ophthalmic surgeries, and thereby preventing the onset of fibrosis [169, 171]. Numerous studies have reported the efficacy of MMC in preventing haze formation after laser surface ablation. Further studies illustrated the role of MMC in haze reduction of eyes with spherical equivalent (SE) greater than −5.00 D.

Hashemi et al. demonstrated the efficacy of a 2-min 0.02% MMC immediately following PRK to inhibit haze formation in 28 myopic patients. The mean SE post-surgery of −7.08 diopters (D) ± 1.11 (SD) improved to a 20/20 vision in 37 eyes within 6 months with no signs of haze reported, and all eyes had a best-corrected visual acuity (BCVA) of 20/40 or greater. Moreover, contrast sensitivity also increased 6 months post-operatively [171] with no chemosis, irregular re-epithelialization defects, or delays observed, except in one eye that developed a large epithelial defect 4 days post-PRK treatment, thereby demonstrating the efficacy of MMC in suppressing corneal opacity following PRK in high-myopia patients [171].

In spite of its efficacy, long-term usage of MMC has raised concerns regarding endothelial toxicity. MMC is a powerful alkalizing agent that suppresses DNA synthesis through forming a covalent linkage. Higher doses could block RNA synthe-

sis, a mechanism known to inhibit corneal haze. Additionally, long-term exposure alleviates stromal loss due to high cellular toxicity, further suppressing conjunctival fibroblasts and retinal pigmented epithelium (RPE) proliferation. Therefore, further clinical trials are required to optimize the MMC dose and minimize undesirable effects with long-term exposure [172, 173].

Scavenging Reactive Oxygen Species

Superoxide, hydrogen peroxide, and singlet oxygen are the main ROS produced in the cornea as a result of various enzymatic and non-enzymatic processes [73]. In the normal physiological condition, antioxidant agents such as superoxide dismutase (SOD) detoxify superoxides by converting them to hydrogen peroxide, which is cleared by glutathione peroxidase (GPx) and catalyzed into water and oxygen [73, 74, 87]. However, increased ROS production following injury and UV exposure has been implicated in causing lipid peroxidation, alterations in cellular proteins and membrane structures, and reduction in the activity of the naturally produced antioxidants following PRK, as in GPx [5, 6, 174] and SOD [67]. A variety of free radical scavengers have been recommended to inhibit the extent of damage by the formation of ROS, and these are further described below.

Cytochrome c Peroxidase

Cytochrome c peroxidase possesses a high affinity (Km 4.5×10^{-6}) for scavenging hydrogen peroxide. Its potency to improve surgical outcomes is due to its antioxidant properties [175, 176]. Primary studies involving cytochrome c peroxidase in mice models have shown an activity similar to GPx, where cytochrome c peroxidase inhibits the peroxidative and peroxide-dependent chain reactions [6, 176].

Vitamin C

Ascorbic acid (vitamin C) is a powerful antioxidant and has been demonstrated to reduce lipid peroxidation when applied topically in a rabbit model [68]. Vitamin C also impairs myeloperoxidase, thereby inhibiting polymorphonuclear (PMN) leukocyte infiltration [68, 177], a source of ROS [65]. Similarly, vitamin C has also been shown to diminish corneal haze significantly in a non-randomized study involving humans, where the late-onset corneal haze did not develop in the 201 eyes treated with vitamin C compared with 11 among 314 untreated eyes [178]. Higher doses of vitamin C, up to 1,000 mg daily for 60 days, is safe and highly recommended to minimize the complications from refractive surgery [179–181]. Further clinical studies are needed to assess the usefulness of using vitamin C to reduce the incidence and severity of corneal haze.

Vitamin E

Alpha-tocopherol (vitamin E), the primary line of defense against ROS and lipid peroxidation, has been investigated for efficacy in reducing corneal haze. Although vitamin E alone has not been shown to reduce corneal haze significantly, topical co-treatment with hydrocortisone in a rabbit model produced promising results [182, 183]. Similarly, clinical trials involving 40 patients (20 vitamin group, 20 control) using a high dose of vitamin A (25,000 IU) and vitamin E (230 mg) over 360 days, exhibited a reduction in re-epithelialization time, thereby reducing ROS-induced damage and decreasing haze formation, and there were no signs of vitamin intoxication with such a high dose [184]. Topical application of vitamin E has also been shown to significantly minimize keratocyte apoptosis after PRK in a rabbit model [182, 183].

Limitation of Traditional Eye Treatments

Traditional therapy application is generally considered the most common approach for the treatment of various ocular diseases [185]. In contrast, these treatments did not achieve the required drug level due to some side effects such as allergic reactions, high ocular pressure, and cataracts [186]. Clinical and experimental research has shown that the frequent use of eye drops over the long term can negatively affect the tear film stability and the surface of the cornea [187]. The excessive use of drugs may also lead to increasing cases of corneal and conjunctival inflammation, as well as developing pathogen resistance [187–189]. The conventional treatments have poor bioavailability, degradation, and a lower desired therapy concentration at the target site because of the efficiency of the eye barriers [190, 191]. One of these barriers is the tear fluid, which covers the eye surface with a thickness of 10 μm and has a rapid turn-over of 2–3 min in humans [185, 192]. Tear fluid consists of three layers: the external layer known as lipid; the aqueous layer, which is an intermediate layer containing proteins, salts, mucin secretions, and metabolic enzymes; then an inner layer with a thickness of 500 nm known as glycocalyx, which involves cell surface mucins and some enzymes such as lysozyme [193]. Tear fluid is the first defense against the external environment, but it dilutes treatment molecules, and thus reduces the bioavailability and the amount of time that the drug remains on the corneal surface. Decreased bioavailability of drugs on the eye surface may reach less than 5%, and this occurs as a result of the rapid renewal rate of the lacrimal fluid with the blinking reflex [192, 194, 195]. Also, glycocalyx hinders the penetration of drug molecules into the cornea. Furthermore, the presence of metabolic enzymes in tear fluid can degrade molecules and reduce the concentration of the drug [196]. However, the conjunctiva and sclera are parts of the eye that are almost impenetrable for drugs to pass through, and thus the cornea is considered the main pathway to transport the drug to a target site in the inner eye [185]. Interestingly, the permeability of drug molecules is very difficult because of the cytoarchitecture of the highly organized corneal layers, the presence of multiple layers and tight junctions in the

corneal epithelium, and various efflux transporters on corneal epithelial cells, as well as the hydrophilic stromal layer. Hence, crossing the corneal barrier represents the major challenge for various traditional drugs [187]. In addition to the above indicated barriers, the pathologies of the posterior segment of the eye also face the vitreous humor barrier, which has a matrix with a high density of collagen fibrils and glycosaminoglycans, as well as the blood-retinal barrier. It consists of many cells, which are tightly joined together, non-fenestrated capillaries, and tight junctions of retinal epithelium that play a key role in blocking certain materials from accessing the retina tissue [197] (Fig. 25.3). Due to the complexity of the ocular barriers and lack of ideal treatment for eye diseases such as corneal haze, fibrosis, and age-related macular degeneration (AMD) [185], there is a clear need to design and introduce modern techniques in the nanocarrier drug delivery system that can overcome limitations of traditional treatments.

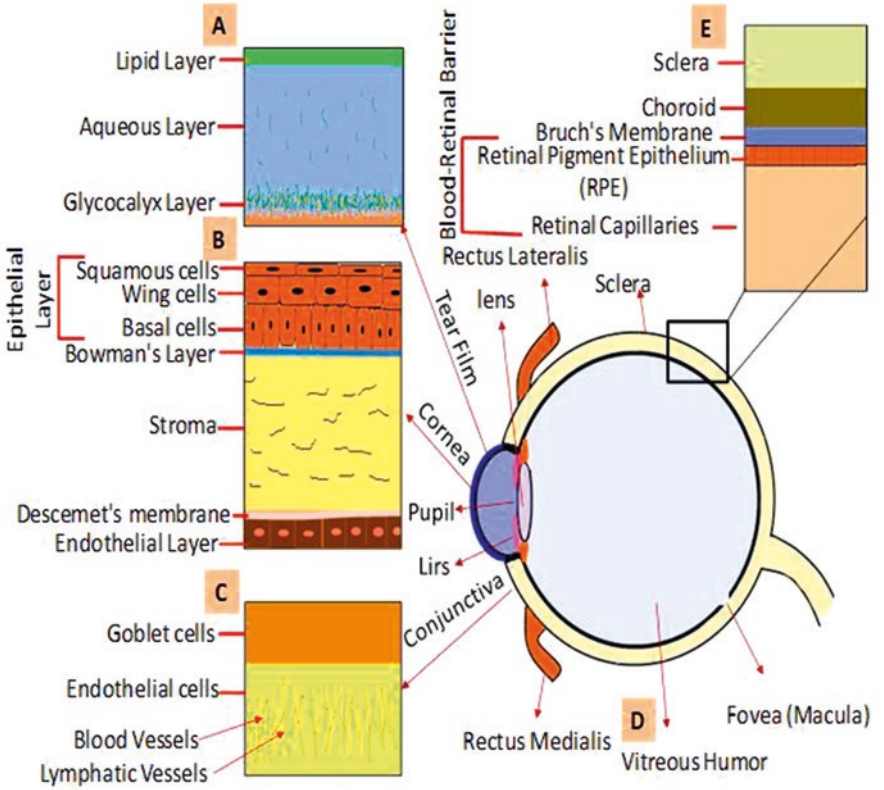

Fig. 25.3 The different ocular barriers. (**A**) Components of tear fluid barrier, which covers the eye surface. (**B**) Corneal tissue barrier with the high-level of organization. (**C**) The structure of the conjunctiva barrier, which stops the crossing of traditional drugs. (**D**) The vitreous humor barrier is a gel that fills the area between the lens and the retina with a complex matrix. (**E**) The structure of blood-retinal barrier that prevents access of drugs to the retina. This image was made using the PowerPoint Drawing Toolkit from MOTIFOLIO

Prospective Treatments

Survivin

Survivin, a 16.5 kDa protein made up of 146 amino acids, is the smallest member of the family of inhibitors of apoptosis [198]. Survivin is encoded by the BIRC5 gene and is an inherent regulator of cell division and cell death [199], and its expression is regulated by transcriptional and post-transcriptional mechanisms [200]. Corneal haze is a consequence of the imbalanced equilibrium between apoptosis and cell proliferation following surgical procedures and is enhanced under the effect of oxidative stress. This abnormal healing leads to varying degrees of corneal opacification. The condition seems to be accelerated by the upregulation of transforming growth factor beta (TGF-β), which promotes differentiation of fibroblasts to myofibroblasts and induction of fibrosis [201]. Survivin ensures continued cell proliferation and survival under stressful conditions [202], and also modulates apoptotic and non-apoptotic cell death [203–205]. Normally, it is expressed in embryonic and fetal cells/organs, but its expression becomes limited in adult tissues [206, 207]. Survivin has also been implicated in the differentiation of epithelial lens cells into lens fiber cells, while its expression is shown to be downregulated in cataractous lenses in the Sparc knockout mice model. This confirms the role of Survivin in maintaining corneal and lens integrity [208–211]. Therefore, if supplied therapeutically, Survivin could be a novel agent for restoring the balance between cell proliferation and death.

Lactoferrin

Lactoferrin (Lf), an antimicrobial and anti-inflammatory protein [212], is naturally present in human tears. Lf constitutes approximately 25% of the total tear protein content by weight [213]; low levels of Lf have been reported to cause dry eye [214], conjunctivitis, trachoma, herpes simplex keratitis, and irritative conjunctivitis [214–216]. Lf was found to appear at a minimum concentration following post-operative cataract surgery [217] and with progressive age [214]. It is highly efficient in scavenging ROS [218], thus reduced levels in tears would probably increase the susceptibly of the eye to infection and oxidative stress [219], and may cause alterations to the corneal epithelial wound-healing pattern. A recent study demonstrated the role of bovine Lf, encapsulated within chitosan nanoparticles, in inhibiting corneal opacity after alkali insult using a bovine *ex vivo* model [162]. Similarly, Lf at a concentration of 1 mg ml^{-1} has been recorded to diminish peroxide formation after sub-threshold UV radiation of 10 mJ sec^{-2} [220]. In this regard, Lf could restore the antioxidant balance following oxidative insult and reduce the development of corneal haze to a lesser extent.

Nanotechnology in Ophthalmology

There are many strategies based on nanotechnology that are likely to have a material impact in ophthalmology, and it seems that nanotechnology has provided new perspectives in the management of eye diseases through overcoming the eye barriers and delivering drugs to both anterior and posterior segments of the eye [221, 222]. Nanotechnology is used widely in medical science and it involves the design and manufacture of various materials at the nanometer scale, at least in one dimension [187]. Nanoparticles (NPs) are classified according to the types of material manufactured, which may include a polymer (polymer NPs), lipid (lipid NPs), or metal (metal NPs) [223]. Interestingly, the distinctive characteristics of nanoparticles, including the small size of molecules, positive charge of some nanoparticle types, ability to permeate into the intracellular compartment of the cell, the higher surface area to volume ratio, and capacity to deliver a large amount of drug with lower toxicity [222, 224], have given nanoparticles great importance in ophthalmology. Nanoparticles have many features such as high bioavailability, permeability, stability of therapy components, sustainable drug release, increased prevalence, targeted delivery, improved solubility and lysis, adhesion enhancement on the corneal surface, and reduced loss of treatment through lacrimal fluid [187, 191, 221]. It has been reported that the biodegradable chitosan nanocarrier in an *in vivo* model can promote the absorption of ocular drugs (indomethacin and cyclosporin A) via the cornea [225]. Also, several studies found that biodegradable nanocarriers such as pluronic for timolol maleate [226], poly (vinyl alcohol) for ciprofloxacin antibiotic [227], and polystyrene (PS) for luteinizing hormone-releasing hormone agonist (LHRH) [228], allow sustainable release of the nanocapsule eye drug and improve the drug bioavailability. Furthermore, other studies show that the use of biocompatible nanocarriers such as alginate loaded with gatifloxacin [229], PLA-PGA loaded with vancomycin [230], and carboxymethyl cellulose loaded with tropicamide [231], can strengthen the drug adhesion and decrease the loss of the drug by tear fluid wash. In this sense, biodegradable and biocompatible nanocarriers are capable of enhancing the pharmacokinetic and pharmacodynamic characteristics of encapsulated nanoparticle eye drugs. In addition, nanoparticles such as chitosan nanoparticles (CH-NPs) loaded with dominant negative protein survivin (SurR9-C84A), poly (D,L-lactide-co-glycolic acid) nanoparticles (PLGA-NPs) loaded with lactoferrin (bLf) or (SurR9-C84A) could enter the eye through the cornea with no toxic side effects, effectively repair corneal tissue after alkali burns, and potentially be used as a treatment to reduce corneal scarring and haze [162].

After refractive surgery (LASER, LASIK, and PRK), various types of drugs are prescribed with a view to avoiding some side effects such as corneal haze, inflammation, keratitis, and dry eye. For instance, corticosteroid anti-inflammatories are given after refractive surgery to avoid some symptoms such as pain, redness, inflammation, inhibitors of plasmin, and antimetabolites [186]. In contrast, the application of corticosteroids in the long term after PRK surgery can cause delayed epithelial healing, intraocular pressure (IOP), and cataracts [232].

It is worth mentioning that corticosteroids can be replaced by non-steroidal anti-inflammatory agents (NSAIDs), tranilast, cysteine, or antioxidants such as vitamin E [233]. NSAIDs are effective in limiting pain, inflammation, and postoperative photophobia. On the other hand, excessive use of certain steroidal and NSAIDs after PRK surgery may increase the incidence of corneal haze and delay corneal re-epithelialization [234]. Moreover, most NSAIDs have limited permeability through the anionic cornea because these drugs are considered weak acids that ionize at tear fluid pH and the decrease in drug pH can promote permeation with a potential increase in ocular irritation cases. Hence, it is difficult to design and produce formulations of NSAIDs that are convenient and efficient at the same time when used topically on the eye [235]. This contradiction has led scientists to nanotechnology to try and find the ideal drug by loading traditional treatments onto nanoparticles in order to improve the efficiency of treatment and reduce the negative side effects after refractive surgery [191]. It was observed that both cyclosporin A-loaded protamine nanoparticles and vitamin A-loaded protamine nanoparticles have adequate characteristics to stimulate the corneal wound healing and rapidly reduce the corneal haze after PRK surgery in *in vivo* mouse models. The results also show that the positive zeta potential and small size of nanoparticles stops any deterioration of the drug during the incubation period with tear fluid and storage, interacting with cells of the cornea, and increasing residence time on the corneal surface by at least 2 h [222]. Another study found that the increased intraocular pressure after glaucoma filtration surgery (GFS) can decrease the optimal hypotensive using mitomycin-C loaded and coated PLGA implant in *in vivo* rabbit models. This methodology may improve surgical outcomes in humans [236]. In ophthalmology, the gene therapy strategy can be used across the cornea with a view to improving structural functions, modulating pathological conditions, decreasing corneal neovascularization cases, avoiding corneal rejection, and limiting herpetic keratitis. Nanotechnology is not limited to loading the nanoparticles with the drug but extended to include loading with the gene therapy and delivering it to the target site. The use of nanoparticle-based gene therapy has contributed to improving cellular uptake, endosomal escape, and even transport to the nucleus [237].

Delivery of medications via nanotechnology-based products seems to have fulfilled the main objectives for the ideal therapy and avoids the side effects after refractive surgery. The main techniques used to treat corneal disorders, especially corneal haze, are summarized below (Fig. 25.4).

Nanoparticles

Nanoparticles are appropriate for the treatment of eye diseases since the eye is a highly complicated organ and possesses physical and anatomical barriers [238]. Notably, several therapeutic molecules could be easily loaded on different types of nanoparticles and used to treat corneal diseases [239]. These nanoparticles are either polymeric, metallic, or hybrid. Metallic nanoparticles involve platinum,

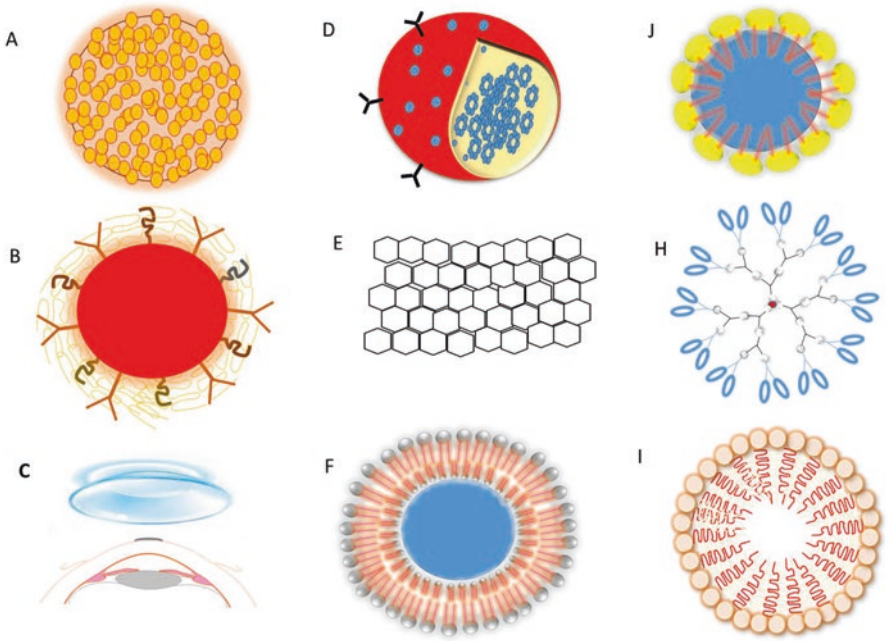

Fig. 25.4 Nanotechnology used for treatment of ocular disorders. (**A**) Nanoparticles; (**B**) polymeric nanoparticles; (**C**) contact lens as a nanodevice; (**D**) representative image of a nanosponge; (**E**) nanotubes; (**F**) liposomes with two phospholipid bilayers; (**J**): nanoemulsion; (**H**) dendrimer; (**I**) polymeric micelles. This image was made using the PowerPoint Drawing Toolkit from MOTIFOLIO

silver, and gold. Platinum nanoparticles exert a potent antioxidant property, although these particles have not been used to treat corneal disorders yet [240]. Silver and gold nanoparticles are widely used as a vehicle due to their desirable characteristics with regard to configuration and lower toxicity [241]. Gold nanoparticles have been successfully loaded with bone morphogenetic protein7 (BMP7) to treat corneal haze; BMP7 attenuates fibrosis and regenerates corneal epithelium [239]. Toxicity tests of silver nanoparticles confirm the relative safety for ocular delivery [242].

Polymeric nanoparticles are generally synthesized from chitosan, polyethylene glycol, and polyethyleneimine. Further study confirms the efficacy of chitosan loaded with gene therapy to treat corneal epithelium [243]. Poly lactic-co-glycolic acid (PLGA) is another type of polymeric nanoparticle involved in ophthalmic formulations; however, PLGA nanoformulations pose a relatively lower safety and toxicity concern. Hybrid nanoparticles are a conjugation of metallic and polymeric nanoparticles; gold nanoparticles loaded with polyethyleneimine (PEI) for gene delivery are an example of hybrid nanoparticles and were reported to stimulate safer and effective delivery to the desired targeted cells and enhance clarity of the cornea [238].

Nanodevices

Additional applications have been recently designed to correct blindness disorders. These devices were developed to improve surgical operation and maintain sustained drug release [244]. Applications of nanodevices include contact lenses, nanosponge, and carbon nanotubes. Contact lenses have an amniotic composition, and are widely used to promote healing of corneal epithelium and reduce scarring [245]. The nanosponge was recently introduced to improve drug solubility and corneal permeation. Nanosponges have a small size and are filled with a specific medication; they possess the capability to adhere to the cell surface and release their therapeutic effect in a controlled and predictable manner [246]. Carbon nanotubes have been recently employed to enhance corneal rigidity and deliver anti-apoptotic genes following corneal epithelial debridement to prevent corneal haze [247]. Carbon nanotubes are biocompatible and safe for treatment of corneal tissues and are also involved in diagnostic procedures [248].

Nanodelivery

A new application is the construction of ophthalmic formulations in order to enhance biodegradation, cellular entrapment capacity, and optimum drug release [249]. Liposomes and dendrimers are considered to be a part of nanodelivery. Liposomes are effective nanocarriers and are classified according to their sizes. They can be used to treat herpes keratitis [250]. Liposomal delivery of short-chain C-6 ceramide boosts optimum drug release, inhibits corneal inflammation, and prevents corneal haze [251]. Dendrimers on the other hand are three-dimensional structures packed with antimicrobial factors to treat keratitis [252]. They were found to have potential antimicrobial action against Gram-positive and Gram-negative bacteria. Polyamidoamine is an example of dendrimers and has been widely used as it possesses desirable characteristics regarding lower toxicity and stability. Nanodendrimers are also involved in gene therapy and bioimaging [253]. In addition to liposomes and dendrimers, nanomicelles and nanoemulsion are classified as nanodelivery tools. Polymeric nanomicelles are used to deliver gene therapies and inhibit corneal apoptosis and undesired complications such as haze formation [254]. Nanomicelles are self-assembled polymers in an aqueous solution characterized by a unique structure with both hydrophilic and hydrophobic segments to maintain sustained and stable release of medication [255]. Generally, polymeric nanomicelles are biocompatible, biodegradable, and safe for ophthalmic drug delivery [256]. Nanoemulsions are tiny oil droplets in water emulsions; they have a translucent appearance and are used for several pharmaceutical formulations. The unique structure of the nanoemulsion enhances bioavailability and high optical clarity as well as long-term stability [257]. The desired configuration of nanoemulsions permits faster diffusion through corneal layers. Restasis, Durezol, Lipimix, and Soothe XP Emollient are different types of nanoemulsions approved by the US Food and

Drug Administration during 2002–2008 [224]. The idea of nanotechnology and delivering therapeutic factors through small particles in a sustained manner has attracted scientists working in the pharmaceutical and ophthalmology fields because of the innovative and promising outcomes. Despite these advances, ideal nanoformulations are still challenging with regard to biodistribution and lower toxicity, and need further investigation.

High Myopia and Choroidal Neovascularization (CNV)

Myopia is defined as a refraction condition where the parallel light rays are focused anterior to the retina [258]. It can be classified into physiological and pathological myopia. Myopia affects a high percentage of individuals worldwide, especially in developed countries, including the USA, Singapore, Taiwan, and China [259].

Pathologic myopia (PM) is a major consequence in eyes with high myopia, represented by progressive globe elongation [260]. PM is considered to be a social and economic issue since it is frequently responsible for visual impairment in young patients [261, 262]. Choroidal neovascularization is the wet form of AMD, and is the main complication of pathologic myopia [263]. However, studies have demonstrated that visual prognosis of the affected patients is influenced by the age of the patient at the time of the onset of the disorder; therefore, patients less than 40 years of age have poorer prognosis than patients above this age [264].

Pathophysiology of Myopic CNV

Myopic CNV has been thoroughly studied and documented in angiogenic studies [265, 266]. However, the mechanism of this disease is still controversial and requires further study [267]. Studies [267, 268] have shown the correlation between the axial length and the incidence of myopic CNV; moreover, the same studies reported the interaction between the axial length elongation and the incidence of chorioretinal atrophy and the temporal crescent. Others have demonstrated a significant correlation between lacquer cracks and myopic CNV [265, 266].

Refractive errors and axial length could be secondary risk factors for development of myopic CNV. It has been found that both the subfoveal and inferior choroid were significantly thin in the affected patients, and because of the thinning choroid at the site of fovea, a hypoxic environment was created, which stimulated the HIF1-α-VEGF pathway, and led to subsequent angiogenesis induction [269–271]. The thinning of the choroid might be a result of mechanical stretching at the site of macula.

Risk Factors of Myopic CNV

Myopic CNV is a multifactorial disease. However, the major risk factors are represented by ocular, genetic, and systemic functions.

The main risk factor is the ocular factor involving patchy retinal atrophy, choroidal thinning, and lacquer cracks [265, 266, 269, 272] at the posterior pole in addition to the angiographic choroidal filling delay [273, 274], and lack of the neovascular surrounding rim. Lacquer cracks were shown to augment the incidence of myopic CNV [259].

Genetic factors are also implicated in the development of myopic CNV. Pigment epithelium-derived factor gene SNP (rs12603825) has been shown to be associated with myopic CNV. Similarly, polymorphism of the complement factor I gene SNP (rs10033900) is significantly correlated with myopic CNV [275, 276]. Although VEGF factor plays a major role in myopic CNV pathogenesis, myopic studies could not demonstrate an interrelation between VEGF gene polymorphism (rs2010963) or other common genes in AMD disease [276].

Systemic risk factors require further investigation. However, common inflammatory factors in AMD such as complement factors CH50 and C3 and C-reactive protein have been found to be predictive risk factors for initiation and progression of myopic CNV [277]. Similar outcomes were obtained with other inflammatory cytokine biomarkers such as interleukins 6 and 8 [278, 279], suggesting the role of inflammation in myopic CNV development.

Treatment of Myopic CNV

Several approaches have been introduced to manage choroidal neovascularization. An early attempt for treatment was using laser photocoagulation; however, this approach was discontinued due to complications following therapy [280, 281]. Following laser coagulation, surgical treatment for myopic CNV was introduced, involving surgical removal of myopic CNV or the neovascular membrane beneath the retina. This approach was shown to improve short-term visual acuity in 50% of the cases [282, 283]. While macular translocations are another surgical approach used to manage myopic CNV, this approach has led to several complications such as choroidal hemorrhage, maculae holes, retinal detachment, and recurrence [284–286].

Photodynamic therapy has been approved to treat myopic CNV [287]. It has shown stabilization in the visual acuity in around 72% at 12 months after treatment; however, there is no significant outcome at 24 months, with young patients demonstrating better results [288–292].

Anti-VEGF therapy is the gold standard approach for managing myopic CNV. It was first published in 2007 followed by off-label use of the medication to treat myopic CNV. Intravitreal treatment with anti-VEGF has improved the functional ocular outcomes [293, 294]. Anti-VEGF treatments have included bevacizumab. Studies

presented a stable and progressive macular recovery after 1 year of treatment, while intravitreal treatment with ranibizumab presents a superior outcome in comparison to photodynamic therapy [295]. Pegaptanib was also evaluated and the outcomes showed selective inhibition of anti-VEGF-165 isoform. However, bevacizumab showed better results regarding mean retinal sensitivity using fewer doses than pegaptanib [287]. Aflibercept (VEGF trap eye) is another anti-VEGF factor, and has shown a higher potency in treating AMD [296]. It also demonstrated an effective outcome during the clinical trial NCT01249664 [297] in treating myopic CNV.

Anti-VEGF molecules were developed to minimize the frequency of injections and include liposomes carriers [298] and cross-linked hydrogel encapsulated bevacizumab. Triamcinolone acetate loaded with thermoreversible gels seem to suppress the expression of VEGF and restore eye function [299, 300].

Conclusion

The ocular surface is exposed to a variety of insults in daily life. In addition to environmental factors like UV exposure and allergens, the cornea is also exposed to oxidative stress from mechanical injuries, infected surgical instruments, and degenerative conditions that initiate a complex wound-healing cascade. Refractive surgery is correlated with the incidence of myopia and subsequent vision loss due to CNV. The currently available medications such as MMC, vitamin E, vitamin C, and Trichostatin A (TSA) show promise in reducing corneal haze. The long-term complications of MMC and TSA have not been evaluated yet; moreover, the toxicity of MMC is concerning. Therefore, a shift in trend towards delivering the naturally occurring and biologically safe medications in the form of nanoformulations is necessary and survivin and lactoferrin could assist in achieving this goal.

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Index

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