Expert Opinion

Ocular novel drug delivery: impacts of membranes and barriers

Jaleh Barar, Ali Reza Javadzadeh & Yadollah Omidi†
† *Tabriz University of Medical Sciences, Research Centre for Pharmaceutical Nanotechnology, Faculty of Pharmacy, Tabriz, Iran*

Background: Ocular drug delivery is an extremely challenging area due to its restrictive barrier functionalities. *Objective*: Drug transport via corneal/non-corneal routes involves several intricate biological processes such as drug penetration across the ocular barriers and transfer to the anterior or posterior chambers, thus the influence of these processes on the pharmacotherapy of the eye should be fully addressed. *Methods*: To pursue the impacts of such impediments in novel drug therapy, recent publications were reviewed regarding advanced strategies such as nanomedicines. *Conclusion*: The ocular barriers are highly specialized and selectively control the inward/outward traverse of compounds, hence a better understanding of these biological obstacles would provide a platform to advance ophthalmic drug therapy towards specified delivery/targeting with minimal adverse consequences.

Keywords: corneal and non-corneal routes, ocular drug delivery, ocular membranes and barriers, nanomedicine

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1. Introduction

The visual cells and transparent tissues perform the perfect vision and ocular functionalities as a result of tight cellular membranes and barriers which control the transport of fluids and solutes [1]. These barriers also effectively hinder the delivery and targeting of ocular therapeutics, thus numerous studies have been devoted to developing efficient strategies to cross such biological impediments.

Topical dosage forms are the mostly utilized medications administered from the anterior segment (i.e., cornea, conjunctiva, sclera, anterior uvea). Most of these medications are given as eye drops that can be easily drained away from the ocular surface, resulting in low bioavailability and failure to reach the posterior segments (retina and vitreous). Drug delivery to the posterior segment is a challenging issue because of the selective functionality of the biological barriers, while the current strategies to treat ocular diseases have had limited successes [1,2]. Drug therapy via intravitreal injection seems to be the remaining option, as reported for treatment of age-related macular degeneration (AMD) with the anti-vascular endothelial growth factor (VEGF) therapies, for example, pegaptanib (Macugen®), ranibizumab (Lucentis®) and bevacizumab (Avastin®) [3,4]. However, this is an invasive strategy which can be exacerbated with repeated injections and may inevitably cause adverse consequences. Systemic administrations are also considered as a suitable approach for some pharmaceuticals that possess appropriate physicochemical and biopharmaceutical characteristics. Ocular diseases therapy via subconjunctival and periocular (sub-Tenon's and peribulbar) routes are deemed to provide prolonged pharmacologic impacts with lower toxicity [5].

Novel nanosystems appear to shed some light on ocular drug therapy by efficiently crossing the relevant barriers with minimal inadvertent side effects [6]. Likewise, smart nanoformulations can be exploited to target the specific biomarkers within

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the retinal pigment epithelium or choroidal vasculature. Modified (e.g., PEGylated) nanomedicines may also be administered intravenously and reach target sites via the bloodstream [7]. For more efficient therapy, futuristic molecular Trojan delivery systems are being developed, exploiting more specific cellular characteristics of carrier-/receptor-mediated transport machineries of the eye. Such battle has already begun as a frontier paradigm using genetically engineered antibodies and modified oligonucleotides [8-10]. Nevertheless, to achieve such goals, better understanding of the biological membranes and barriers of the ocular system is clearly demanded. This review provides an overview of the main biological aspects of the ocular systems towards novel therapies such as nanomedicines.

2. Mechanisms of topical ocular drug absorption

Local drug delivery into the eye cul-de-sac involves the corneal and/or non-corneal routes, where the medications may be carried away by the lacrimal fluids. The corneal route represents the main absorption path for most ophthalmic therapeutics. However, corneal absorption is also considered to be a rate-limited process due to presence of the corneal epithelium [1,11]. The second path involves penetration across the conjunctiva and sclera into the intraocular tissues; however, this path appears to be less productive due to presence of the local capillary beds that remove the drug from target sites into the general circulation. Despite this drawback, poor corneal permeability compounds such as timolol maleate, gentamicin and prostaglandin $PGF_2\alpha$ were shown to reach the intraocular section through a diffusion across the conjunctiva and sclera [12-15]. Thus, the absorption mechanism depends mostly on the physiochemical characteristics of the compounds and the biological membranes and barriers of the target tissue [16,17].

3. Ocular membranes and barriers

After passing the tear film and lacrimal fluid, to reach the desired target sites, drugs are faced with several membranous barriers located in the cornea, conjunctiva, iris-ciliary body and retina (see **Figure 1**), in which the epithelial and/or endothelial cells are sealed by the tight junctional constituents [18].

3.1 Tear film

A healthy ocular surface (cornea and conjunctiva) is largely dependent on the amount and composition of the tear film, which is tightly controlled by regulation of the orbital glands and ocular surface epithelial secretions [19]. The tear film contains various factors such as nutrients, electrolytes, proteins, lipids and mucin [19]. Among them, the expressed mucins by human ocular surface epithelia (MUC1, 4, 5AC, 7 and 16) are heavily glycosylated and display antiadhesive properties, providing a protective barrier for the cell membrane, and prevent cell–cell and cell–protein interactions [20]. The tear film as a buffered aqueous fluid (pH ~ 7.2 – 7.5) displays a fast restoration time of 2 – 3 min and most administered eye drops/solutions are washed away within the first 15 – 30 seconds, resulting in poor bioavailability (< 5%) [21]. Thus, more robust medications such as gel-forming timolol (Timoptol®-LA and Timoptol®-XE, used to treat glaucoma) [22], thermosensitive *in situ* gel-forming systems [23], or mucoadhesives [24] are needed to exert prolonged pharmacologic impacts within the local target tissues.

3.2 Corneal route: epithelial and endothelial barriers

As demonstrated in **Figure 2**, the cornea is a highly specialized, clear and avascular tissue that consists of several layers: corneal epithelium, basement membrane, Bowman's layer, stroma, Descemet's membrane and endothelium [18]. The corneal epithelium barrier selectively controls the traverse of ocular drugs (in particular hydrophilic compounds), whereas the stroma and endothelium pose less control on trans-corneal permeation [16,25]. The stroma consists of hydrated collagen and proteoglycans as an organised matrix with interspersed cells (keratocytes) [26]. This biostructure may function as a barrier to highly lipophilic drugs.

The corneal endothelium is a monolayer of polygonal cells, most of which are hexagonal in shape (20 µm diameter and 4 – 6 µm thickness). These cells play a key role in maintaining corneal transparency through their transport, synthetic and secretory functions. The abundance of endothelial intracellular organelles (e.g., mitochondria) indicates its high metabolic activity. The localization of some crucial plasma membrane transporters at one side of these polarized cells highlights their specialized transport functionality [27,28]. They form a cellular barrier between the stroma and aqueous humor, where its selective carrier- and/or receptor-mediated transport functionality support maintenance of the anterior chamber [18]. These membranes also provide selective gates for hydrophilic pharmaceuticals and macromolecules and control their traverse to the anterior segment. In fact, the 'leaky tight junctions' of the corneal endothelium result in the passage of macromolecules between the aqueous humor and stroma [29]. The fluid transport mechanism across the corneal endothelium appears to be mediated by either the local osmotic gradients across basolateral and apical cell membranes, or the electro-osmotic driving force due to the transport of bicarbonate from the stroma to the aqueous side, together with the functionality of the Na^+ pump within the leaky tight junctions [29]. Nonetheless, association of the paracellular route and the receptor/carrier-mediated transport systems (e.g., endocytosis) with the traverse of macromolecules is yet to be fully investigated. The leakiness of the corneal endothelia correlates with its low bioelectrical properties (20 – 60 $\Omega.cm^2$) [29-32], while brain capillary endothelial cells display significantly higher electrical resistance (> 1500 $\Omega.cm^2$) [33,34]. In comparison, immortalized human

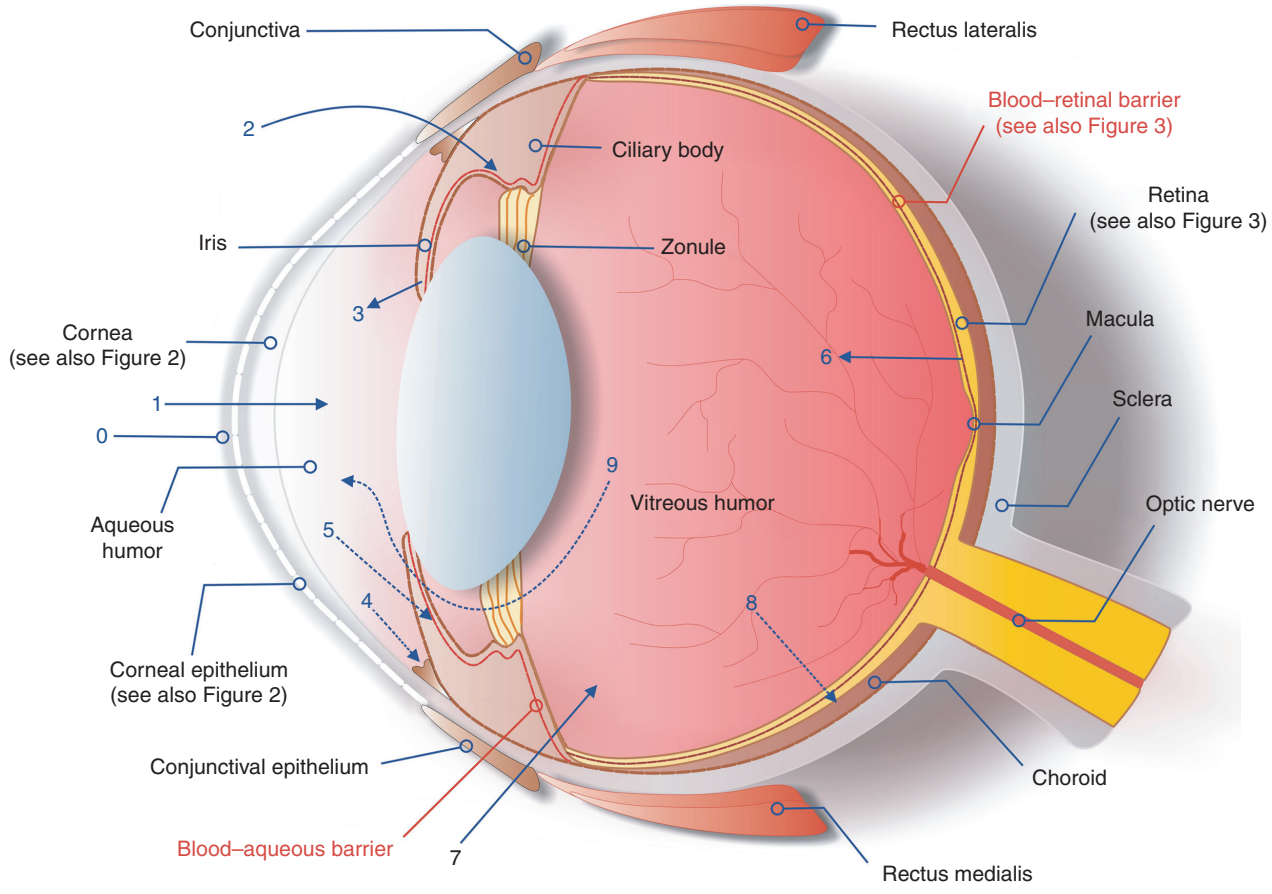

Figure 1. Schematic illustration of the eye and its biological barriers. Tear film provides the foremost physiologic impediment against installed drugs (0). The cornea is the main route for drug transport to the anterior chamber (1). The conjunctival and scleral route provides a passage for macromolecules and hydrophilic drugs (2). Small compounds penetrate from the iris blood vessels into the anterior chamber after systemic administration (3). The administered drugs can be carried away from the anterior chamber either by aqueous humor outflow (4) or by venous blood flow after diffusing across the iris surface (5). The retinal pigment epithelium and the retinal capillary endothelium are the main barriers for systemically administered drugs (6). Intravitreal injection to reach the vitreous (7). Drugs can be removed from the vitreous through the blood–retinal barrier (8) and/or by diffusion into the anterior chamber (9).

corneal epithelial cells [35] and rabbit primary corneal epithelial cultures [36] display approximately 500 and 5000 ($\Omega.cm^2$), respectively. On the whole, the topically applied drug diffuses into the aqueous humor and to the anterior uvea after crossing the corneal impediment, but these pharmaceuticals fail to reach the retina and vitreous at sufficient therapeutic concentrations [37].

3.3 Non-corneal route: conjunctiva and sclera

The non-corneal route, the so-called conjunctival/scleral pathway, is a competing and parallel route of absorption. It is a minor absorption pathway compared to the corneal route, but for a few compounds its contribution is significant.

The conjunctiva is a thin and transparent membrane lining the inside of the eyelids and covering the anterior surface of the sclera, extending to the edge of the cornea (the limbus). The mucous membrane of the conjunctiva consists of three layers: i) an outer epithelium which is a permeability barrier; ii) substantia propria, containing nerves, lymphatics and blood vessels; and iii) submucosa, which provides a loose attachment to the underlying sclera (tenon's capsule). The more firmly adhering segment lining the inside of the eyelids is called the tarsal or palpebral conjunctiva [21]. Due to its rich vasculature nature, the existence of goblet cells and transdifferentiation potential, this tissue is different from the cornea and is considered as of great importance to the non-corneal route for ocular drug delivery (e.g., macromolecular nanomedicines). Further, the conjunctival epithelium plays a key protective role by the tight junctional barrier at the apical surface of the epithelium, with the bioelectrical resistance over 1500 $\Omega.cm^2$ [38], which is an important functionality for the permeation of hydrophilic substances and delivery of macromolecules [39,40]. A markedly large amount of the administered pharmaceutical is usually carried away by the systemic circulation while crossing the conjunctiva and the remaining drug penetrates across the sclera to

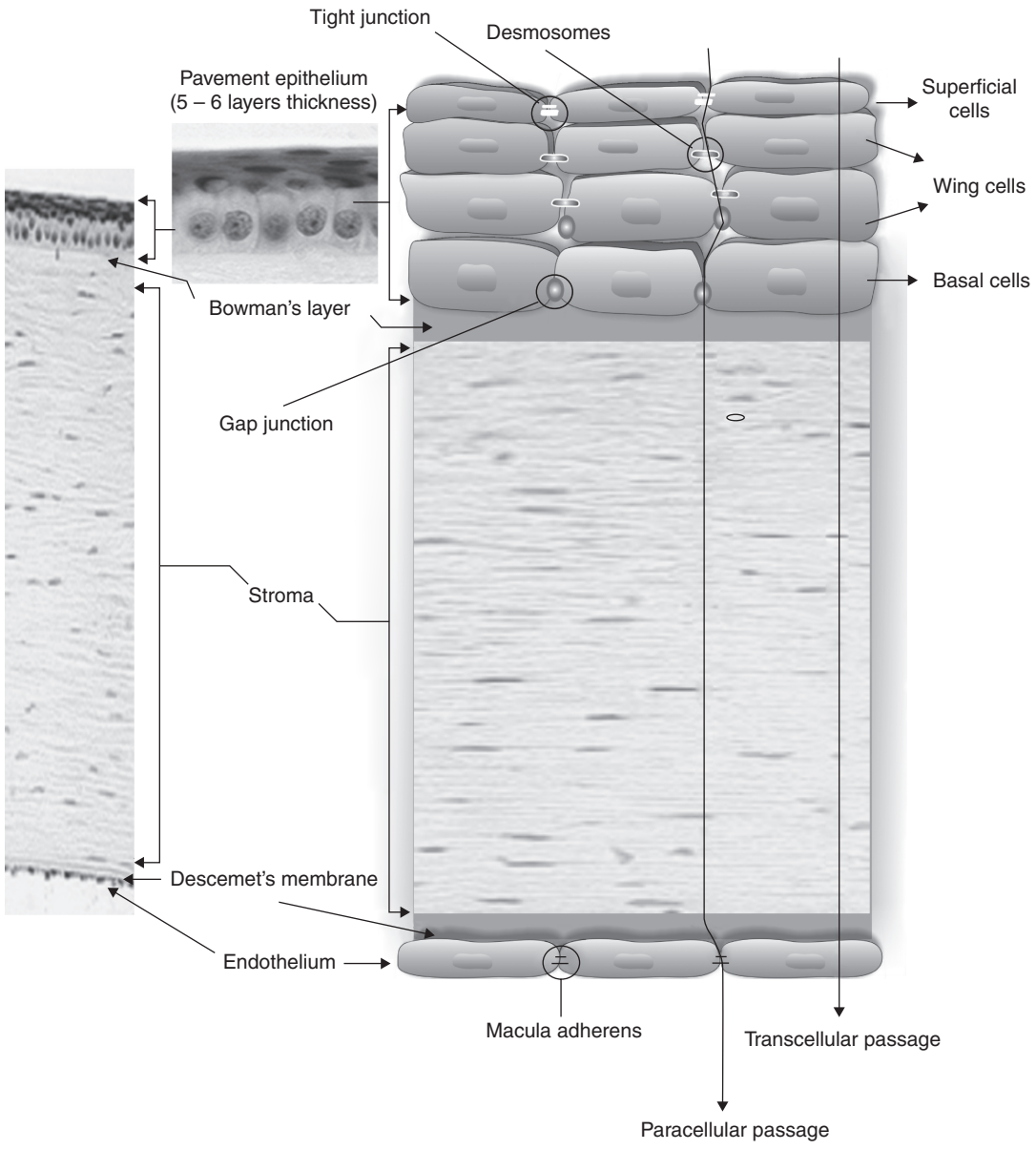

Figure 2. The cornea and its cellular organization of various transport-limiting layers. The outer superficial epithelial cells, possessing tight junctions, display the tightest monolayer. The inner endothelial cells, displaying macula adherens, are more permeable.

reach the posterior parts (uveal tract, retina, choroid and vitreous humor) [41-43]. Various transporters were shown to be expressed in conjunctival epithelium. Among them, neutral and cationic amino acids transporter ($ATB^{O,+}$), nucleoside transporter (CNT2) and peptide transporter (PepT1) can be exploited for transporting the associated drugs (e.g., acyclovir) [44-46]. The functional expression of efflux pumps including P-glycoprotein (P-gp) and multi-drug resistance protein (MRP1) have been reported [47,48], but the role of the vesicular transport machineries (e.g., clathrin-coated pits and caveolae) in the conjunctival epithelium is yet to be fully examined.

The sclera is continuous with the cornea and extends posteriorly from the limbus. Structurally, the sclera is very similar to the corneal stroma, containing numerous channels and consisting mainly of collagen and mucopolysaccharides [49,50]. The poorly vascularized sclera is significantly more permeable than the cornea, but less permeable than the conjunctiva. The sclera has been shown to be exploited for antibody delivery [51].

In general, ophthalmic drugs can be absorbed from conjunctiva and delivered to the eye via the sclera. However, drainage loss through blood vessels of the conjunctiva can greatly impact the conjunctival/scleral pathway. Therefore,

this non-corneal route is considered to be non-productive for most ophthalmic drugs, although it should be noted that the conjunctival epithelium is the most viable route for ocular delivery of peptides and oligonucleotides [49].

3.4 Iris, ciliary body and aqueous humor flow

The iris, ciliary body and choroid represent the vascular uveal coat of the eye, where the iris anterior is immersed in the aqueous humor. The ciliary body is formed by several biological regions, including non-pigmented ciliary epithelium, pigmented ciliary epithelium, stroma and ciliary muscle. It is fenestrated and leaky capillaries confer intercommunication of the anterior and posterior chambers [52], at which the aqueous humor is secreted into the posterior chamber and flows through the pupil into the anterior chamber. By mechanisms of diffusion, ultrafiltration and active transport, the aqueous humor is derived from plasma within the capillary network of the ciliary. Of these, the active transport processes account for the majority of aqueous humor production, where water soluble substances of larger size or higher charge are actively transported through the cellular membrane requiring expenditure of energy such as Na^+, K^+-ATPase and glycolytic enzymes [2].

3.5 Vitreous body and fluid flow

The space between the lens and the retina is filled by the clear and avascular connective tissue of the vitreous (~ 4 ml and ~ 4 g in adults) [53]. The gel-like composition of the vitreous contains water (99.9%), collagen, hyaluronic acid and ions [54,55]. Drug movement in the vitreous is largely dependent on its diffusion rate; and unlike the vitreous composition, the convective flow fails to affect the drug diffusion [2]. The intravitreal route appears to be the major route of drug administration to the posterior segment, in which the diffusivity potential of the vitreous depends on the pathophysiological state and molecular weight of the administered drugs [2,56].

3.6 Blood–aqueous barrier

Two discrete cell layers (the endothelium of the iris/ciliary blood vessels and the non-pigmented ciliary epithelium) form the blood–aqueous barrier (BAB) in the anterior part of the eye (see **Figure 1**), whose functionality controls the traverse of solutes between the posterior and anterior chambers [57,58]. Both of these cell layers display tight junctional complexes, by which the inadvertent non-specific passage of solutes into the intraocular milieu is impeded. This results in maintenance of the transparency and chemical composition of the ocular fluids [58,59]. However, the barrier functionality of BAB is not complete even when it is intact [60]. Injected horse radish peroxidase (HRP; 40 kDa) was shown to reach the aqueous humor through the fenestrated capillaries of the ciliary but not via the iris blood vessels, which control the permeation of plasma proteins into the aqueous humor [61].

Traverse of substances from the aqueous humor into systemic circulation through the iris microvasculature endothelia seems to be less restricted. Hence, drugs dissolved in the aqueous humor can easily penetrate into the anterior surface of the iris, are absorbed by the iris pigments and thereafter they are washed from the anterior chamber away by passage into the iris blood vessels [62,63]. The small and lipophilic drugs were shown to enter the uveal blood circulation via BAB, from where they are eliminated more rapidly than larger and more hydrophilic drugs, which are eliminated by aqueous humor turnover only [60]. The passage of drugs from the anterior segment to the posterior segment appears not to be an efficient strategy because of the continuous drainage of the aqueous humor (i.e., a turnover of 2.0 – 3.0 ml/min). Thus locally used ophthalmic therapies fail to provide an efficient pharmacological effect in the posterior segment (e.g., retina and vitreous). Novel therapeutic strategies such as intravitreal injections and subconjunctival implants seem to grant only limited successes [2].

3.7 Retina

The retina, the light -sensitive part of the eye, is a thin film of tissue which covers the entire of inner wall of the eye. The retina is formed from neural cells as well as glial cells (i.e., Müller cells, astrocytes, microglial cells and oligodendroglial cells) [26]. The outermost part of the retina is a single layer of pigmented cuboidal epithelial cells, the so-called retinal pigment epithelium (RPE) [26]. Although the functions of the RPE and the retina are tightly coupled, RPE and accordingly the blood–retinal barrier (BRB) will be our focus due to their important roles in ocular drug therapy.

3.8 Blood–retinal barrier

The BRB is located in the posterior part of the eye and consists of two types of cells, including the retinal capillary endothelial (RCE) and RPE cells that form the inner and outer BRB, respectively (see **Figure 3**). Specialized transport processes within RPE, along with the robust barrier restrictiveness of RPE, control the traverse of nutrients/compounds selectively, at which only selected nutrients are exchanged between the choroid and retina [2,37]. The polarized RPE cells display predominant apical localization of Na^+, K^+-ATPase that regulates intracellular Na^+ and K^+ homeostasis [64,65]. The inner BRB covers the lumen of retinal capillaries and selectively protects the retina from the blood circulating molecules. Unlike the fenestrated choroidal capillary endothelial cells, the RCE cells possess intercellular tight junctions (see **Figure 3**) [66]. Regardless of lipophilic substances permeation across the RCE cells, this barrier displays poor permeability to proteins and small hydrophilic compounds [18,60].

Substantial delivery and sufficient pharmacological effects of drugs within the vitreous and the retina necessitate systemic or intravitreal drug administration. Systemic application, via oral or intravenous administration, requires high doses of

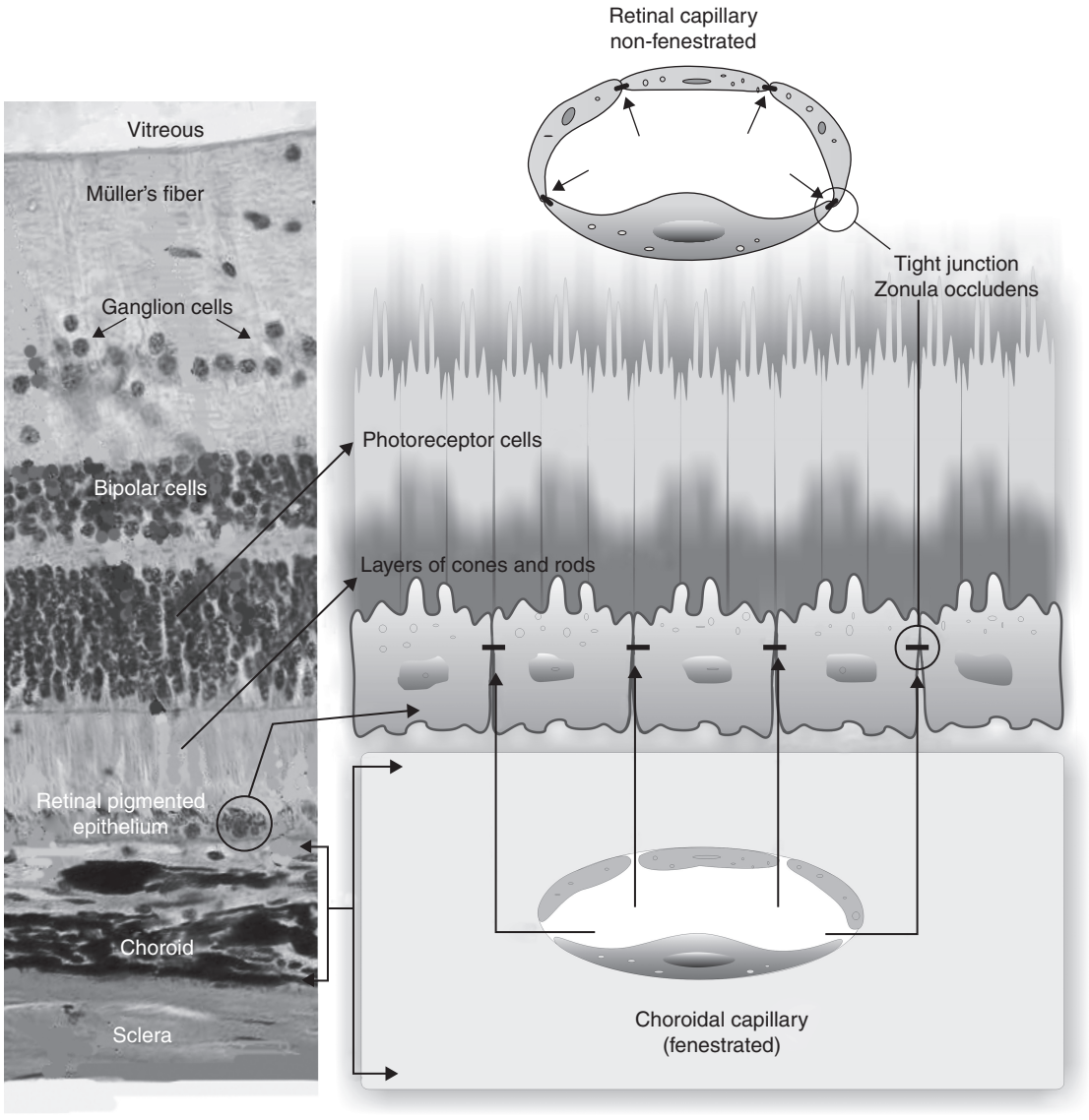

Figure 3. The retina and its cellular organization of various transport-limiting layers. The outer layer of retinal pigmented epithelium displays a tight barrier due to the presence of tight junctions (zonula occludens). The inner layer of retinal capillary endothelial cells possessing tight junctions are non-fenestrated compared to the choroidal capillary endothelial cells, which are fenestrated.

the drug, since blood flow and restrictive functionality of BRB allow only a very small fraction of the drug to reach the posterior segment. Thus a large amount of the drug is disseminated in the entire body, leading to inadvertent adverse reactions [67].

4. Membrane transport systems

Cell membranes impose a barrier to the free movement of molecules through the membranous lipid bilayer and associated transport machineries. A solute, based upon molecular properties, is transported across the cell membranes by passive diffusion and/or carrier/receptor-mediated transport [68]. Nearly all ocular tissue displays the Na^+/H^+ exchanger, Na^+/HCO_3^- symporter and Cl^-/HCO_3^- exchanger that are involved in the regulation of intracellular pH [69,70]. The Na^+/H^+ exchanger is present at the basolateral membranes of both epithelial and endothelial cells, while the Na^+/HCO_3^- transporter is predominantly localized at the basolateral side of the corneal endothelium and is faintly expressed in the corneal epithelium. This implies that the distribution pattern of these channel transporters at the apical and basolateral membrane vary upon the cellular needs and physiologic functions [71,72]. **Figures 4** and **5** schematically represent the currently known influx/efflux transport machineries of the corneal epithelium and the retinal outer/inner barriers, respectively; the reader is directed to the following reviews for further details [18,63,73]. The functional expression,

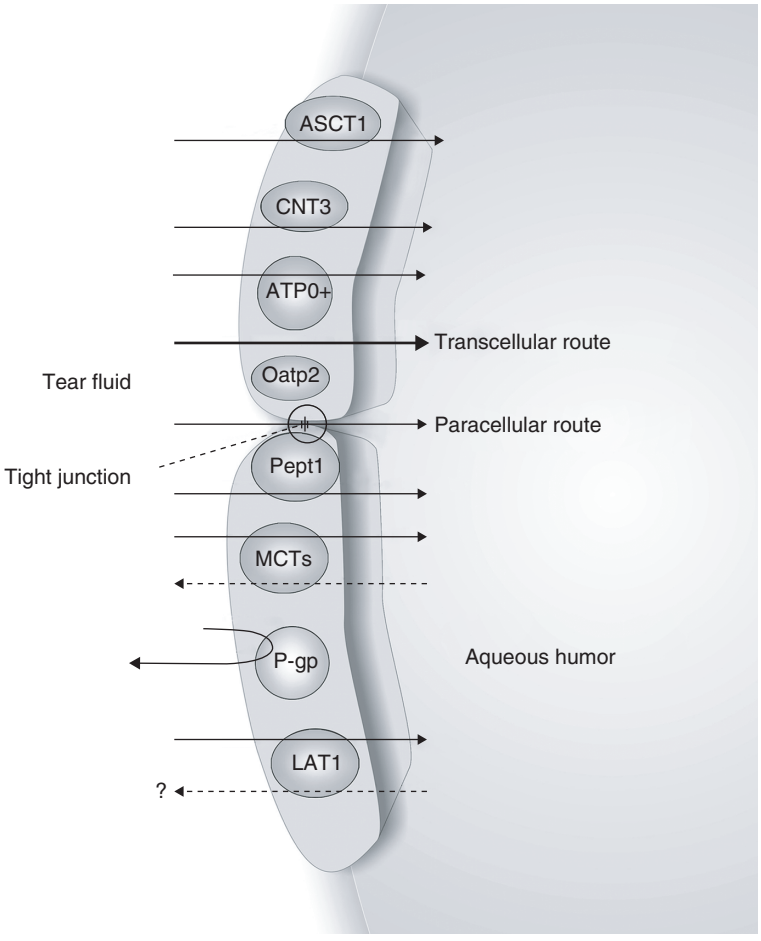

Figure 4. Schematic representation of the currently known carrier-mediated transport machineries in the corneal epithelium.
ASCT: Na^+-dependent small neutral amino acid transporter (L-alanine); ATB0+: Concentrative amino acids transporter (carnitine, valacyclovir and valganciclovir); CNT3: Concentrative nucleoside transporter 3 (thymidine); LAT1: Large neutral amino acids transporter (L-phenylalanine); MCTs: Monocarboxylate transporters (lactate, pyruvates and ketones); Oatp2: Organic anion transporting polypeptides 2 (unknown); Pept1: Proton-coupled oligopeptide transporter (POT) di/tri peptide transporter 1 (valacyclovir, valganciclovir, glycylsarcosine and cephalexin); P-gp: P-glycoprotein (verpamil and fluorescein).

transport direction, membrane distribution pattern and exchange potentials of many of these transporters need to be fully investigated.

4.1 Influx and efflux transporters

The influx and efflux transport machineries are functionally expressed in the main membranous barriers of the eye, that is the cornea, conjunctiva, iris–ciliary body and the retina (see **Figures** 4 and 5) [63]. The uni/bidirectional influx transporters such as monocarboxylate transporters (MCTs), glucose transporters (e.g., Glut1), amino acid transporters (LAT1 and LAT2) and peptide transporters (Pepts) supply the essential nutrient requirements of the cells [68]. The LAT1 transporter of the brain capillary endothelial cells functions bidirectionally with greater efflux activity [74]. However, its functional directionality in the corneal/retinal barriers has yet to be fully investigated, as have most of carrier-mediated transporters in the eye. For detailed information, the reader is directed to [46,63,73,75-80].

Among the ATP -binding cassette (ABC) superfamily, the P-glycoprotein (P-gp) and multi-drug resistance associated proteins (MRPs) play a key role in the unidirectional efflux of substances. Human and rabbit corneal epithelium were shown to significantly express P-gp [81] and MRPs [82]. Similarly, these efflux pumps have been identified in different tissues of the eye, such as the retinal capillary endothelial cells [83], the retinal pigmented epithelial cells [84], the ciliary non-pigmented epithelium [85], the conjunctival epithelial cells [47] and the iris and ciliary endothelial cells [47]. On the basis of the current knowledge about the functional expression of efflux transporters in ocular tissues, useful modification of drug delivery strategies to increase ocular bioavailability and to harness the ophthalmic diseases in a more efficient way are expected to be forthcoming.

4.2 Endocytosis pathway

Specialized receptors exist at the ocular barriers to control the passage of xenobiotics. The endocytosis pathway via

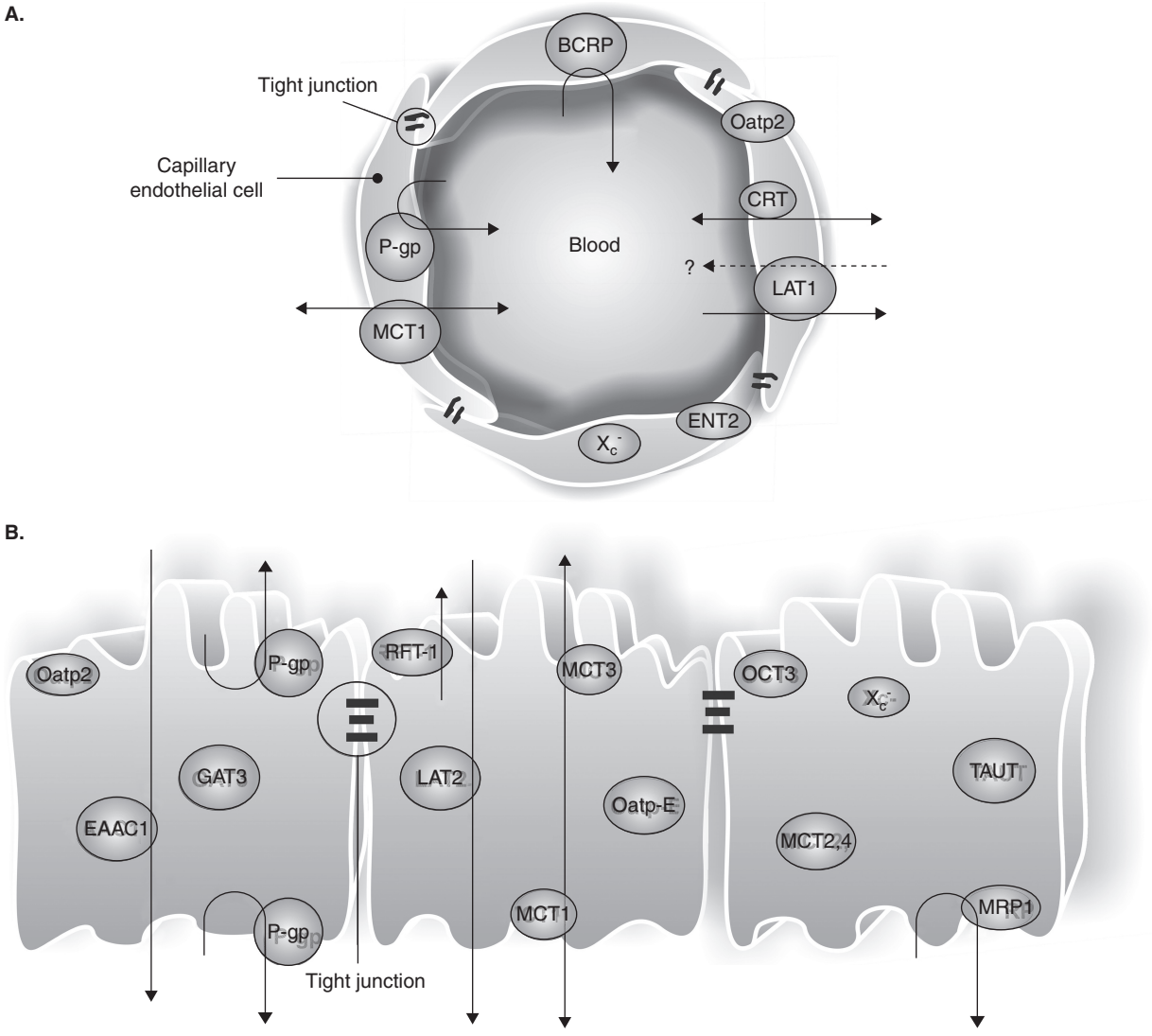

Figure 5. Schematic demonstration of the currently known transport machineries in the cellular barriers of the retina. **A.** The inner endothelial cells of the retina. **B.** The outer pigmented epithelial cells of the retina.
BCRP: Breast cancer-related protein; CRT: Creatine transporter; EAAC: Glutamate transporter; ENT2: Equilibrative nucleoside transporter 2 (thymidine); GAT3: GABA transporter; LAT1: Large neutral amino acids transporter (L-phenylalanine); MCTs: Monocarboxylate transporters (lactate, pyruvates and ketones); MRP: Multi-drug resistance proteins; Oatp2: Organic anion transporting polypeptides 2; OCT: Organic cation transporter; P-gp: P-glycoprotein (verpamil and vincristin); RFT: Reduced folate/thiamine transporter; TAUT: Taurine transporter; X_c^-: Glutamate/cysteine exchange transporter.

clathrin-coated and/or caveolae (non/smooth coated) vesicles are accounted for ocular receptor mediated transports. The expression of clathrin and the integral protein of caveolae domain (caveolin-1) have been reported within the ocular tissues [86-90]. Using cultured human retinal pigment epithelial cells (ARPE-19) and a mouse model, Mo *et al.* reported the involvement of caveolae-mediated endocytosis pathways in the uptake of albumin nanoparticles containing Cu, Zn superoxide dismutase gene [91]. However, Qaddoumi *et al.* showed that the endocytosis of the poly(lactic-co-glycolic) acid (PLGA) nanoparticles in primary cultured rabbit conjunctival epithelial cells occurs mostly independently of clathrin- and caveolin-1-mediated pathways, despite the transcriptome and protein expression of clathrin [92]. However, the transport of albumin in the rabbit lens epithelial cells revealed the involvement of a transcellular transport mechanism employing both clathrin and caveolae-mediated endocytosis/transcytosis pathways [90]. The detection of insulin and insulin-like growth factor (IGF)-1 receptors in the corneal and conjunctival epithelial cell membranes [93], together with the role of transferring in nanoparticle delivery [94], reveal that more investigations are required to resolve the ambiguity of macromolecular (e.g., peptide/protein) trafficking in the eye.

5. Ophthalmic therapy paradigms

Several factors affect topical ocular drug therapy, including drainage of the instilled solution, lacrimation and tear turnover, metabolism, tear evaporation, non-productive absorption/adsorption, limited corneal area and poor corneal permeability, and binding by the lacrimal proteins [2]. Effective ocular drug absorption requires sufficient corneal penetration and prolonged contact time with the corneal tissue. Hence, various approaches such as iontophoresis, prodrugs, ion pair formation, sustained-release formulations, mucoadhesives and cyclodextrins have been exploited to enhance ocular drug absorption. Nonetheless, most of the currently used medications for treatment of the ocular diseases (e.g., glaucoma, conjunctivitis, keratitis and uveitis) were shown to have bioavailability problems [2].

Treatment of the retinal pathologies (e.g., fungal and bacterial endophthalmitis, viral retinitis and proliferative vitreal retinopathies) requires systemic administration, although it has limited success, primarily due to the exclusion of the organ from systemic circulation. For example, foscarnet sodium or ganciclovir is used to treat cytomegalovirus (CMV) retinitis and endophthalmitis as intravenous [95] or intravitreal injections [96]. Of these, the intravitreal injection appears to be the mainstay treatment of posterior segment infections/diseases [2], even though it may associate with patient non-compliance, endophthalmitis, cataracts, astigmatism and retinal detachment [97,98]. Thus, to avoid such complications, intravitreal implants (e.g., Vitrasert®) have been developed. Nevertheless, the scleral implants and the subconjunctival administrations appear to confer limited success, mainly because of the repeated need for surgical intervention associated with the presence of a foreign device within the eye [2].

6. Novelties in ocular drug delivery and the implementation of nanomedicines

The biological characteristics of the eye render this organ impervious to foreign substances and thus, for the attainment of an optimal concentration at the intended ocular tissue of action through circumventing the ocular barriers, colloidal nanoparticle drug carriers have been devoted a great deal of attention [99]. Piloplex®, consisting of pilocarpine ionically bound to poly(methyl) methacrylate-co-acrylic acid, is a nano-scaled colloidal carrier system effectively used in glaucoma patients as twice-daily instillations. Multidimensional mechanisms appear to be involved for the pharmacologic action of ocular nanosystems, including extending the time of drug residency in the cornea/conjunctiva, sustaining drug release from its carrier, reducing the precorneal drug loss and targeting the desired biomarker [99-101]. Thus, it is highly desirable to exploit bioadhesive materials for the formulation of nanosystems to be retained in the cul-de-sac after topical administration. Various biodegradable and non-biodegradable carriers have been used, for example, poly(lactic acid), PLGA, chitosan, poly(isobutyl cyanoacrylate) and Eudragit RS100 or RL100 [99]. Erodible nanosystems are superior because the self-eroding process of the hydrolyzable polymer exerts less harm on tissue [102,103]. For example, PLGA colloidal nanoparticles were exploited to deliver gene-based therapeutics to the retinal pigment epithelial cells [104]. The sustained-release nanosuspension of piroxicam and methylprednisolone acetate were formulated using Eudragit to control the endotoxin-induced uveitis in rabbits [105,106]. For the treatment of chronic ocular diseases (e.g., CMV retinitis), localized prolonged nanomedicines can be used effectively as a safer alternative to the frequent injections that may cause cataract development, retinal detachment, endophthalmitis and vitreous hemorrhage [100].

A variety of innovative drug delivery systems (implants) have been so far introduced for ophthalmic applications, including: Ocusert® (1974) for 1 week constant release of pilocarpine from conjunctiva; Vitrasert® (1996) for 6 months constant release of ganciclovir from the pars plana area of vitreous; Retisert® (2005) for 2.5 years constant release of fluocinolone acetonide. Further, intravitreally injectable biodegradable (Posurdex®) and non-biodegradable (Medidur®) implants are currently undergoing Phase III Clinical trials [107-109]. In September 2006, the global bio-nanotech company pSivida announced the initiation of a Phase II clinical trial of Mifepristone as an eye drop treatment for steroid-associated elevated intraocular pressure [107], for the formulation of which a nanocarrier has possibly been used. More recently, a branched PEGylated anti-vascular endothelial growth factor (VEGF) aptamer (pegaptanib sodium, marketed as Macugen®) was approved by the FDA for the treatment of neovascular AMD, which demonstrated the first oligonucleotide aptamer nanomedicine. It suppresses the pathological angiogenesis in the neovascular AMD by specifically targeting the extracellular VEGF, resulting in inhibition of angiogenesis, reduction of permeability of the vascular bed and diminution of inflammation [4]. Ranibizumab is a recombinant humanized monoclonal antibody fragment (marketed as Lucentis®) that targets VEGF-A, an important mediator in the development of choroidal neovascularization, and reduces neovascularization and leakage in the wet AMD [4]. Ranibizumab (48 kDa) is a markedly smaller molecule than the RhuMab VEGF (bevacizumab, Avastin®, 148 kDa) and is in early clinical testing for the treatment of the choroidal neovascularization via the intravitreal route [4]. Unlike RhuMAb VEGF, ranibizumab is able to penetrate the retina and enter the subretinal space after intravitreal injection because of the notable size difference.

Artificial vesicles such as liposomes, niosomes and discomes have been successfully utilized as vehicles for ophthalmic drugs (e.g., oligonucleotides, acetazolamide, pilocarpine HCl, cyclopentolate and timolol maleate), resulting in improved ocular bioavailability. Of these, positively charged nanostructures seem to be preferentially captured at the negatively charged corneal surface and slow down drug elimination by lacrimal

flow [100,110]. Using laser-targeted delivery (LTD), it is likely to release and activate the encapsulated drug within the heat-sensitive liposomes injected intravenously [111]. By virtue of being encapsulated, the drug is confined to the liposomes and shielded from the general metabolism.

Photodynamic therapy (PDT) with verteporfin for choroidal neovascularization associated with retinal pigment epithelium detachment AMD [112], and the combination of PDT with the aforementioned nanomedicines [113,114], have revealed promising results. These medications are unable to completely cure AMD, but they significantly decelerate the progression of the lesion growth in a proportion of patients [3]. Ocular gene therapy has reached clinical trials (for inherited retinal degeneration), which possibly marks the culmination of decades of investigations [115]. The eye, as a valuable model system for gene therapy, is a unique, highly compartmentalized organ for efficient delivery of small volumes of viral (e.g., adeno/lenti-viral vectors) [116-118] or non-viral (e.g., PEGylated nanoliposomes and niosomes) [7,119-121] vectors. Among them, PEGylated non-viral nucleic acid nanostructures prevent their interaction with undesired biomolecules and provide promising results [7]. Significant recent progress in the mapping and cloning of retinal disease genes has provided great potential for gene therapy in the eye, for example gene replacement in inherited retinal degeneration (Leber's congenital amaurosis due to defects in the gene encoding the enzyme RPE65) [122,123].

7. Expert opinion and final remarks

In ocular drug therapy, the need for the safe application of medications to the posterior segment is deemed to be even more important than surface delivery. Treatment of intricate posterior segment diseases crucially necessitates safe drug delivery to the retina, the choroid, or the ciliary body. Systemic delivery and devices inserted into the vitreous are valuable strategies, as are biodegradable/non-biodegradable controlled-release implants inserted into both aqueous and vitreous. Moreover, in recent years there has been a dramatic increase in understanding of the pathobiology of ocular diseases at cellular/molecular level. There now exists a large number of drugs under development [124]. For ocular drug therapy, this state of high flux resulted in a few advanced therapeutics such as Visudyne®, Macugen® and the angiostatic anecortave acetate (Retaane®), which is administered as a periocular injection every 6 months [4,125].

It is also possible to perceive nano-scaled technologies in practice, providing a promising platform for improved non-invasive ocular drug delivery. However, further developments need to be accomplished to render the nanosystems more effective. The primary practical approach to provide nanomedicines with the necessary site adherence and site retention to achieve carrier and drug targeting in topical ocular therapy is to endow them with the ability to be a bioadhesive system, perhaps by the utilization of natural biopolymers such as hyaluronic acid. The mutual use of penetration-enhancers, along with nanomedicines, without compromising the stability of the system, could also provide higher ocular bioavailability. Bioadhesive nanosystems can maximize ocular drug absorption by prolonging the drug residence time in the cornea and conjunctiva and minimize precorneal drug loss, resulting in increased patient compliance. For development of the ocular bioadhesive systems as localized sustained released medications, non-biodegradable systems appear to be adequate to treat perforations and ulcerations. For long-term use, however, ideally these systems would be non-toxic, biodegradable adhesives with site specificity and minimal immunogenicity, yet improving bioavailability by enhancing absorption (particularly for protein/peptide-based macromolecules) or inhibiting the metabolizing enzymes.

The unique bioarchitecture of the eye means that it is considered to be a perfect organ for gene therapy because it is difficult for the delivery vector to escape to systemic sites. To date, ocular pathologies have been tackled with 12 trials (Phase I/II) focused on different conditions, including retinitis pigmentosa, glaucoma, diabetic macular edema and AMD, while a total of 1347 gene therapy clinical trials are in development [126]. This highlights the growing interest in gene therapy of the ocular diseases, for which futuristic genomedicines are deemed to become more effective therapeutics by exploiting molecular Trojan delivery systems for the safe shuttling of genomedicines (e.g., Antisense, ribozyme and siRNA) and targeting the desired biomarkers [127,128]. There is much excitement surrounding the potential of the short interfering RNA (siRNA), resulting in siRNA moving remarkably rapidly towards applications; there exist a total of 11 clinical trials for its implementation and two these trials are involved with ocular disease [126]. LTD and PDT seem to be promising methodologies to deliver and to activate therapeutic and diagnostic agents to the retina and choroid. However, their successful application largely depends on the appropriateness of the agent. Perhaps a combination of these techniques with gene therapy could benefit ocular diseases. Encapsulated cell technology (ECT) and cell therapy appear to have treatment potential for ocular diseases. ECT implants consist of living cells encapsulated within a semipermeable polymer membrane and supportive matrices, which are genetically engineered to produce a specific therapeutic substance to target a specific disease or condition. Once implanted, it allows the outward passage of the therapeutic product [129]. It is anticipated that the biological properties of the eye would undergo the desired alterations through the application of these technologies. However, for implementation of cell therapy technology in the human eye, validation of such techniques will be a critical step. Furthermore, the cellular and subcellular/molecular aspects of the target tissues should be fully addressed and ocular disease-related biomarkers should be exclusively

clarified. Possibly, high-throughput screening technologies (e.g., DNA/protein array and phage display screening methodologies) would facilitate investigations towards specific targeting.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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Affiliation

Jaleh Barar[1], Ali Reza Javadzadeh[2] & Yadollah Omidi[†3]

[†]Author for correspondence

[1]Assistant Professor of Cellular & Molecular Pharmaceutics, Tabriz University of Medical Sciences, Research Centre for Pharmaceutical Nanotechnology, Faculty of Pharmacy, Tabriz, Iran

[2]Clinical Associate Professor of Ophthalmology, Tabriz University of Medical Sciences, Ophthalmology Department, Nikookari Eye Hospital, Faculty of Medicine, Tabriz, Iran

[3]Assistant Professor of Pharmaceutical Nanobiotechnology, Tabriz University of Medical Sciences, Research Centre for Pharmaceutical Nanotechnology, Faculty of Pharmacy, Tabriz, Iran

Tel: +98 411 3376149; Fax: +98 411 3376149;

E-mail: yomidi@tbzmed.ac.ir