



Recent advances and strategies for nanocarrier-mediated topical therapy and theranostic for posterior eye disease

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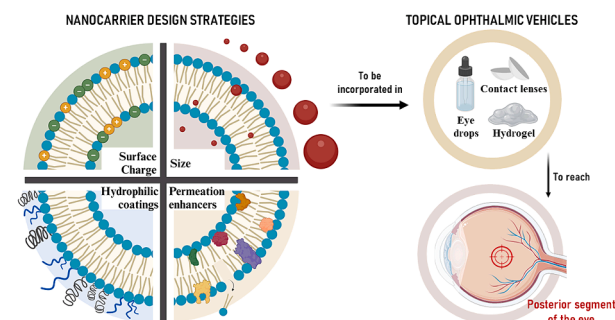
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GRAPHICAL ABSTRACT

Nanocarrier-mediated topical therapies and theranostic are valuable strategies for targeting posterior ocular tissues. This review exalts safe-by-design approaches in the development of tuneable nanocarriers able to surpass the main ocular barriers.

NANOCARRIER-MEDIATED THERAPIES FOR THE TREATMENT OF AGE-RELATED RETINAL DISEASES



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ABSTRACT

Posterior eye disorders, such as age-related macular degeneration, diabetic retinopathy, and glaucoma, have a significant impact on human quality of life and are the primary cause of age-related retinal diseases among adults. There is a pressing need for innovative topical approaches to treat posterior eye disorders, as current methods often rely on invasive procedures with inherent risks. Limited success was attained in the realm of topical ophthalmic delivery through non-invasive means. Additionally, there exists a dearth of literature that delves into the potential of this approach for drug delivery and theranostic purposes, or that offers comprehensive design strategies for nanocarrier developers to surmount the significant physiological ocular barriers.

This review offers a thorough and up-to-date state-of-the-art overview of 40 studies on therapeutic loaded nanocarriers and theranostic devices that, to the best of our knowledge, represent all successful works that reached posterior eye segments through a topical non-invasive administration. Most importantly, based on the

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successful literature studies, this review provides a comprehensive summary of the potential design strategies that can be implemented during nanocarrier development to overcome each ocular barrier.

1. Introduction

The world is facing severe demographic changes, and the prevalence of age-related retinal diseases (ARRD) is increasing globally, mirroring the aging of the world population. By 2020, the Vision Loss Expert Group (VLEG), together with the Global Burden of Disease (GBD) study, estimated that 206 million people were suffering from moderate to severe visual impairment and that the cases of blindness surpassed 33 million [1], with an increase of about 7 million per year [2–4]. Furthermore, most visual disorders occur in the population aged 50 years and older (80%), and the female gender registers higher incidence rates given their longer life expectancy [5]. Age-related macular degeneration, diabetic retinopathy, and glaucoma are the frontline ARRD responsible for visual impairment among adults, affecting mainly the posterior segment of the eye [2]. Treating ocular conditions within this region poses challenges, and it is often dependent on invasive approaches that come with significant limitations, such as potential complications and risk of infection, retinal detachment, vitreous hemorrhage, and cataracts [6]. Besides the adverse consequences on human quality of life, ocular disorders also have profound socioeconomic effects, with an estimated global annual cost of US\$3,000 billion (€520 billion in Europe) [2]. Altogether, age and gender-related visual loss are societal challenges demanding immediate action when it comes to prevention, early detection, and, most importantly, innovative treatment approaches.

The human eye (Fig. 1) is a globe-shaped structure with unique anatomy and physiology [7]. In general, an adult eye is around 24 mm in diameter and may be categorized into two primary regions: anterior and posterior [3,7,8]. The anterior region represents the eye's frontal part and entails the lachrymal system, lens, anterior and posterior chambers, ciliary body, cornea, conjunctiva, iris, and eyelids [8–10]. Meanwhile, the eye's posterior portion covers the area from the lens zonula and ciliary muscle to the optic nerve, including specific structures such as the retinal pigmented epithelium, neural retina, sclera, choroid, and the vitreous humor [9,10].

Despite the ocular accessibility for therapeutic administration, the emergence of several physiological and anatomical barriers (e.g., tear turnover mechanisms, physical–chemical barriers of ocular cell membranes, and blood ocular barriers) has always been a challenge to ocular therapeutic delivery [11,12]. For example, systemic delivery is the less effective ophthalmic route of administration given the existence of specialized blood–ocular barriers. These display selective permeability that restricts the penetration of molecules to intraocular tissues (1% a 2%), causing a poor therapeutic outcome in posterior eye disorders [8,9]. As a result, alternative routes, such as intravitreal or periorcular (subconjunctival, suprachoroidal, or transscleral) injections, are often selected to reach posterior regions [13]. Even though intraocular injections are effective and allow high drug doses in the posterior segment of the eye, they are intrusive procedures that can result in severe complications [6]. Also, the growing life expectancy and aging of the population do not align with complex and invasive administrations requiring technical expertise.

In these circumstances, topical application stands out for its great convenience, as a well-accepted delivery route for the medical management of ocular disorders, particularly those that impact the front area of the eye, such as dry eye syndrome, elevated intraocular pressure (IOP), conjunctivitis, keratitis, and corneal dystrophies [6,14]. To access the tissues located in the posterior portion of the eye, topically applied therapeutics must face three main obstacles: the precorneal region, the corneal barrier, and noncorneal absorption [9]. Considering the limitations imposed by the previously mentioned barriers, only a very short percentage ($\leq 5\%$) of topically administered therapeutic agents have conditions to effectively reach the aqueous humor [7,15]. As such, current ophthalmic topical formulations are tailored to deliver small drug molecules and developed for maladies affecting the anterior portion of the eye [12,13,16]. To get beyond these limitations, innovative drug delivery systems have emerged using nanomedicine approaches.

Nanomedicine brought new opportunities and promising tools for disease prevention, diagnosis, monitoring, and treatment [17]. The

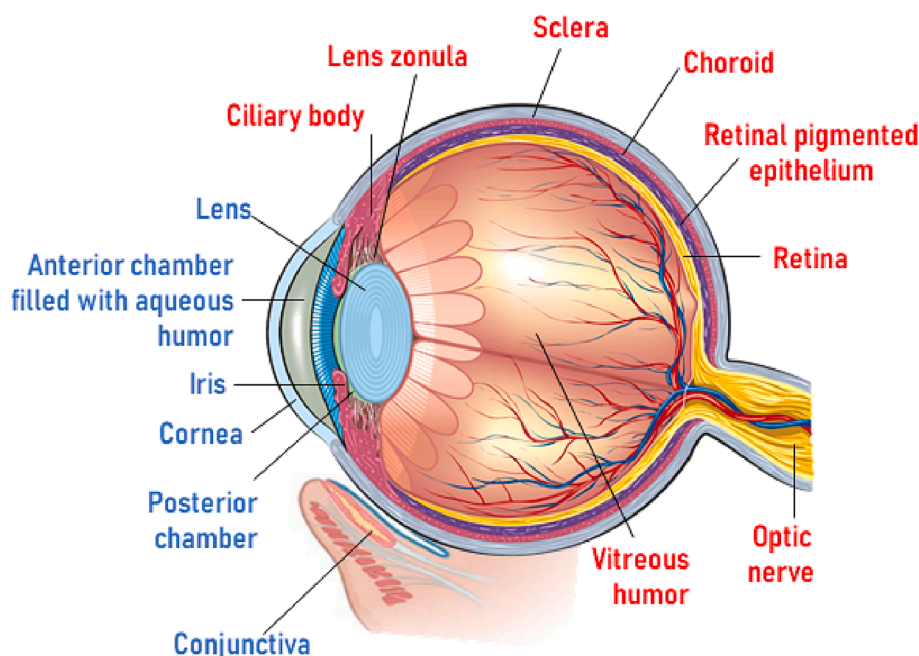


Fig. 1. Diagram depicting the anatomical structures of the eye. The blue and red ocular compartments correspond to the anterior and posterior areas, respectively.

research conducted in this subject focuses on four main topics: diagnostic imaging (nanodiagnosis), drug delivery (nanotherapy), regenerative medicine, and diagnosis coupled to treatment (theranostics) [18]. The global nanomedicine market is anticipated to keep expanding, reaching \$350.8 billion by 2025; drug delivery stands as one of the major driving forces for this trend [19]. To go beyond the inherent hurdles of conventional therapies, nanotechnology-based strategies using nanocarriers hold great promise in therapeutic agents' delivery. In the particular case of vision sciences, ophthalmic nanocarriers offer the capability to: (i) enhance therapeutic targeting through surface functionalization; (ii) enhance the duration and efficacy of therapeutic effects; (iii) modulate release profiles and maintain therapeutic levels at the target sites; (iv) protect therapeutic payloads and preserve their activity until they reach their target; (v) reduce administration frequency as well as the therapeutic dosage delivered, further mitigating adverse side effects resultant from therapeutic activity or off-target interactions; and (vi) penetrate deeper and overcome the different anatomic barriers of the eye [20]. This review focuses on topical nanotechnology-based approaches for therapeutic delivery to the posterior segment of the eye. Up to date, several review articles have addressed therapeutic delivery to the posterior portion of the eye using nanomedicine approaches [14,21–28]. Few, however, have discussed the topical route of administration [10,29,30]. The differentiating factor of the present review is that, unlike the existing literature that offers a broader discussion on delivery methods to the posterior portion of the eye, ours provides a comprehensive list of detailed design strategies that can be implemented during nanomedicine development (i.e., composition, size, charge, deformability, surface modification with targeting moieties or functional coatings, permeation enhancers). The proposed strategies consider the anatomical, morphological, and physicochemical properties of the ocular barriers. Furthermore, another important goal of this review is to provide researchers in the field with a set of tools that will help them make an informed decision when selecting a therapy delivery system. These tools will enable the chosen system to feature the most convenient design and properties to overcome the major ocular barriers to the desired site. Additionally, the inclusion of therapeutic-loaded nanocarriers in topical vehicles as a tool to prolong their residence time at the eye's surface and enhance posterior ocular delivery will also be addressed. The review will also cover innovative topical theranostic devices designed for ocular disorders, with a particular focus on emerging strategies targeting the posterior eye's region, while referring interesting results obtained in the anterior region.

In summary, this review fills a literature gap by focusing on topical administration for delivering therapeutics to the posterior eye. It addresses recent theranostic approaches and specific design strategies for nanocarrier developers to overcome key biological barriers unique to the eye's structure and function. Moreover, it emphasizes the significance of adhering to applicable guidelines and regulatory standards to ensure a rational design process.

The goal of this review is not to detail the different types of nanocarriers or describe their synthesis methods as reported elsewhere [31,32]. Instead, it intends to highlight the role of different types of organic and inorganic nanocarriers in delivering therapeutic agents to the eye. As so, this review provides a comprehensive state-of-the-art overview (Table 1) of nanodelivery systems reaching the posterior portion of the eye through topical application.

Forty studies were revised, and, to the best of our knowledge, they represent the main works using solely the topical route of administration to reach the posterior segment of the eye. The key criterion for selecting the studies included in the revision was the *in vivo* assessment in animal models (regardless of the animal) that measurably confirmed the detection of therapeutic compounds in posterior eye tissues. It should be noted, however, that rodents, which are commonly used to study topical drug delivery to the eye, because they are readily available and similar to humans in many aspects, still have anatomical differences. Their smaller eyes, thicker corneas, and faster tear turnover rates affect drug

penetration and distribution [33,34]. Additionally, discrepancies in ocular surface composition and vitreous humor volume pose challenges in extrapolating findings in rodents to humans [35]. Additionally, alternative models, such as rabbits, pigs, or non-human primates, also presented in Table 1, may better mimic human ocular anatomy and physiology for certain aspects of ocular drug delivery research. Studies focusing solely on *in vitro* assays or alternative administration routes were excluded from Table 1.

2. Ocular barriers encountered upon topical ophthalmic therapeutic administration

When considering topical ophthalmic delivery, therapeutic agents can take one of two major permeation routes, with several elimination routes to systemic circulation (Fig. 2) [29,76]. The first is the corneal route, which includes the tear film, cornea, anterior chamber, lens (therapeutics can cross it or go around it by possible parallel diffusion routes), posterior chamber, vitreous chamber, and retina. The second is the non-corneal (or conjunctival) route and comprises the tear film, conjunctiva, sclera, choroid, and retina [76]. Both the tear film and the retina are common structures to each route. The subsequent sections will provide a detailed review of the various eye compartments, their barriers, and possible strategies to successfully overcome them.

2.1. Barriers to therapeutic absorption common in corneal and conjunctival routes

Fig. 3 depicts the barriers encountered during therapeutic absorption via the corneal and the conjunctival route, which are then explained in the text.

2.1.1. Tear film turnover

The precorneal region represents the outermost eye barrier and is the first one to be surpassed after topical therapeutic administration. In this region, the presence of dynamic barriers, such as tear turnover or blinking mechanisms (Fig. 2), cause a partial drug clearance that ultimately results in poor drug bioavailability. In addition, tear film selectivity also compromises the delivery and penetration of therapeutic agents along mucosal surfaces [77].

The tear film (pH $\approx$ 7.4) has a thickness of approximately 8 μ m and is constituted by a lipid layer and a muco-aqueous layer (Fig. 3) [24,78]. The lipid layer is the first line of defense against foreign agents and is mainly composed of a mixture of lipids (triglycerides, cholesterol, free fatty acids, waxes, and others) secreted by the Meibomian, Zeiss, and Moll glands [24,27]. The lipophilic environment within this area limits the hydrophilic drugs' diffusion to the corneal interface and prevents the evaporation of the lachrymal fluid. Subsequently, the muco-aqueous layer helps to moisten and hydrate the eye surface, accounting about 90% of the overall volume of the tear film [24]. The fluid, mainly composed of monovalent and divalent inorganic ions such as Na⁺, K⁺, Cl⁻, HCO₃⁻, and proteins, is secreted by the lachrymal gland [24]. Finally, mucus is a biological barrier that covers and protects exposed surfaces of the body [79,80]. This hydrophilic gel, rich in negatively charged mucins, maintains epithelial hydration but also has a selective permeability that allows the exchange of water, nutrients, and gases with the underlying epithelium while restricting the diffusion of pathogens and other particles (for detailed information, see section 2.3) [80,81].

2.1.2. Blood-retinal barrier (BRB)

The blood-retinal barrier (BRB) is a highly specialized barrier with selective permeability formed by an inner (iBRB) and outer (oBRB) layer [26]. The retinal capillary endothelial (RCE) cells and the retinal pigmented epithelium (RPE) constitute the iBRB and oBRB, respectively (Fig. 3) [26,27]. In both layers, the cells are closely associated by tight junctions that restrict the penetration of molecules to intraocular tissues

Table 1

State-of-the-art overview of successful ocular drug delivery systems reaching the posterior segment of the eye following topical application.

Nanocarrier	Topical vehicle	Composition	Therapeutic agent	Physicochemical parameters	Strategies to reach posterior eye segment	Experimental model	Ref.
Liposomes	Eye drops	• PEG15H • PEG-12 glyceryl dimyristate • BAC	TA (Corticosteroid)	Size: 187.8 nm PDI: 0.6 ζ Potential: +0.554 mV E.E.: 35%	• Coating with PEG15H • BAC: –Penetration enhancer –Close to neutral surface charge • Reduced size (<300 nm)	• <i>In vitro</i>: HCF cell line • <i>In vivo</i>: rabbits	[36]
Liposomes	Eye drops	• SPC • CHOL • CHS	TA (Corticosteroid)	Size: 135.46 $\pm$ 4.49 nm PDI: 0.21 $\pm$ 0.03 ζ Potential: +17.98 $\pm$ 3.21 mV E.E.: 90.66 $\pm$ 3.21%	• Coating with CHS: –Overall cationic charge –Mucoadhesive properties –Hydrophilic permeation enhancer • Reduced size (<300 nm)	• <i>In vitro</i>: HCEC and ARPE-19 cell lines • <i>In vivo</i>: mice	[37]
Liposomes	Eye drops	• SPC • CHOL • CHS	TA (Corticosteroid)	Size: 176 nm PDI: N.D. ζ Potential: +41.1 mV E.E.: 74%	• Coating with CHS: –Overall cationic charge –Mucoadhesive properties –Hydrophilic permeation enhancer • Reduced size (<300 nm)	• <i>In vivo</i> : rats	[38]
Liposomes	Eye drops	• EPC • CHOL	EDV (Antioxidant)	Size: 92.6 $\pm$ 1.5 nm PDI: ζ Potential: +7.4 $\pm$ 0.4 mV E.E.: 41.4 $\pm$ 0.3%	• Close to neutral surface charge • Reduced size (<300 nm)	• <i>In vitro</i>: HCEC and NHC cell lines • <i>In vivo</i>: Mice	[39]
Liposomes	Eye drops	• DSPC or EPC • DCP or SA • CHOL	Coumarin-6 (Fluorescent probe)	Size: 114.0–561.0 nm PDI: N.D. ζ Potential: –76.9 to +25.9 mV E.E.: N.D.	• DCP: –Negative charge • SA: –Overall cationic charge –Mucoadhesive properties • DSPC: –Rigid lipid ($T_m \approx 55^\circ\text{C}$) diffuses better through than fluid lipids (EPC) • Reduced size (<300 nm)	• <i>In vivo</i> : rats, rabbits and monkeys	[40]
Liposomes	Eye drops	• EPC • DCP • CHOL • PLL	Coumarin-6 (Fluorescent probe)	Unmodified Liposomes: Size: 94.6 nm PDI: 0.064 ζ Potential: –40.2 mV E.E.: N.D. PLL-modified liposomes: Size: 97.2 to 286.3 nm PDI: 0.068 to 0.726 ζ Potential: –39.5 to +15.7 mV N.D.	• PLL: –Cationic polymer –Mucoadhesive and muco-penetrating properties depending on [PLL] concentration • Reduced size (<300 nm)	• <i>In vivo</i> : mice	[41]
Liposomes	Eye drops	• TMAG • DLPC • DOPE • DC-CHOL	pDNA for β -galactosidase (Plasmid vector)	N.D.	• TMAG: –Overall cationic charge –Mucoadhesive properties • DOPE: –Fusogenic properties	• <i>In vivo</i> : rats	[42]
Liposomes	Eye drops	• DSPC • CHOL • PVA or PVA-R	DF (Anti-inflammatory)	Size: 176.7 nm PDI: 0.037 ζ Potential: –0.5 mV E.E.: 98.9%	• PVA-R: –Mucoadhesive properties • Reduced size (<300 nm)	• <i>In vivo</i> : rabbits	[43]
Liposomes	Eye drops	• EPC • Brain PS • CHOL • Vit E • Annexin-A5	BVZ (Anti-VEGF)	Size: 163 $\pm$ 73 nm PDI: 0.203 ζ Potential: –7.2 $\pm$ 0.6 mV E.E.: 25%	• EPC and Brain PS: –Confer negative charge • Annexin- A5: –Corneal permeation enhancer • Reduced size (<300 nm)	• <i>In vitro</i>: HCE cell line • <i>In vivo</i>: rats and rabbits	[44]
Liposomes	Eye drops	• DPPC • DOPS • CHOL • Annexin-A5	TGF- β 1 (Polypeptide member of the TGF- β superfamily of cytokines)	Size: 157 nm PDI: 0.176 ζ Potential: –28.83 $\pm$ 0.9 mV E.E.: 30%	• DOPS: –Confers negative charge • Annexin- A5: –Corneal permeation enhancer • Reduced size (<300 nm)	• <i>In vivo</i> : rabbits	[45]
Solid lipid nanoparticles	Eye drops	• SA • PC • STC	TBM (Antibiotic)	Size: 80.01 $\pm$ 11.10 nm PDI: 0.155 $\pm$ 0.03 ζ Potential: –25.7 $\pm$ 0.20 mV E.E.: 2.5% w/w	• SA: –Confers an overall negative charge • STC: –Penetration enhancer • Reduced size (<300 nm)	• <i>In vivo</i> : rabbits	[46]

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Table 1 (continued)

Nanocarrier	Topical vehicle	Composition	Therapeutic agent	Physicochemical parameters	Strategies to reach posterior eye segment	Experimental model	Ref.
Solid lipid nanoparticles	Eye drops	• Compritol®888 ATO • HSPC • Tween®80 • PEG-600	KTZ (Antifungal)	Size: 126.35 ± 2.2 nm PDI: 0.28 ± 0.02 ζ Potential: -3.19 mV E.E.: $70.19 \pm 1.94\%$	• Coating with PEG-600: –Hydrophilic and muco-penetrating –P-gp efflux inhibitors • Tween® 80: – Penetration enhancer – P-gp efflux inhibitors • Reduced size (<300 nm)	• <i>In vitro</i>: ARPE-19 and RCE cell lines • <i>In vivo</i>: rabbits	[47]
Solid lipid nanoparticles	Eye drops	• Compritol®888 ATO • P188 • Tween®80 • Glycerin • CHS	IN (Anti-inflammatory)	Size: 265 ± 8 nm PDI: 0.30 ζ Potential: $+27 \pm 1.2$ mV E.E.: $91.5 \pm 3.2\%$	• Coating with CHS: –Overall cationic charge –Mucoadhesive properties –Hydrophilic permeation enhancer • P188: –Hydrophilic and muco-penetrating • Tween® 80: – Penetration enhancer – P-gp efflux inhibitors • Reduced size (<300 nm)	• <i>In vitro</i>: excised corneas and scleral tissue with retinal pigmented epithelium from rabbits • <i>In vivo</i>: rabbits	[48]
Solid lipid nanoparticles		• Compritol®888 ATO • PEG400 • HSPC • P188	ATS (Statins)	Size: 256.3 ± 10.5 nm PDI: 0.26 ± 0.02 ζ Potential: 2.65 mV E.E.: $73.1 \pm 1.52\%$	• PEG 400 and P188: –Hydrophilic and muco-penetrating • PEG 400: – Inhibits P-gp efflux pumps • Neutral surface charge • Reduced size (<300 nm)	• <i>In vitro</i>: HCLE, R28, ARPE-19 and RCE cell lines • <i>In vivo</i>: rabbits	[49]
Solid lipid nanoparticles	Eye drops & gel	SLN composition: • Compritol®888 ATO • GMS • P188 • Tween®80	TA (Corticosteroid)	Size: 187.5 ± 1.8 nm PDI: 0.35 ± 0.09 ζ Potential: -33 ± 2.5 mV E.E.: $95.1 \pm 1.3\%$	• Gelling agents –Increase corneal residence time • Tween® 80: – Penetration enhancer – P-gp efflux inhibitors • P188: –Hydrophilic and muco-penetrating • Overall negative charge • Reduced size (<300 nm) • Overall negative charge	• <i>In vitro</i>: isolated rabbit corneas • <i>In vivo</i>: rabbits	[50]
Nanostructured lipid carriers	Eye drops	• Compritol®888 ATO • Miglyol® 812 • P188	PEA (Anti-inflammatory)	Size: 264.5 ± 0.19 nm PDI: 0.200 ± 0.035 ζ Potential: -37.1 ± 0.02 mV E.E.: $82.3 \pm 0.82\%$	• Reduced size (<300 nm) • Overall negative charge • P188: –Hydrophilic and muco-penetrating	• <i>In vivo</i>: rats	[51]
Nanostructured lipid carriers	Eye drops	• Compritol®888 ATO • Miglyol® 812 • P188	PEA (Anti-inflammatory)	Size: 208.6 ± 10.2 nm PDI: 0.18 ζ Potential: > -20 mV E.E.: N.D.	• Reduced size (<300 nm) • Overall negative charge • P188: –Hydrophilic and muco-penetrating	• <i>In vivo</i>: rabbits	[52]
Nanostructured lipid carriers	Eye drops	• Compritol®888 ATO • Miglyol® 812 • P188 • Tween® 80 • Propylene Glycol	IN (Anti-inflammatory)	Size: 227 ± 11 nm PDI: 0.23 ζ Potential: -12.2 ± 2.3 mV E.E.: $99.8 \pm 0.2\%$	• Tween® 80: – Penetration enhancer – P-gp efflux inhibitors • P188: –Hydrophilic and muco-penetrating • Reduced size (<300 nm)	• <i>In vivo</i>: rabbits	[48]
Nanostructured lipid carriers	Eye drops	• Precirol®ATO5 • P188 • Squalene® • MO	TA (Corticosteroid)	Size: 173.30 ± 0.32 nm PDI: 0.10 ± 0.02 ζ Potential: -46.70 ± 0.91 mV E.E.: $94.82 \pm 1.12\%$	• MO: – Fusogenic properties • P188: –Hydrophilic and muco-penetrating • Reduced size (<300 nm)	• <i>In vivo</i>: mice	[53]
Nanostructured lipid carriers	Eye drops	• Precirol®ATO5 • CO • mPEG-2000-DSPE • P188 • Tween® 80	AmB (Antifungal)	Size: 218 ± 5 nm PDI: 0.3 ± 0.02 ζ Potential: -50 to -55 mV E.E.: $92.7 \pm 2.5\%$	• mPEG-2000-DSPE and P188: –Hydrophilic and muco-penetrating • mPEG-2000-DSPE: –Inhibits P-gp efflux pumps • Tween® 80: – Penetration enhancer – P-gp efflux inhibitors • Reduced size (<300 nm)	• <i>In vitro</i>: ARPE-19 cell line • <i>In vivo</i>: rabbits	[54]
Emulsomes	Eye drops	• DPPC • GMS • CHOL	TA (Corticosteroid)	Size: 131.17 ± 3.17 nm PDI: 0.198 ± 0.021 ζ Potential: $-24.2 \pm$	• Overall negative surface charge • Reduced size (<300 nm)	• <i>In vitro</i>: SIRC cell line • <i>In vivo</i>: mice and rabbits	[55]

(continued on next page)

Table 1 (continued)

Nanocarrier	Topical vehicle	Composition	Therapeutic agent	Physicochemical parameters	Strategies to reach posterior eye segment	Experimental model	Ref.
Nanoemulsion	<i>In situ</i> gel	• MCT • EPC • Vit E • P188 • Penetratin	Lutein (Carotenoid)	2.12 mV E.E.: $71.56 \pm 4.19\%$ Size: 109.8 nm PDI: 0.079 ± 0.008 ζ Potential: $+16.9$ mV E.E.: N.D.	• <i>In situ</i> gel: – Prolongs the residence time of P-NE at the ocular surface • Penetratin: – CPP that acts as a permeation enhancer, – Confers an overall cationic charge • P188: – Hydrophilic and muco-penetrating • Reduced size (<300 nm)	• <i>In vitro</i>: ARPE-19 cell line • <i>In vivo</i>: rats, mice and rabbits	[56]
Nanoemulsion	Eye drops	• EPC or HSPC • MCT • CO • DCP or STE • CHS or P407	Coumarin-6 (Fluorescent probe)	Unmodified Size: 89.25 nm PDI: 0.123 ζ Potential: -34.6 ± 7.46 mV CS-modified Size: 94.49 nm PDI: 0.104 ζ Potential: -14.9 ± 6.10 mV P407-modified Size: 110.6 nm PDI: 0.194 ζ Potential: -42.7 ± 8.25 mV	• STE: – Overall cationic charge – Mucoadhesive properties • Coating with CHS: – Overall cationic charge – Mucoadhesive properties – Hydrophilic permeation enhancer • P407: – Gelling agent – Neutral charge • Reduced size (<300 nm)	• <i>In vivo</i>: mice	[57]
Dendrimers	Eye drops	• PAMAM • Cyclic RGD • Penetratin • PEG	N.D.	E.E.: $\approx 98\%$ Size: 19.16 ± 0.41 nm PDI: N.D. ζ Potential: $+6.74 \pm 0.37$ mV E.E.: N.D.	• Penetratin: – CPP that acts as a permeation enhancer, – Confers an overall cationic charge • RGD: – High specificity towards integrin $\alpha\beta_3$ (involved in neovascularization) • Coating with PEG: – Hydrophilic and mucopenetrating – P-gp efflux inhibitors • Reduced size (<300 nm)	• <i>In vitro</i>: HCE, HUVEC and NHC cell lines • <i>In vivo</i>: mice	[58]
Dendrimers	Eye drops	• PAMAM-COOH	DEX (Corticosteroid)	Size: 131.20 ± 19.43 nm PDI: N.D. ζ Potential: -52.23 ± 2.54 mV E.E.: 100%	• PAMAM-COOH: – Overall negative charge • Reduced size (<300 nm)	• <i>In vitro</i>: ARPE-19 cell line • <i>In vivo</i>: rats	[59]
Dendrimers	Eye drops	• PAMAM • Penetratin	pRFP (Fluorescent plasmid)	Size: 154.8 ± 8.9 nm PDI: 0.167 ± 0.007 ζ Potential: $+29.9 \pm 1.0$ mV E.E.: N.D.	• Penetratin: – CPP that acts as a permeation enhancer, – Confers an overall cationic charge • Reduced size (<300 nm)	• <i>In vitro</i>: NHC and SDHCEC cell lines • <i>In vivo</i>: mice	[60]
Cyclodextrin	Eye drops	• (2-hydroxypropyl)-β-cyclodextrin • Hypromellose • Caffeine	AXT (RTKi)	E.E.: $8.494 \pm 132.1\%$ (w/v%)	• β-cyclodextrin: – Hydrophilic – Penetration enhancer	• <i>In vivo</i>: rabbits and non-human primates	[61]
Cyclodextrin	Eye drops	• γ-cyclodextrin	DEX (Corticosteroid)	Size: 20.4 ± 10.3 μ m	• γ-cyclodextrin: – Hydrophilic – Penetration enhancer • Reduced size (<300 nm)	• <i>In vivo</i>: rabbits	[62]
Cyclodextrin	Eye drops	• γ-cyclodextrin	IRB, CAN (Antagonists of angiotensin II receptor)	N.D.	• γ-cyclodextrin: – Hydrophilic – Penetration enhancer	• <i>In vivo</i>: rabbits	[63]

(continued on next page)

Table 1 (continued)

Nanocarrier	Topical vehicle	Composition	Therapeutic agent	Physicochemical parameters	Strategies to reach posterior eye segment	Experimental model	Ref.
Polymeric micelles	Eye drops	• PEG • PPG • PCL	AFL (VEGF-A)	Size: 64.5 nm PDI: N.D. ζ Potential: N.D. E.E.: 47.3%	• Coating with PEG: –Hydrophilic and muco-penetrating –P-gp efflux inhibitors • Reduced size (<300 nm) • Coating with MPEG: –Hydrophilic and muco-penetrating –P-gp efflux inhibitors • Reduced size (<300 nm) • Tween® 80: – Penetration enhancer – P-gp efflux inhibitors • P40S: –Hydrophilic and muco-penetrating –Inhibits P-gp efflux pumps • Close to neutral surface charge • Reduced size (<300 nm) • Vitamin E TPGS: –Absorption enhancer by inhibiting P-gp efflux pumps • Povidone K 90: –Enhances formulation viscosity which increases retention time at the ocular surface • Hydrophilic corona of nanomicelles: –Can use scleral aqueous channels/pores and minimize washout into systemic circulation • Reduced size (<300 nm)	• <i>In vitro</i>: ARPE-19 cell line • <i>In vivo</i>: mice	[64]
Polymeric micelles	Eye drops	• MPEG-hexPLA	CsA (Immuno suppressant)	Size: 54 ± 1 nm PDI: 0.229 ± 0.008 ζ Potential: N.D. E.E.: 4.9 ± 0.2 mg/mL	• Coating with MPEG: –Hydrophilic and muco-penetrating –P-gp efflux inhibitors • Reduced size (<300 nm) • Tween® 80: – Penetration enhancer – P-gp efflux inhibitors • P40S: –Hydrophilic and muco-penetrating –Inhibits P-gp efflux pumps • Close to neutral surface charge • Reduced size (<300 nm) • Vitamin E TPGS: –Absorption enhancer by inhibiting P-gp efflux pumps • Povidone K 90: –Enhances formulation viscosity which increases retention time at the ocular surface • Hydrophilic corona of nanomicelles: –Can use scleral aqueous channels/pores and minimize washout into systemic circulation • Reduced size (<300 nm)	• <i>In vivo</i>: rats	[65]
Polymeric micelles	Eye drops	• P40S • Tween® 80	DEX (Corticosteroid)	Size: 14.5 ± 0.4 nm PDI: N.D. ζ Potential: 0.23 mV E.E.: 100%	• Coating with PEG: –Hydrophilic and muco-penetrating –P-gp efflux inhibitors • Reduced size (<300 nm) • Tween® 80: – Penetration enhancer – P-gp efflux inhibitors • P40S: –Hydrophilic and muco-penetrating –Inhibits P-gp efflux pumps • Close to neutral surface charge • Reduced size (<300 nm) • Vitamin E TPGS: –Absorption enhancer by inhibiting P-gp efflux pumps • Povidone K 90: –Enhances formulation viscosity which increases retention time at the ocular surface • Hydrophilic corona of nanomicelles: –Can use scleral aqueous channels/pores and minimize washout into systemic circulation • Reduced size (<300 nm)	• <i>In vivo</i>: rabbits	[66]
Polymeric micelles	Eye drops	• Vit E TPGS • Oc-40 • Povidone K 90	RAP (Antibiotic)	Size: 10.84 ± 0.11 nm PDI: 0.05 ζ Potential: –0.789 mV E.E.: 100 ± 1.2%	• Coating with PEG: –Hydrophilic and muco-penetrating –P-gp efflux inhibitors • Reduced size (<300 nm) • Tween® 80: – Penetration enhancer – P-gp efflux inhibitors • P40S: –Hydrophilic and muco-penetrating –Inhibits P-gp efflux pumps • Close to neutral surface charge • Reduced size (<300 nm) • Vitamin E TPGS: –Absorption enhancer by inhibiting P-gp efflux pumps • Povidone K 90: –Enhances formulation viscosity which increases retention time at the ocular surface • Hydrophilic corona of nanomicelles: –Can use scleral aqueous channels/pores and minimize washout into systemic circulation • Reduced size (<300 nm)	• <i>In vitro</i>: D407 and rPCEC cell lines • <i>In vivo</i>: rabbits	[67]
Polymeric nanoparticles	Eye drops	• PLGA • P188	FMT (Corticosteroids)	Size: 149.1 ± 3.5 nm PDI: 0.079 ± 0.008 ζ Potential: –34.3 ± 1.6 mV E.E.: 99.8 ± 0.2%	• Coating with PEG: –Hydrophilic and muco-penetrating –P-gp efflux inhibitors • Reduced size (<300 nm) • Tween® 80: – Penetration enhancer – P-gp efflux inhibitors • P40S: –Hydrophilic and muco-penetrating –Inhibits P-gp efflux pumps • Close to neutral surface charge • Reduced size (<300 nm) • Vitamin E TPGS: –Absorption enhancer by inhibiting P-gp efflux pumps • Povidone K 90: –Enhances formulation viscosity which increases retention time at the ocular surface • Hydrophilic corona of nanomicelles: –Can use scleral aqueous channels/pores and minimize washout into systemic circulation • Reduced size (<300 nm)	• <i>In vitro</i>: HET-CAM • <i>In vivo</i>: pigs	[68]
Polymeric nanoparticles	Eye drops	• PLGA • Surface: CHS, GCHS or Tween® 80	Coumarin-6 (Fluorescent probe)	Unmodified Size: 224.5 nm PDI: 0.068 ζ Potential: –41.3 mV E.E.: N.D. CS, GCS and Tween® 80 Size: 332.7, 518.6 and 240.7 nm PDI: 0.339, 0.296 and 0.129 ζ Potential: –9.34, +39.9, –44.6 mV E.E.: N.D.	• Coating with PEG: –Hydrophilic and muco-penetrating –P-gp efflux inhibitors • Reduced size (<300 nm) • Tween® 80: – Penetration enhancer – P-gp efflux inhibitors • P40S: –Hydrophilic and muco-penetrating –Inhibits P-gp efflux pumps • Close to neutral surface charge • Reduced size (<300 nm) • Vitamin E TPGS: –Absorption enhancer by inhibiting P-gp efflux pumps • Povidone K 90: –Enhances formulation viscosity which increases retention time at the ocular surface • Hydrophilic corona of nanomicelles: –Can use scleral aqueous channels/pores and minimize washout into systemic circulation • Reduced size (<300 nm)	• <i>In vivo</i>: mice	[69]
Polymeric nanoparticles	Eye drops	• CHS • HA	EPOβ	Size: 289 ± 3 nm PDI: 0.126 ± 0.085 ζ Potential: 39 ± 1 mV E.E.: 38.4 ± 0.3%	• Coating with CHS: –Overall cationic charge –Mucoadhesive properties –Hydrophilic permeation enhancer • HA: –Affinity to CD44 cell receptors • Reduced size (<300 nm) • PLGA: –Overall negative charge • Tween® 80: – Penetration enhancer – P-gp efflux inhibitors • Reduced size (<300 nm) • HA: –Overall negative charge –Affinity to CD44 cell	• <i>In vitro</i>: ARPE-19 and HaCaT cells • <i>In vivo</i>: rats	[70]
Polymeric nanoparticles	Eye drops & <i>In situ</i> gel	NPs composition: • PLGA • Tween® 80	RLZ(Benzothiazole)	Size: 148.2 ± 1.4 nm PDI: 0.116 ± 0.011 ζ Potential: –30.0 ± 0.6 mV E.E.: 94.2 ± 0.8%	• Coating with CHS: –Overall cationic charge –Mucoadhesive properties –Hydrophilic permeation enhancer • HA: –Affinity to CD44 cell receptors • Reduced size (<300 nm) • PLGA: –Overall negative charge • Tween® 80: – Penetration enhancer – P-gp efflux inhibitors • Reduced size (<300 nm) • HA: –Overall negative charge –Affinity to CD44 cell	• <i>In vivo</i>: mice	[71]
BSA nanoparticles	Eye drops	• BSA • HA	APA (RTKi)	Size: 222.2 ± 3.56 nm PDI: 0.22 ζ Potential: –37.3 ± 1.8 mV	• Coating with CHS: –Overall cationic charge –Mucoadhesive properties –Hydrophilic permeation enhancer • HA: –Affinity to CD44 cell receptors • Reduced size (<300 nm) • PLGA: –Overall negative charge • Tween® 80: – Penetration enhancer – P-gp efflux inhibitors • Reduced size (<300 nm) • HA: –Overall negative charge –Affinity to CD44 cell	• <i>In vitro</i>: RCE cell line • <i>In vivo</i>: rats	[72]

(continued on next page)

Table 1 (continued)

Nanocarrier	Topical vehicle	Composition	Therapeutic agent	Physicochemical parameters	Strategies to reach posterior eye segment	Experimental model	Ref.
MPP	Eye drops	● P407	LE & KAL821 (Corticosteroid & RTKi)	E.E.: 69 ± 1% LE-MPP & KAL-821-MPP Size: 240 & 160 nm PDI: N.D. ζ Potential: Neutral E.E.: N.D.	receptors ● Reduced size (<300 nm) ● P407: –Gelling agent –Neutral charge ● Reduced size (<300 nm)	● <i>In vivo</i> : rabbits and mini-pigs	[73]
Nanocomposite (Liposome@LDH)	Eye drops	● HSPC ● CHOL ● Pristine LDH ● GS	DEXP (Corticosteroid)	Size: 187.7 ± 7.9 nm PDI: 0.241 ζ Potential: +24.33 ± 2.56 mV E.E.: 69 ± 1%	● LDH: –Overall cationic charge –Mucoadhesive properties ● Clathrin-mediated endocytosis ● Active transport by PepT-1 ● Reduced size (<300 nm)	● <i>In vitro</i> : HCEC and NHC cell lines ● <i>In vivo</i> : rabbits	[74]
Gold Nanoparticles	Eye drops	● HAuCl ₄ ● HA	N.D.	Size: 15–20 nm PDI: N.D. ζ Potential: N.D. E.E.: N.D.	● HA: –Overall negative charge-Affinity to CD44 cell receptors ● Reduced size (<300 nm)	● <i>In vivo</i> : mice	[75]

AFL: Aflibercept; AmB: Amphotericin B; APA: Apatinib; ARPE-19: Arising retinal pigment epithelia cell line derived from the normal eyes of a 19-year-old male; ATS: Atorvastatin; AXT: Axitinib; BAC: Benzalkonium chloride; BSA: Bovine serum albumin; BVZ: Bevacizumab; CAN: Candesartan; CHOL: Cholesterol; CHS: Chitosan; CO: Castor oil; Compritol®888 ATO: blend of different esters of behenic acid with glycerol or glyceryl behenate; CPP: Cell penetrating peptide; CsA: Cyclosporin A; D407: Human retinal pigment epithelial cells; DCP: Dicytlyphosphate; DEX: Dexamethasone; DEXP: Dexamethasone disodium phosphate; DF: Diclofenac; DLPC: 1,2-dilauroyl-sn-glycero-3-phosphocholine; DOPE: 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine; DOPS: 1,2-dioleoyl-sn-glycero-3-phospho-L-serine (sodium salt); DPPC: 1,2-dihexadecanoyl-sn-glycero-3-phosphocholine or Dipalmitoyl phosphatidylcholine or 1,2-dipalmitoyl-sn-glycero-3-phosphocholine or 1,2-Dipalmitoyl-3-sn-phosphatidylcholine or 1,2-Dipalmitoyl-L-lecithin; DSPC: 1,2-distearoyl-sn-glycero-3-phosphocholine or 1,2-dioctadecanoyl-sn-glycero-3-phosphocholine; DSPE-PEG2000: Conjugate of 1,2-distearoyl sn-glycero-3-phosphoethanolamine and polyethylene glycol (2000) monostearate; EDV: Edaravone; EE: Encapsulation efficiency; EP: Epalrestat; EPC: egg phosphatidylcholine; EPOβ: Epoetin beta; FMT: Fluorometholone; GCHS: Glycol chitosan; GS: glycylsarcosine; HA: Hyaluronic acid; HAuCl₄: gold (III) chloride hydrate; HCEC: Human corneal epithelial cells; HCF: Primary human corneal fibroblasts; HCLE: Human cornea limbal epithelial; HET-CAM: Hen's egg-chorioallantoic membrane test; HSPC: Hydrogenated soybean phosphatidylcholine or Phospholipon® 90H or L-α-phosphatidylcholine, hydrogenated (Soy); HUVEC: Human umbilical vein endothelial cells; IN: Indomethacin; IRB: Irbesartan; KTZ: Ketoconazole; LE: Loteprednol etabonate; LDH: Layered Double Hydroxides; Miglyol®812: caprylic/capric triglycerides; mPEG-hexPLA: Methoxy poly(ethylene) glycol-hexylsubstituted poly(lactides); MPP: Mucus penetrating particles; N.D.: Not defined; NHC: Human conjunctival epithelial cells; NPs: Nanoparticles; Oc-40: Octoxynol-40; P188: Poloxamer 188 or Pluronic F68® or Kolliphor P188® or Lutrol F68®; P407: Poloxamer 407 or Pluronic F-127; P40S: Polyoxyl 40 stearate; PAMAM: Poly(amidoamine); PC: 1,2-diacyl-sn-glycero-3-phosphocholine or Phosphatidylcholine or Diacylphosphatidylcholine or lecithin or Epikuron® 200 or Phospholipon®90 G; PCL: Polycaprolactone; PDI: Polydispersity index; pDNA: Plasmid DNA; PEG15H: Polyethylene glycol (15)-hydroxystearate or Macrogol 15 hydroxystearate or Polyoxyl 15 Hydroxystearate or Solutol HS® 15 or Kolliphor® HS15 or PEG-660 stearate; PEG400: Polyethylene glycol (400) monostearate; PEA: Palmitoylethanolamide; PEG: poly(ethyleneglycol); PEG2000-SA: poly(ethyleneglycol) monostearate; PLL: Poly-L-Lysine; PPG: Poly(propylene glycol); pRFP: Red fluorescent protein plasmid; PVA: Poly(vinyl alcohol); PVA-R: Poly(vinyl alcohol) derivatives bearing a hydrophobic anchor (C16H33–S–) at the terminal of the molecule; Precirol®5 ATO: Glyceryl palmitostearate; Tween® 80: Polysorbate 80; PLGA: Poly(lactic-co-glycolic acid); R28: Retinal neuronal; RAP: Rapamycin; RCE: Rabbit corneal epithelial cell lines; rPCECs: Rabbit primary corneal epithelial cells; RTKi: Receptor tyrosine kinase inhibitor; SA: Stearic acid or octadecanoic acid or n-Octadecanoic acid; SDHCEC: Spontaneously derived human corneal epithelial cells; SIRC: Statens Seruminstitut Rabbit Corneal cell line; SPC: soybean phosphatidylcholine; STC: Sodium taurocholate; STE: stearylamine; TA: Triamcinolone acetonide; TBM: Tobramycin; TGF-β: Transforming growth factor beta; Tm: Phase transition temperature; TMAG: N-(alpha-trimethylammonioacetyl)-didodecyl-D-glutamate; VEGF: Vascular endothelial growth factor; Vit E: Vitamin E or Tocopherol or α-tocopherol; Vit E TPGS: Vitamin E tocopherol polyethylene glycol succinate.

(1–2%) and are responsible for a poor therapeutic outcome in posterior eye disorders [82,83]. Furthermore, the expression of efflux transporters such as P-gp also represents an obstacle to therapeutic delivery across the BRB, given the rapid drug clearance from the extravascular space (Fig. 2) [84,85].

The iBRB regulates the microenvironment of neuroretina by blocking the diffusion of hydrophilic compounds across retinal capillaries [82]. Furthermore, the iBRB is covered by an underlying layer of Müller cells, astrocytes, and pericytes. These cells can sense variations in the extracellular fluid and relaying this information as signals to the RCE cells [82,86]. The oBRB is composed of a monolayer of RPE cells located atop the iBRB that restricts the diffusion of solutes from the sclera/choroid to the retina [86]. Given the limited permeability of the intercellular junction complex surrounding endothelial and epithelial cells, therapeutics are more likely to diffuse transcellularly across the BRB [87,88].

2.2. Barriers to therapeutic absorption via the corneal route

2.2.1. Corneal barriers

The human cornea is the major barrier encountered upon topical

therapeutic administration and includes three main layers of alternating polarity: the corneal epithelium, stroma, and endothelium, separated by Bowman's and Descemet's membranes, respectively (Fig. 3) [89]. Recently, a fourth layer, located among the Descemet's membrane and the stroma, was identified and termed as the Dua's layer (Fig. 3) [90]. At this level, the epithelium of the cornea is the primary barrier because it forms intercellular tight junctions around the superficial epithelial cells that act as selective barriers for small molecules and limit the diffusion of macromolecules and hydrophilic compounds [3,91]. The polarity of the compound, and its molecular weight, determine its transport across the cornea. Depending upon the partition coefficient (logP), the therapeutic agent can diffuse via paracellular or transcellular route. logP is an indicator of drug distribution between aqueous and lipid phases [92]. Additionally, logP is a reliable tool to predict drug absorption, distribution, metabolism, and elimination processes, as well to assess their toxic and/or therapeutic effects [92].

Thus, lipophilic therapeutic agents (logP > 0) will likely benefit from the transcellular pathway to diffuse across the epithelium of the cornea, while small hydrophilic therapeutic agents (logP < 0) would benefit the paracellular route [29].

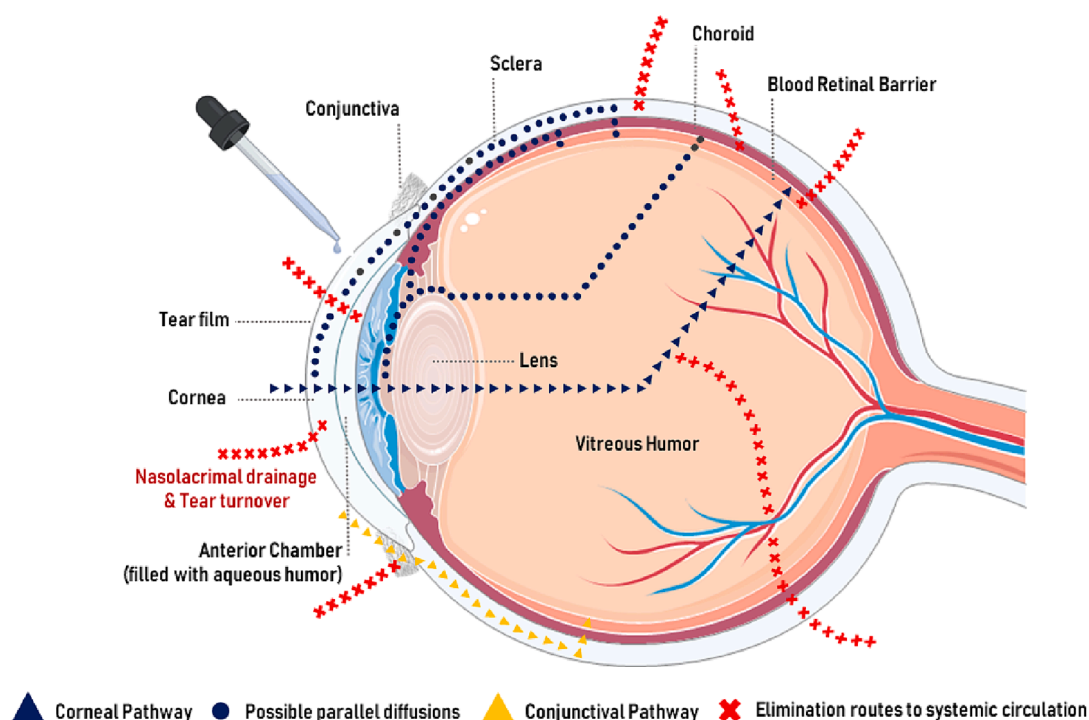


Fig. 2. Graphical representation of the two principal topical ophthalmic routes for therapeutics absorption and potential parallel diffusion courses. The primary pathways by which therapeutic agents enter the circulatory system are also shown.

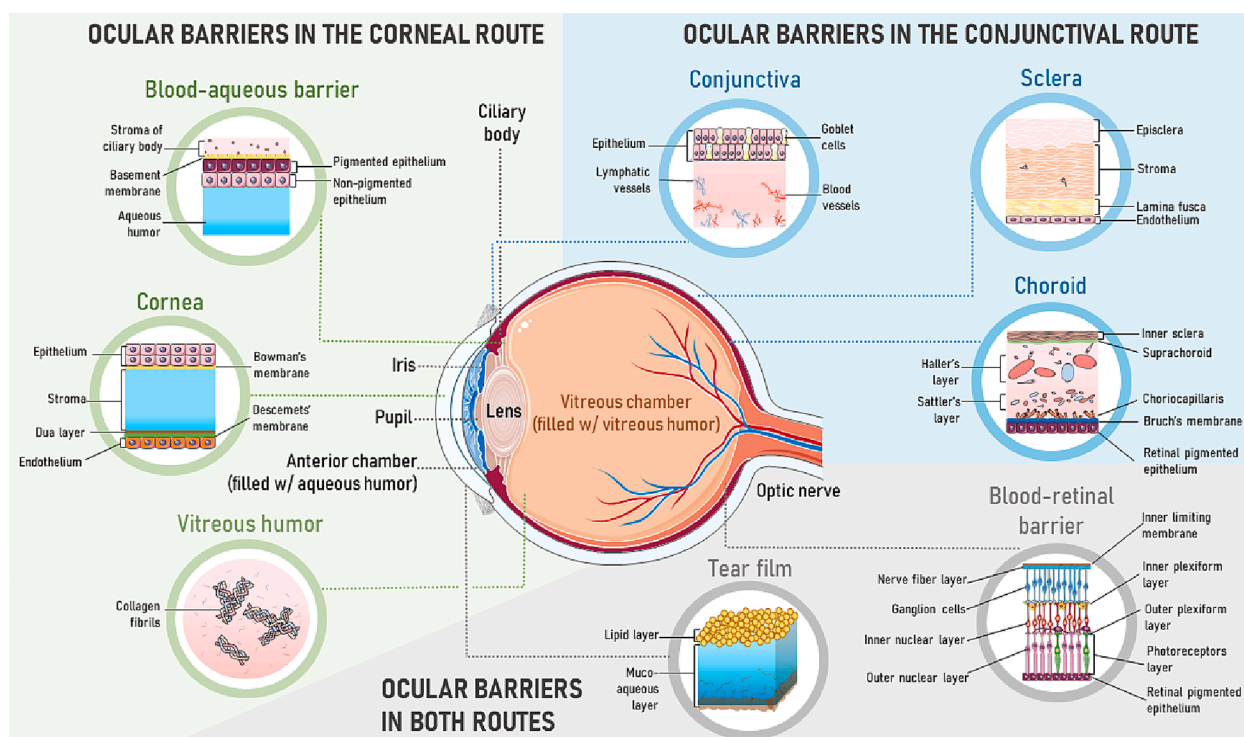


Fig. 3. Graphical illustration of the primary ocular barriers encountered upon topical ophthalmic therapeutic delivery in each major permeation route.

2.2.2. Anterior and posterior chambers

The anterior eye chamber, situated between the cornea and the iris, and the posterior eye chamber, placed between the ciliary body and iris, are filled with aqueous humor ($\approx 200\text{--}300\ \mu\text{L}$ total volume) [76,88]. Despite its reduced protein content, aqueous humor is chemically comparable to blood plasma [88]. The main role of this water-like fluid

is to grant nutrients and antioxidants to the lens and cornea, but it also aids in the preservation of the IOP and the eye's spherical shape [88,93].

The epithelial cells of the ciliary body continuously secrete aqueous humor, which flows from the posterior to the anterior chamber and is subsequently drained via the trabecular meshwork into Schlemm's canal. It then exits the eye by the uveoscleral pathway and enters the

systemic circulation [76]. The turnover of the aqueous humor affects the bioavailability and distribution of the drugs in the vitreous chamber (Fig. 2) [94].

2.2.3. Blood-aqueous barrier

The eye's posterior and anterior portions are protected by the blood-ocular barriers. The blood-aqueous barrier (BAB), located in the anterior part of the eye, is constituted by the non-pigmented ciliary epithelium and endothelial cells of blood vessels in the iris (Fig. 3) [9,23]. The tight junctions of the non-pigmented epithelium represent a barrier to the paracellular movement of large molecules from the blood to the aqueous humor [95]. The existence of fenestrations within the ciliary epithelium confers selective permeability to BAB, and also low transepithelial resistance [95,96]. Iridial vessels are well sealed with tight junctions, but have greater permeability than retinal capillaries (about 10 times leakier) [96].

2.2.4. Vitreous humor

The vitreous humor (≈ 4 mL) is a gel-like fluid that fills the vitreous chamber (Fig. 3), and it is constituted mainly by water, type-II collagen, hyaluronic acid, proteoglycans, and some hyalocyte cells [26,97]. This highly viscous component covers the space between the lens and the retina, accounting for approximately 80% of the tissue volume of the eye [97]. The primary role of the vitreous humor is helping to avoid retinal detachment but also to stabilize and resist to rapid changes of size by fluid flows [88,98].

Moreover, the vitreous humor is regarded as an obstacle in the retinal transportation of therapeutics (Fig. 2), mainly because it can affect their diffusional properties [97]. The vitreous humor has an overall net negative charge resulting from hyaluronan. In addition, it has been described that the vitreous structure and its gel-like properties, significantly change with age, turning more fluid and heterogeneous, which might alter the diffusional properties of therapeutics [88,99].

2.2.5. Inner limiting membrane

The inner limiting membrane (ILM) is a thin membrane (≈ 4 μm) that separates the retina and the vitreous humor (Fig. 3) and is the basement membrane for Müller cells [100]. It is mainly constituted by collagen IV, laminin, nidogen-1, and heparan sulfate proteoglycans, but its composition and thickness vary with age and disease state [97,100]. It is possible for the ILM to obstruct retinal penetration, and its size and charge seem to be critical parameters of this barrier [94,97]. The membrane shows a negative net charge and a pore size of ≈ 10 –100 nm, indicating a greater attraction to hydrophilic therapeutic agents [26,94].

2.3. Nanomedicine design strategies to surpass corneal barriers

Delivering nanomedicines and therapeutic substances to the posterior segment of the eye via topical application poses challenges and requires thoughtful consideration. As mentioned in Section 2, nanomedicines follow one of two ocular routes, each facing specific barriers hindering their diffusion to posterior tissues. To overcome these barriers, nanomedicines will need different design strategies. In the next section, we will present several design strategies for nanomedicines (i.e., drug-loaded nanocarriers) in a step-by-step approach conceived individually for the major ocular barriers. The proposed methodologies will be supported by a state-of-the-art overview (Table 1) and a compilation of literature results (Figs. 5 and 6) detailing successful works of nanomedicines for topical eye administration with the aim of reaching the eye's posterior region. If nanomedicines are absorbed via the corneal pathway, design strategies to overcome corneal barriers, as depicted in Fig. 4, should be considered. However, it is important to notice that the same strategies can address obstacles encountered along the conjunctival and corneal pathways.

The lipophilic environment in the precorneal region, which restricts

the diffusion of hydrophilic drugs to the corneal interface and stops the lachrymal fluid from evaporating, is the first obstacle that therapeutic agents must overcome, as previously mentioned. Thus, using nanocarriers loaded with hydrophilic therapeutic agents may improve their delivery as long as the hydrophilic-lipophilic balance of these nanocarriers is carefully considered. Moreover, nanocarriers *per se* are powerful tools to avoid the rapid drug clearance resultant from pre-corneal dynamic barriers (Fig. 6-I), and several features can be tuned and optimized during their development. To address the issue of low therapeutic bioavailability resulting from tear recycling or blinking processes, one potential strategy is tailoring the nanocarrier by including mucoadhesive agents (Fig. 4-1). The tear film adhesive properties can be exploited to extend the time a substance remains in the eye. Various agents like chitosan (Fig. 5-III), carboxymethylcellulose, hydroxypropyl methylcellulose, hyaluronic acid, polyethylene glycol, and polyvinyl alcohol were tested for their suitability in ocular applications, as indicated by the data presented in Table 1 [94]. Another option to increase the interaction with mucin molecules is to take advantage of their intrinsic negative charge conferred by carboxyl and sulfate groups (Fig. 4). For example, in the case of lipid nanocarriers, their mucoadhesive properties may be increased by varying their composition. Utilization of cationic phospholipids (e.g., surfactants generated from dioctadecyldimethylammonium, stearylamine, and others) increases the electrostatic bonds among lipids and mucins' negatively charged groups [101,102]. Also, an overall positive surface charge confers stability to the formulation, which may increase ocular retention time [103,104]. Yet, given the net anionic charged mucus, the drug nanocarriers' size must be properly evaluated because cationic charges increase residence time (i.e., increase mucoadhesion) but may decrease mucopenetration. Nevertheless, it is feasible to improve drug penetration into mucus by adjusting the dimensions of its nanocarrier (Fig. 4-1). When the hydrodynamic diameter is too large (>1 μm), nanocarriers cannot pass through the small canaliculi of mucus, being retained on its surface. The ideal size for deeper penetration is between 200–500 nm, and nanocarriers with a diameter ≤ 100 nm are incapable of reaching areas as deep as those with intermediate size [105]. If the previously mentioned strategies are not applicable, incorporating nanocarriers into topical vehicles like hydrogels (Fig. 6-V) or contact lenses can be an interesting alternative [94,106]. Beyond prolonging the duration of hydrophilic compounds that remain in the conjunctival sac or on the surface of the cornea, these vehicles also allow for the loading of larger amounts of such compounds (Fig. 4-1).

Another important aspect to consider when developing a nanomedicine is the biphasic environment encountered through the corneal layers [107]. Lipophilic therapeutic compounds are more prone to diffuse in the corneal epithelium, but their diffusion is inhibited in the stroma. In this inner layer, hydrophilic agents with low molecular weight (up to 500 kDa) and sizes below 20 nm are more prone to permeate to posterior intraocular tissues [91]. Thus, as shown in Fig. 4-1.1, it is necessary to have a nanomedicine with amphiphilic qualities for therapeutic medicines to spread across both the stroma (hydrophilic) and the epithelium and endothelium (lipophilic) [107]. Using nanocarriers loaded with therapeutic agents to enhance their delivery is thus dependent on the proper hydrophilic-lipophilic balance of these nanocarriers, and on their size and/or deformability to overcome both the lipophilicity requirements and size constraints imposed by corneal barriers. Following this line of thought, lipid-based nanocarriers (e.g., liposomes, cubosomes, hexosomes, spongosomes), polymer-based nanocarriers (e.g., polymersomes), and hybrid combinations of polymers and lipids could be interesting options. All these nanocarriers are constituted by amphiphilic molecules (e.g., phospholipids with a hydrophilic headgroup and a fatty acid lipophilic chain or co-block polymers with hydrophilic and lipophilic portions) that, when dispersed in an aqueous solution, can self-assemble. This feature allows the encapsulation and diffusion of drugs and other therapeutic agents with varying polarity or lipophilicity levels. Another important feature of

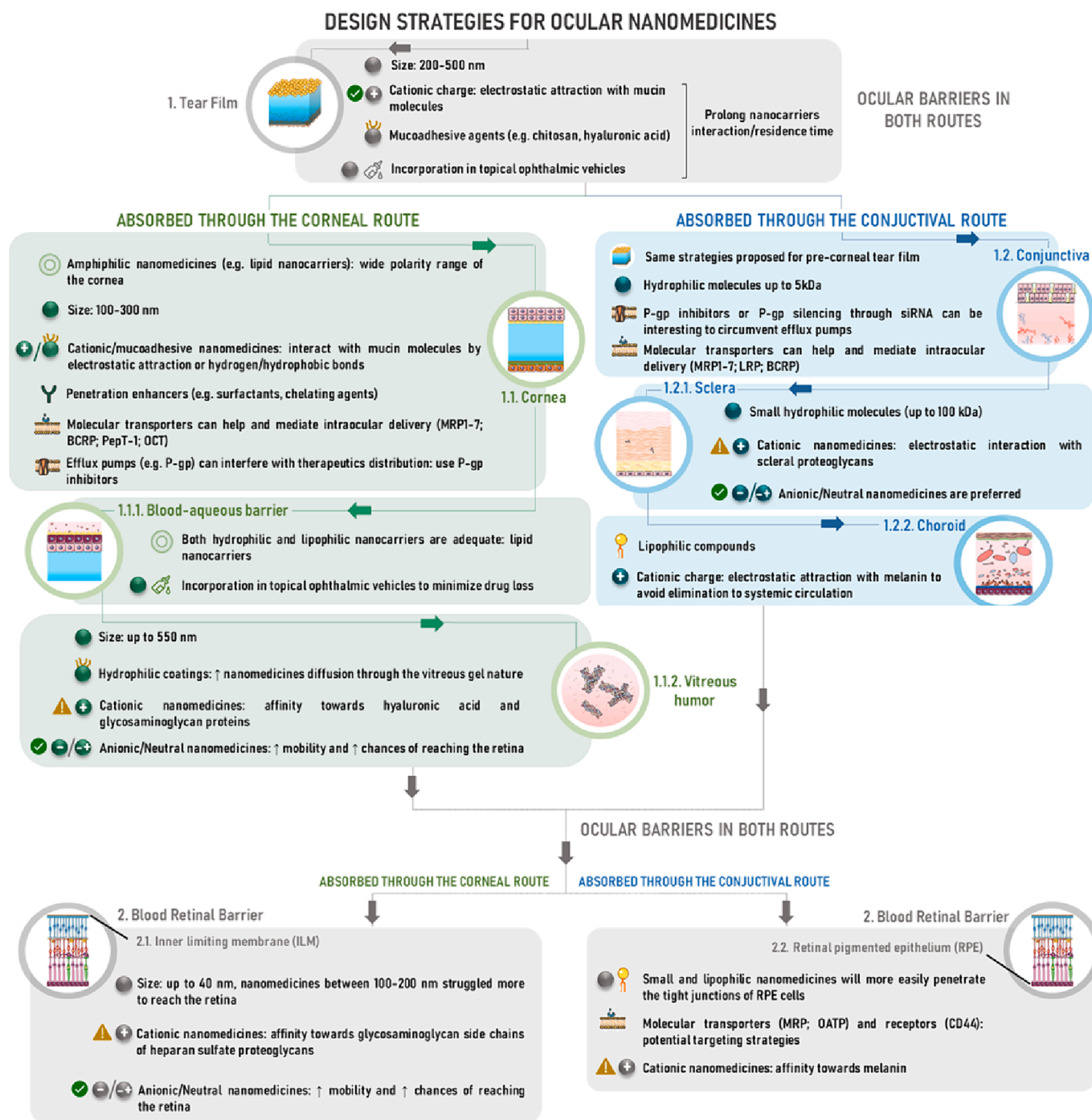


Fig. 4. Personalized design strategies for each ocular barrier encountered both in corneal or conjunctival therapeutic absorption and that should be adopted in the early stages of nanomedicines development. The arrows indicate the most probable pathways of therapeutic absorption to the posterior segment of the eye following topical administration.

these nanocarriers that may improve their corneal permeation and diffusion along tight junctions is their soft-matter composition. This property improves nanocarrier deformability and, thus, their competence to pass through pores that are tighter than their diameter. Besides deformability, the mean diameter of nanocarriers may also be tuned to enhance their therapeutic performance (Fig. 4-1.1) [108]. Indeed, nanomedicines with small sizes are more prone to cornea permeation and association given their larger surface area [49]. Inokuchi and co-workers assessed the influence of size in therapeutic topical delivery targeting intraocular tissues [40]. They found that a reduction in size promoted a higher accumulation of nanocarriers in the retina of mice,

particularly those ranging between 100–200 nm (Fig. 5-I) [40]. Moreover, it was shown that the uptake of polymeric nanocarriers by primary-grown rabbit epithelial cells of conjunctiva was contingent upon their size [109]. Smaller nanocarriers (≈ 100 nm) exhibited enhanced uptake compared with their larger counterparts (≈ 800 nm and $10 \mu\text{m}$) [109]. Nevertheless, a size-dependent disposition study, has proven that nanocarriers up to 20 nm were rapidly cleared out from periocular tissues after subconjunctival injection [110,111]. The findings are consistent with the comprehensive summary provided in Table 1, where the majority of the examined nanomedicines exhibited sizes of 100–300 nm, with just a small number falling below 20 nm. Overall, the available

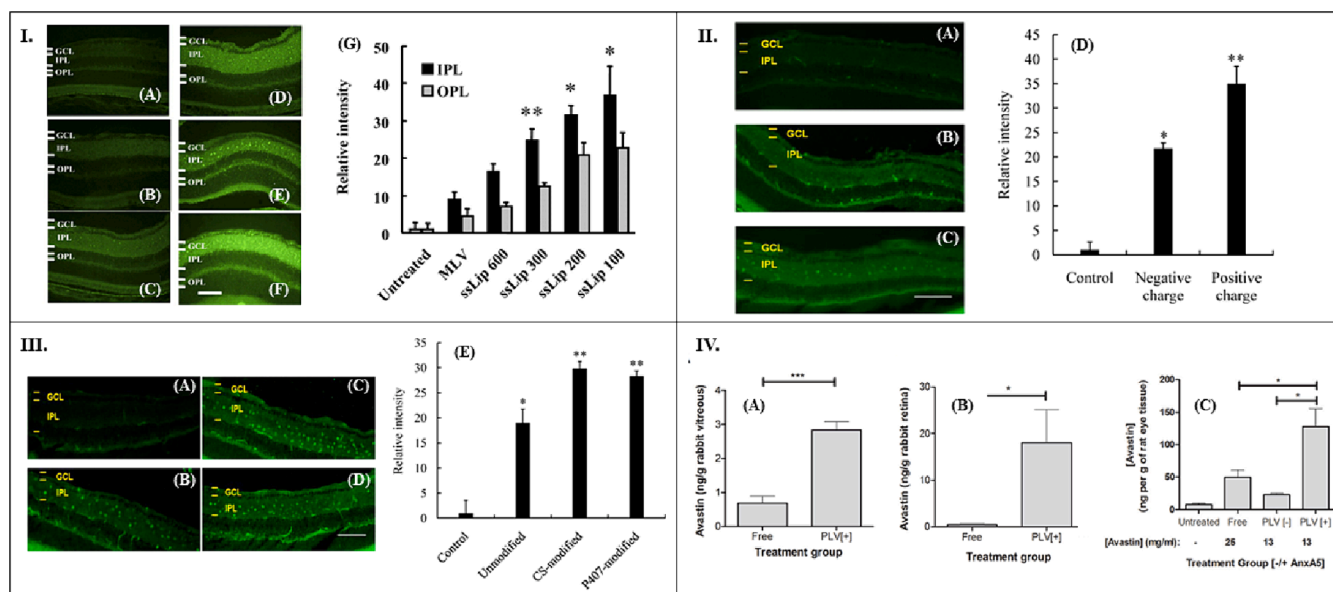


Fig. 5. *In vivo* representative studies of therapeutic-loaded nanocarriers that were able to reach the retina after topical instillation using different animal models. The figure outlines the impact of different factors on delivery efficiency: Section I (A)–(G) illustrates the impact of liposomal particle size on delivery efficiency to the retina of mice. A reduction in particle size correlates with an increase in nanocarrier retention in the retina. Epifluorescence microscopic images of the retina in (A) untreated eye, and 30 min after eye drop administration of (B) MLV; (C) ssLip 600; (D) ssLip 300; (E) ssLip 200, and (F) ssLip 100. (G) Variation of median fluorescence intensity in the IPL and OPL after administration of liposomes of different particle sizes. Particle sizes of MLV, ssLip 600, ssLip 300, ssLip 200, and ssLip 100 were 6490.0, 561.0, 300.6, 174.8, and 116.8 nm, respectively. ($n = 4$). * $P < 0.05$, ** $P < 0.01$ versus each OPL. Scale bar, 50 μm [40]. Section II (A)–(D) demonstrates the influence of lipid emulsion surface charge on delivery efficiency to the retina of mice. Charged lipid emulsions evidenced higher distribution in retinal tissues compared to uncharged emulsions. Epifluorescence microscopic images of the retina 30 min after eye drop administration. (A) Untreated (control), (B) DCP-modified emulsion, (C) SA-modified emulsion, and (D) Accumulated fluorescence intensity in the IPL after eye drop administration. ($n = 4$). * $P < 0.05$ versus control. ** $P < 0.05$ versus negative charge emulsion. Scale bar, 100 μm [57]. Section III (A)–(E) shows the effect of chitosan (CS) and Poloxamer 407 (P407) surface modification on delivery efficiency to the retina of mice. CS and P407 surface modification improves the ocular diffusion to intraocular tissues. Epifluorescence microscopic images of the retina 30 min after eye drop administration. (A) Untreated (control), (B) Unmodified lipid emulsion, (C) CS-modified lipid emulsion, (D) P407-modified lipid emulsion, and (E) Accumulated fluorescence intensity in the IPL after eye drop administration. $n = 3$. * $P < 0.05$ versus control. ** $P < 0.05$ versus unmodified. Scale bar, 100 μm [57]. Section IV (A)–(C) displays the impact of using a corneal permeation enhancer (Annexin-A5 – AnxA5) on delivery efficiency of Avastin-loaded phospholipid vesicles (PLV) to the rabbit retina. A single PLV dose (25 mg/mL Avastin) was topically administered in the presence of AnxA5 (PLV [+]), once per day for five consecutive days. A second population of rabbits were topically dosed with the highest commercially available concentration of unencapsulated Avastin (25 mg/mL). ($n = 6$, $\pm$ SE). A significantly greater concentration of Avastin was detected in the (A) vitreous and (B) retina of the rabbit eye after dosing with PLV [+] than dosing a greater concentration of unencapsulated Avastin (two tailed unpaired T-test, * $p < 0.05$, *** $p < 0.001$ where indicated); (C) Corroboration that the concentration of Avastin detected in the posterior segment of the rat eye after dosing with PLV [+] was significantly higher than dosing with PLV [–] or dosing a greater concentration of unencapsulated Avastin (One-way ANOVA with Tukey's multiple comparison test, * $p < 0.05$ and ** $p < 0.01$ where indicated) [44]. All data is expressed as mean $\pm$ S.D. DCP: Dicylphosphate; GCL: ganglion cell layer, INL: inner nuclear layer, IPL: inner plexiform layer, MLV: multilamellar vesicles; ONL: outer nuclear layer, OPL: outer plexiform layer, PL: photoreceptors layer, RPE: retinal pigment epithelium; SA: Stearylamine; ssLip: sub-micron sized liposomes. Reproduced with permission [40,44,57]. Copyright © 2014, Wiley; Copyright © 2013, Elsevier; Copyright © 2010, Association for Research in Vision and Ophthalmology.

data evidence that the mean size of nanomedicines is a critical parameter and influences the extent of absorption/permeation, and it also impacts the distribution along the different ocular barriers.

As already reported above, it is feasible to take advantage of the negatively charges of mucins on the corneal epithelium to expedite corneal penetration [107]. However, transcorneal diffusion may also be improved using penetration enhancers (Fig. 4-1.1) like chelating agents, surfactants, cell-penetrating peptides, fatty acids, cyclodextrins, and mucoadhesive polymers [112,113]. Their modes of action differ, and penetration can be enhanced by causing morphological alterations or disrupting the epithelium's integrity, loosening of the tight junctions by Ca^{2+} extraction, promoting endocytosis, among others [113]. However, these mechanisms come with an increased possibility of infection [114]. In Table 1, several studies report the application of permeation enhancers (Fig. 5-IV) as a strategy to increase absorption to intraocular tissues, attesting their efficacy. Particular emphasis should be paid to a recent work that emphasized the prospective use of anionic phospholipid binding protein (annexin-A5) in the delivery, by topical administration, of biopharmaceutical drugs to the eye's posterior portion [44]. Annexin-A5 showed promising results in phosphatidylserine-enriched

nanocarriers, increasing the bevacizumab delivery across corneal epithelium *in vitro* models and *in vivo* corneas of rats and rabbits (Fig. 5-IV) [44].

Finally, it is worth noticing the existence of several molecular transporters (MRP, multidrug resistance-associated proteins; BCRP, breast cancer-resistance proteins; PepT-1, peptide transporter 1; OCT, organic cation transporter proteins and MCT, monocarboxylate transporters) and efflux pumps (MDR, multidrug resistance proteins, MRP1-3, and BCRP) that are expressed at the corneal epithelium surface [30,115]. These transporters have the prospective to facilitate the distribution of nanomedicines throughout the ocular compartment (Fig. 4-1.1), but they also have the opportunity to enhance their efflux. The most concerning efflux pump is the phagocytic glycoprotein (P-gp or MDR-1); however, several reported P-gp inhibitors can be employed and favor the transfer of therapeutics from the epithelium to the endothelium of the cornea (Fig. 4-1.1) [115]. When nanomedicines overcome the corneal barrier, they will then struggle to be distributed across the aqueous humor and blood-aqueous barrier without moving toward other off-target directions.

The complexes of tight junctions localized around the non-

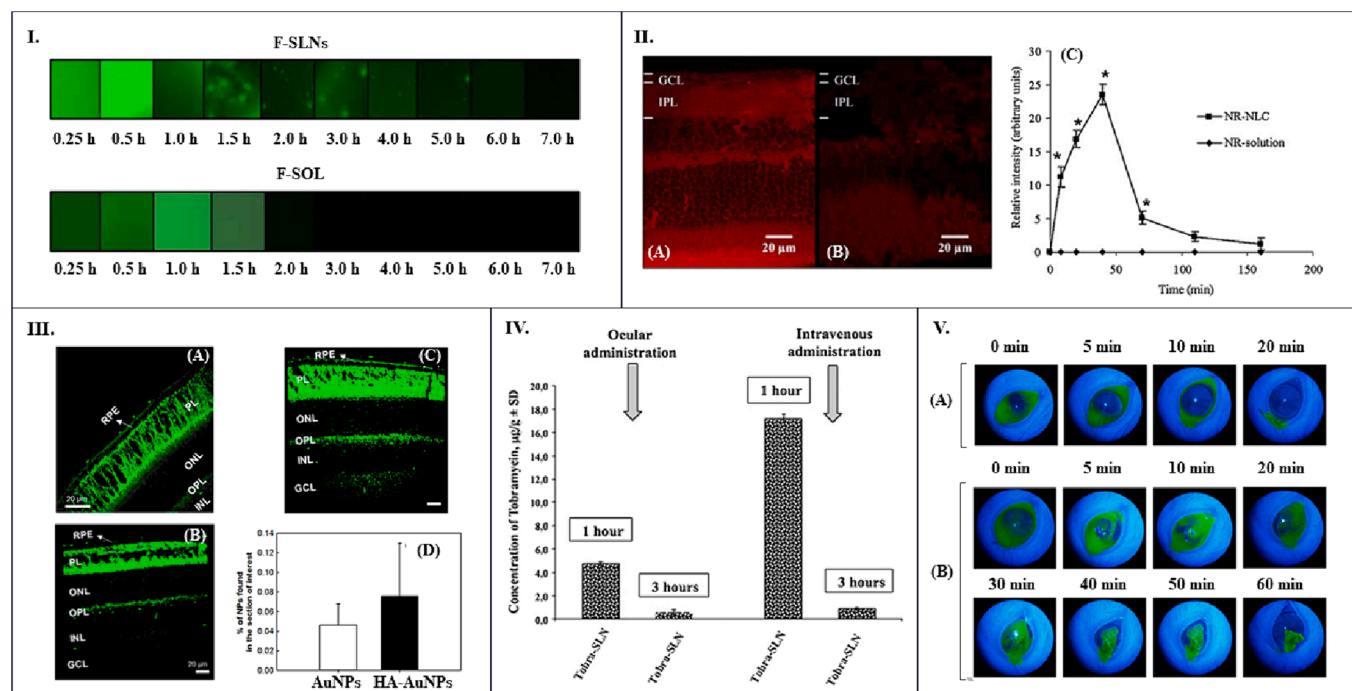


Fig. 6. *In vivo* representative studies of therapeutic-loaded nanocarriers reaching the retina (II-IV) after topical instillation using different animal models. Beyond their efficacy in targeting posterior tissues, some of these works highlight nanocarriers' potential in increasing ocular retention time (I and V) while addressing the advantages of incorporating therapeutic-loaded nanocarriers in topical ophthalmic vehicles. Section I shows the capacity of nanocarriers in prolonging ocular residence time. Fluorescence images (100x) of lacrimal fluid samples collected at different time points following topical instillation of free-fluorescein solution (F-SOL) and fluorescein-labelled SLNs (F-SLNs) in the rabbit eye [49]. Section II (A)-(C) displays the importance of nanocarriers as delivery systems to intraocular tissues. Comparison of Nile-red solution (NR-solution) and Nile-red loaded nanostructured lipid carrier (NR-NLC) delivery efficiency after eye drop administration. Fluorescence images of the retina 40 min post-treatment with: (A) NR-NLC and (B) NR-solution; (C) Accumulated fluorescence intensity variation in the IPL after eye drop administration of NR-NLC and NR-solution. ($n = 3$), * $P < 0.05$ [53]. Section III (A)-(D) demonstrates the role of surface modification in retinal delivery. Confocal microscopy images of retinal sections from (A) non-treated mice, (B) animals treated with gold nanoparticles (AuNPs), and (C) animals treated with hyaluronic acid-modified gold nanoparticles (HA-AuNPs). (D) Resultingly, $\approx 50\%$ more HA-AuNPs were detected in the retina within 5 min. ($n = 3$) [75]. Section IV reinforces the ability of therapeutic delivery to the posterior segment of the eye through the use of topically applied nanocarriers. Comparative tobramycin concentrations in the retina 1 h and 3 h after topical and intravenous administration [46]. Section V (A)-(B) demonstrates the importance of topical ophthalmic vehicles as strategies to enhance the retention time at the surface of the eye. Comparative study of the retention time of (A) Penetratin-modified nanoemulsion (P-NE) and (B) Penetratin-modified nanoemulsion gel (P-NE GEL) at the corneal surface of rats [56]. All data is expressed as mean $\pm$ S.D. GCL: ganglion cell layer, INL: inner nuclear layer, IPL: inner plexiform layer, ONL: outer nuclear layer, OPL: outer plexiform layer, PL: photoreceptors layer, RPE: retinal pigment epithelium; SLN: solid lipid nanoparticles. Reproduced with permission [46,49,53,56]. Copyright © 2016, Elsevier; Copyright © 2020, Springer; Copyright © 2011, Elsevier; Copyright © 2020, Taylor and Francis. Reproduced under terms of the CC-BY 4.0 license [75]. Copyright © 2021, MDPI.

pigmented ciliary body epithelium and the iridial vessels are the major challenge imposed by the BAB [88,96]. Hence, the passage of large hydrophilic macromolecules to the aqueous humor is limited [27], as it can be for nanocarriers with hydrophilic coatings. However, the existence of fenestrations within the non-pigmented epithelium confers higher permeability to both hydrophilic molecules and nanocarriers, as well as lower transepithelial resistance [96]. Furthermore, despite being more closely sealed, the iridial vessels have demonstrated higher permeability than the retinal capillaries of the BRB [95,96]. A feasible strategy to mediate nanomedicines distribution to their target sites is through their modification with active targeting agents or functional moieties. As detailed in Table 1, most studies take advantage of passive targeting strategies such as reduced diameter, overall cationic/anionic net surface charge, or deformable properties. However, several studies also include active targeting methods. A commonly used approach is the incorporation of hydrophilic moieties in nanomedicines composition. For instance, chitosan is a hydrophilic cationic polymer known for its increased interaction with the corneal epithelium and enhanced mucoadhesive properties. Moreover, chitosan has the ability to decrease the transepithelial electrical resistance and open tight junctions, which promotes a higher penetration of the ocular tissues [37,38,57,69,70]. Polyethylene glycol (PEG) is another widely used coating agent. It is an inert hydrophilic polymer that acts as a protective shield around

nanocarriers, avoiding their recognition by opsonins and further macrophage uptake. PEG has muco-penetrating properties and inhibits P-gp efflux pumps [36,47,49,55,65,66]. Some reports in Table 1 also show that active targeting agents were employed for nanomedicines to reach posterior ocular tissues. One promising approach that showed successful results was the use of hyaluronic acid (HA), a natural hydrophilic glycosaminoglycan with a negatively charged surface. HA has affinity towards CD44 cell receptors, expressed in both corneal and retinal tissues. Furthermore, its negative charge may facilitate mobility through the vitreous humor while minimizing accumulation in pigmented tissues [73,76]. Another active targeting strategy reported was the use of Annexin-A5. This protein specifically targets cells expressing phosphatidylserine on their surface and demonstrated that can enhance corneal permeation by mediating endocytosis, thereby improving the delivery of nanomedicines to intraocular tissues [44,45].

Drugs or other therapeutic agents administered topically may find their distribution to posterior tissues automatically conditioned by the aqueous humor flowing in the opposite direction [88]. Therapeutic agents may be washed or diluted in the aqueous humor and removed via Schlemm's canal if the penetration occurs by the corneal pathway before it reaches the retina [93]. However, as the aqueous humor is expelled by the ciliary muscle into the supraciliary space and subsequently into the choroid and suprachoroidal clefs, the uveoscleral outflow may be a

potential alternative pathway for therapeutics to reach the posterior portions of the eye. As referred above, an interesting strategy to minimize drug loss and frequent drug administrations would be to lengthen the duration of drugs retention in the eye by merging the therapeutic loaded nanocarriers and ophthalmic vehicles (Fig. 4-1.1.1). This synergy allows to increase drug bioavailability by up to 50% and avoids rapid clearance through tear fluids [116]. Another effective and broader approach (Table 1) is to develop formulations with mucoadhesive properties or cationic charges to increase their retention at the eye's surface. Upon traversing the cornea and entering the aqueous humor, nanomedicines disperse across the adjacent intraocular tissues and vitreous fluid. When nanomedicines reach the vitreous humor, the diffusional properties of therapeutic agents will be conditioned. To improve mobility and improved likelihood of reaching the retina, it is preferable to focus on the development of nanomedicines with anionic or neutrally charged properties (Fig. 4-1.1.2) [27,94]. Indeed, studies performed in bovine vitreous humor showed that neutrally or negatively charged nanocarriers with mean sizes of 100 to 500 nm displayed higher diffusion coefficients than cationic and larger ones [117]. Contrarily, cationic nanocarriers' diffusion across the vitreous would be hindered, given their binding affinity towards hyaluronic acid and glycosaminoglycan proteins [106]. It seems paradoxical though that the cationic charge required to increase nanomedicines retention at the eye surface is detrimental to their diffusion through the humor vitreous. However, it was found that a threshold of net positive charge (up to +15 mV) assures an effective diffusion through the vitreous humor [118]. Käs Dorf et al. used oligopeptides with increasing charges (+7 to +23 mV) and realized that up to +15 mV nanomedicines showed good mobility properties through the gel-like fluid [118]. When the net charge is increased to higher values (+19 and +23 mV), the diffusion is blocked [118]. Furthermore, the diameter of nanomedicines also influences their mobility properties, and, as so, this is an important aspect to consider [94]. The vitreous has a mesh size of ≈ 550 nm [117], and nanomedicines up to that value diffuse freely through the gel-like fluid (Fig. 4-1.1.2). Oppositely, larger formulations will struggle more and probably be retained within the vitreous humor structure [94]. Nanocarriers' motion inside the vitreous humor is also influenced by its gel nature, which suffers great changes with age, becoming less fluid and heterogeneous [99]. Therefore, despite the lack of studies to support this assumption, in elderly patients the threshold of net positive charge may be stricter as diffusion is expected to be slower. Nevertheless, modifying the surface of the nanocarrier with hydrophilic coatings (Fig. 5-III) may increase its diffusion through collagen fibers and avoid their adhesion (Fig. 4) [107].

To finalize, nanomedicines need to surpass the ILM at the vitreous-retina interface to access the retina. Even though the information about therapeutic delivery across the ILM is sparse, there are important factors to consider during nanomedicines' development. For instance, glycosaminoglycan side chains of heparan sulfate proteoglycans confer a net negative charge to the ILM, which can be a critical parameter in nanomedicines design [94,100]. Cationic nanocarriers will face more difficulties in permeating to the retina than neutral or anionic ones (Fig. 4-2.1) [100,119]. Furthermore, the ILM is a hydrophilic membrane, and the diffusion of small and soluble molecules is facilitated [94,120]. Even though literature reports a mesh size between 10–100 nm, a well-established size-range that favors the permeation of nanocarriers through this barrier has not been defined yet [94]. For instance, in small rodents, intravitreally injected nanocarriers up to 325 nm were able to overcome the ILM, but the extrapolation of such results to larger species must be carefully analyzed [121]. More recently, Peynshaert et al. conducted an interesting study that, in disagreement with previously tested models, used a bovine explant of an intact vitreoretinal interface to evaluate retinal drug delivery [122]. The authors found that nanocarriers with 40 nm of diameter struggled less to reach the retina than particles ranging between 100–200 nm (Fig. 4-2.1) [122]. Overall, small non-cationic nanomedicines with hydrophilic coatings seem to be

the most appropriate for effective diffusion through the ILM [94]. However, the deformability of nanomedicines as well as the charge tuning threshold play a role in meeting the opposite requirements for passing the previous barriers and the ILM (Fig. 5-II).

Considering the strategies proposed for nanomedicines to reach posterior eye tissues by the corneal pathway and gathering the information compiled in Table 1, it is possible to conclude that the success of topically administrated nanomedicines through this route is contingent on the conjugation of several elements, namely: (i) deformable soft-matter based nanocarriers and/or reduced size (≤ 300 nm); (ii) balance the surface charge in accordance with the eye barrier; (iii) use of permeation enhancers, and (iv) use of agents with hydrophilic coatings and mucoadhesive properties.

2.4. Barriers to therapeutics absorption through the non-corneal route

2.4.1. Conjunctiva barrier

The conjunctiva is a highly vascularized transparent structure that accounts for almost one-third of the entire eyeball [10,24]. Tight connections enclose the cells of the conjunctival epithelium, much as in the cornea (Fig. 3), which limits the passage of several compounds [123]. Nevertheless, the space between the intercellular junctions is wider, which makes this compartment more permeable to hydrophilic compounds and larger biomolecules (< 20 kDa) such as proteins, peptides, and siRNA [107,124]. The conjunctival epithelium is also covered by a tear film layer (Fig. 3) whose main components (e.g., mucus, electrolytes, and fluids) are secreted by epithelial goblet cells [10,88]. The stroma, which is positioned just below the epithelium and in close proximity to the inner sclera, is a well-vascularized region. This means that it has many lymphatic and blood capillaries, which may potentially cause unintended off-target absorption of therapeutic agents (Fig. 2). Consequently, this might lead to the loss of the desired therapeutic effect at the intended location [89,125].

2.4.2. Sclera

The sclera is an avascular elastic tissue composed of irregular collagen fibers that covers the whole eye structure. It is composed of the episclera, scleral stroma, lamina fusca, and scleral endothelium (Fig. 3). Furthermore, the sclera is responsible for maintaining the IOP and the eye shape during ocular movement, and can also protect by preventing the permeation of exogenous agents into intraocular tissues [9]. The permeation via the sclera is considerably higher than through the cornea, and therapeutic agents can diffuse through perivascular spaces or spaces between the collagen fibers and through the aqueous media rich in mucopolysaccharides. Scleral permeability is mainly affected by molecular radius (up to 100 kDa); however, lipophilicity and charge also have an impact [94,107]. Underneath the sclera is the choroid, a highly innervated tissue situated in the space between the sclera and the retina.

2.4.3. Choroid

The choroid comprises three layers: suprachoroid, vascular layer, and Bruch's membrane (Fig. 3) [88]. The last layer limits the diffusion of hydrophilic therapeutic agents while simultaneously facilitating the ongoing transfer of metabolites and solutes between the choroidal blood circulation and the retina [10,23]. The thickness of Bruch's membrane varies with age and certain ocular conditions [88]. For example, age-related macular degeneration leads to chronic inflammation of Bruch's membrane due to the accumulation of extracellular debris, which triggers oxidative stress processes [88,107]. Although absorption of therapeutics through the conjunctiva/sclera is higher than through the cornea, the highly vascularized nature of choroid results in some therapeutic agent being lost to the blood circulation before reaching retinal tissues (Fig. 2) [29]. The pigmented nature of choroid and the existence of efflux transporters (e.g., P-gp or MRP) also conditions therapeutic diffusion towards the retina [91].

2.5. Nanomedicine design strategies to surpass non-corneal barriers

Nanomedicines can be absorbed through the conjunctival route after topical eye administration. In this case, nanomedicine design strategies to bypass the non-corneal barriers (Section 2.4) should be planned, as shown schematically in Fig. 4.

The main difficulty in the conjunctival route lies in the highly vascularized stroma. Typically, a significant portion of the administered therapeutic is removed from the body's circulation, leading to a reduction in the drug dose available at the posterior region. (<5%) [78]. Nevertheless, the higher surface area of the conjunctiva and the wider space between its epithelial tight junctions promote an enhanced absorption of hydrophilic and larger molecules (up to 5 kDa) compared to the cornea [29,94]. It is worth noticing that the leaky structure is not the only obstacle in therapeutic absorption through the conjunctiva. The presence of efflux pumps (e.g., P-gp) can also hinder the passage of therapeutic agents. As so, P-gp inhibitors (e.g., cyclosporin, ketoconazole, verapamil, and others) or P-gp silencing through siRNA delivery (Fig. 4-1.2) can be effective approaches to circumvent these proteins and increase ocular bioavailability (Table 1 [46,47,49,51,53,56,57,63–65, 69,74,126]). Additionally, the conjunctiva expresses molecular transporters that can help and mediate intraocular delivery, such as: MRP1-7, lung resistance proteins (LRP), and BCRP (Fig. 4-1.2) [127,128]. Furthermore, above the conjunctival surface also exists a tear film layer; thus the strategies discussed in Section 2.3, to prolong the therapeutic substances on the ocular surface may be equally used. The remaining nanomedicines which are not systemically cleared out from the conjunctiva diffuse to the highly permeable sclera.

The larger porous spaces within the sclera collagen network favor the passage of small hydrophilic molecules up to 100 kDa (Fig. 4-1.2.1), providing higher permeability than the cornea [12,29]. Scleral permeability is less sensitive for lipophilic compounds that can enter into the posterior eye region directly by lateral scleral diffusion, further penetrating Bruch's membrane and the RPE [88,129,130]. Charge is also a limiting factor for therapeutic delivery through the sclera [129]. Scleral proteoglycans are anionic at physiological pH and using cationic molecules may restrict their diffusion [27,129]. Hence, anionic or neutrally charged nanocarriers are preferred (Fig. 4-1.2.1). When the sclera is surpassed, therapeutic agents will cross directly to anterior intraocular tissues or the choroid.

The choroid barrier properties to therapeutic permeation are mainly attributed to Bruch's-choroid complex, offering more resistance than the sclera. The choriocapillaris are highly irrigated and the blood circulation within this layer can compromise therapeutic retinal permeation. In detail, the vascularized nature of choroid can promote the rapid washout of small nanomedicines to the blood circulation and reduce therapeutic concentrations within this structure [27,30]. Furthermore, the choroid is a densely pigmented tissue, which can affect therapeutic distribution [130]. As already reported, melanocytes are negatively charged, and using cationic compounds with lipophilic properties seems a good opportunity to increase melanin binding and affinity towards the choroid (Fig. 4-1.2.2) [131]. However, the best strategy to avoid bioaccumulation in choroidal tissues is to employ anionic nanomedicines with hydrophilic properties.

To access the inner retina, nanocarriers must penetrate the tight junctions around RPE cells. Their diffusion depends on several physicochemical properties, e.g., molecular weight, lipophilicity, ionization, or protein binding affinity. It is documented that small lipophilic compounds will more easily penetrate through tight junctions than large and hydrophilic molecules [130]. The transport of hydrophilic compounds typically occurs through the paracellular route, and the permeation is largely limited [93]. However, several transporter families (e.g., MRP; BCRP; OATP, and others) and receptors (e.g., folate, transferrin, and CD44 receptors) overexpressed at the surface of RPE cells can be used as potential targeting strategies (Fig. 4-2.2) for drug and gene therapies and to promote the delivery of non-lipophilic molecules transcellularly

[26,93,107]. The permeation of non-lipophilic compounds may be increased through their binding affinity towards melanin (Fig. 4-2.2). The pigment is overexpressed in RPE cells and its main functions are to protect the retina from overexposure to light and to act as an antioxidant by scavenging reactive oxygen species [85,131]. As already referred, melanin has a mostly negative surface charge, namely due to the presence of carboxylic groups. This charge may be used to modulate therapeutic permeation [26]. For example, cationic formulations bind to melanin and slow down the therapeutic agent release; however, a strong binding affinity can be counterproductive and lead to poor efficacy and drug accumulation [85,130]. Encouraging results (Table 1) of topical delivery to posterior eye tissues confirm the importance of adopting these design strategies (Fig. 6 II-IV).

Overall, the non-corneal pathway is more permeable to large and hydrophilic compounds; however, the threat of therapeutic leakage to blood circulation increases. As so, to reach posterior tissues through conjunctival delivery, it would be beneficial to: (i) load therapeutics into the nanocarriers; (ii) use nanocarriers with reduced size (≤ 300 nm); (iii) use agents with mucoadhesive properties; (iv) formulate nanocarriers with anionic or neutral surface charges; (iv) functionalize nanocarriers with targeting ligands and take advantage of receptors overexpressed at the surface of RPE cells; (v) use viscosity enhancers; and (vi) incorporate therapeutic-loaded nanocarriers in ophthalmic vehicles.

3. Topical vehicles for incorporation of ocular nanomedicines

The rapid washout of therapeutics from the eye surface and their entry to the bloodstream are the primary disadvantages related to the use of topical ophthalmic administration [116]. The pre-corneal clearance leads to poor therapeutic outcomes in posterior eye maladies and, as such, invasive strategies are often required. As an alternative, nanomedicines have been recently incorporated in topical vehicles such as hydrogels or contact lenses (CL) as a strategy to prolong their residence at the eye surface [132,133]. The non-invasiveness and comfort provided by these systems significantly increase patient compliance, avoiding frequent therapeutic administrations and potential side effects resulting from conventional topical regimens. Moreover, prolonging the therapeutic agents at the ocular surface will improve their bioavailability (up to 50%), which will ultimately enhance their efficacy [116].

Hydrogels are three-dimensional hydrophilic polymeric networks that can be synthesized by physical or chemical cross-linking methods using natural or synthetic polymers [133,134]. Ophthalmic gels can be divided into two main categories: (i) eye drop gels, which exist in the form of viscous solutions; and (ii) *in-situ* gels, which are liquid under storage conditions but undergo a sol-to-gel transition when applied to the eye [135]. *In-situ* gels are typically stimuli-responsive and their transition occurs as a response to biological (e.g., antibodies or enzymes), chemical (e.g., pH or ionic strength), or physical external stimulus (e.g., temperature, light, magnetic or electrical fields, and blinking speed) [135,136]. Hydrogels have the capacity to swell in aqueous environments and retain large amounts of water, which makes them suitable vehicles for the delivery of hydrophilic compounds [133]. Furthermore, their viscosity and good transparency properties allow the maintenance of therapeutic drug levels for longer periods of time at the target sites without affecting visual acuity [134]. Despite the scarcity of studies reporting successful delivery of nanocarriers loaded with therapeutic agents to the posterior pole of the eye using topical ophthalmic vehicles (Table 1), a few noteworthy ones must be discussed. For example, Ying Ge and co-workers conducted a research using thermo-sensitive *in-situ* gels with lutein-loaded nanoemulsions (IG-L-NE) that were posteriorly modified with penetratin (P) for the treatment of AMD [56]. The results showed that IG-L-NE-P inhibits apoptosis and the production of ROS and is effective in delivering lutein to the retina without symptoms of eye irritation [56]. As mentioned above, *in-situ* gels undergo a sol-to-gel transformation after contacting with the eye surface, increasing the retention of therapeutic agents in the pre-corneal

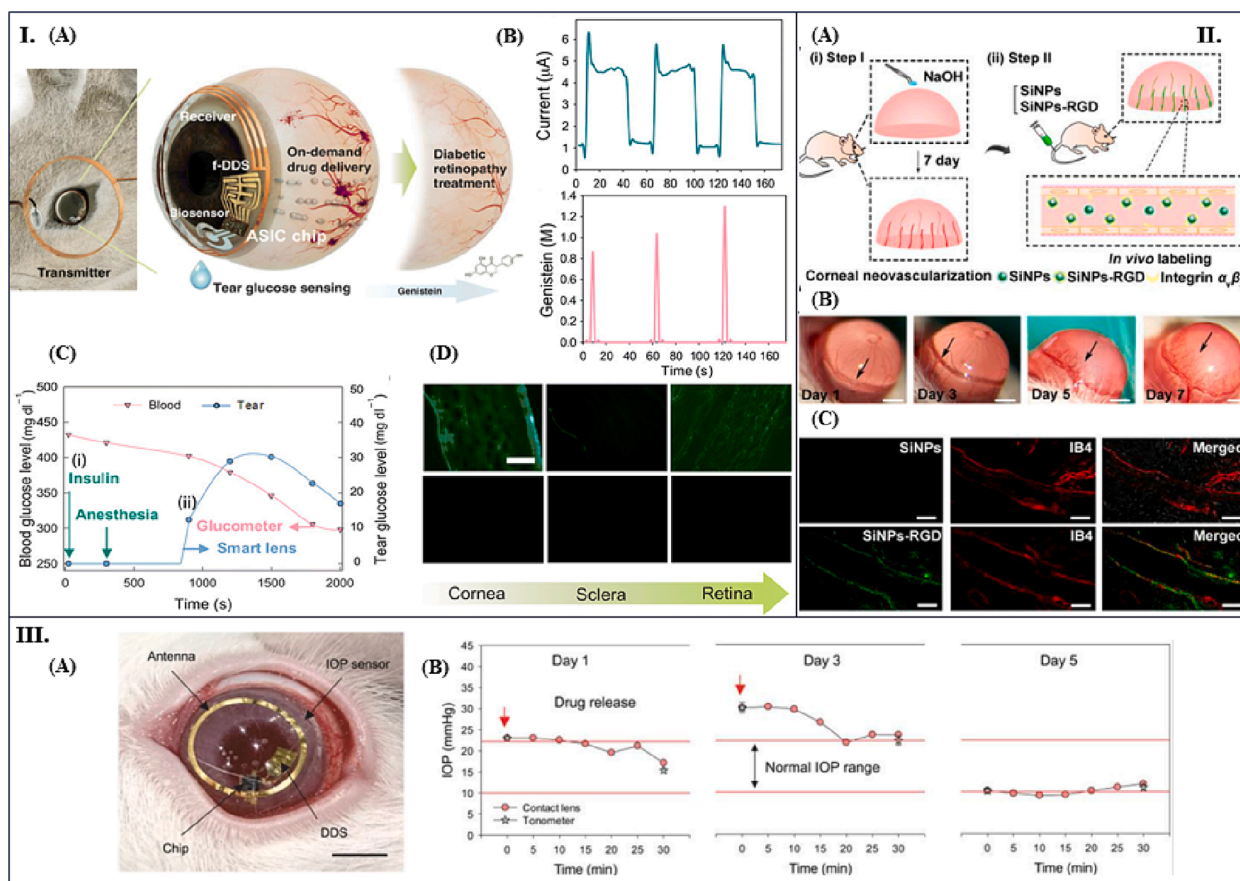


Fig. 7. Compilation of *in vivo* studies using theranostic devices to diagnose and treat ocular conditions in the anterior and posterior segments of the eye. Section I (A)–(D) shows a wireless theranostic CL able to monitor glucose levels in rabbit eyes' tear film and deliver therapeutic concentrations of genistein to the retina. (A) Schematic representation of the theranostic smart CL; (B) Demonstrates the ability of smart CL to selectively release genistein from the drug reservoirs after applying an electrical potential. Changes in the current of the smart CL and evidence of genistein release in a pulsatile manner. (C) Real-time monitoring of glucose levels in diabetic rabbits using smart CL. Glucose was measured in the blood and the tear (i) after injection of insulin and anesthesia and (ii) after wearing the smart CL, the sensor detected an increase of glucose concentration in the tear (30.53 mg dl^{-1} , tear glucose), and then it decreased (16.72 mg dl^{-1}) due to the insulin effect, which was in line with the blood glucose levels determined by a glucometer. The blood glucose level was measured every 5 min with a commercial glucometer. (D) Absorption of genistein *in vivo*. Fluorescence images of cryo-sectioned cornea, sclera, and retina of rabbits wearing genistein-loaded smart CL (top row) and smart CL without genistein. Scale bar, 0.1 mm. Section II (A)–(C) displays the binding affinity of silicon nanoparticles (SiNPs) and cyclo-(Arg-Gly-Asp-d-Tyr-Cys) (c-(RGDyC))-conjugated SiNPs (SiNPs-RGD) to angiogenic blood vessels in mice corneas. (A) Schematic representation of the experiment. (B) Corneal neovascularization progress following NaOH injury burn from day 1 to day 7. Scale bar, 5 mm. (C) Fluorescence images of mice corneas 4 h after intravenous injection of SiNP (upper row) and SiNPs-RGD (lower row). The binding of SiNP/SiNPs-RGD to angiogenic blood vessels was monitored *in vivo*. Scale bar, 25 μm . Section III (A)–(B) demonstrates a smart CL capable of controlling IOP in glaucoma and releasing timolol after electrical triggering on alternative days over a period of 5 days. (A) Fully assembled theranostic smart CL in rabbit's eye. Scale bar, 5.5 mm. (B) IOP levels on day 1 were high (above 22 mmHg), and timolol was released leading to IOP reduction to the normal range values. On day 3, IOP levels were still above the normal range and timolol was released again and decreased IOP to near the normal range. The IOP was within the normal range on day 5 and no timolol was released from the gold channels of smart CL. Reproduced with permission: [144,145,147]. Copyright © 2020, American Association for the Advancement of Science under CC BY-NC 4.0 license; Copyright © 2018, American Chemical Society; Copyright © 2022, Springer Nature under CC BY 4.0 license.

region and significantly improving their bioavailability. The developed system complies with such features and seems to extend the residence time of lutein and its penetration through the different tissues and fluids of the eye [56]. In another study, triamcinolone acetonide-loaded SLN (TA-SLN) were included in *in-situ* gels (IG-TA-SLN) to enhance TA delivery to deeper ocular tissues [50]. *In vivo* studies on transcorneal permeability and TA biodistribution revealed that IG-TA-SLN increased permeability through rabbit corneas and was able to deliver higher TA concentrations to both anterior and posterior tissues when compared to TA-SLN suspension [50].

Besides hydrogels, the incorporation of therapeutic agents in CL has been also gaining attention for the treatment of ocular conditions [116]. Although primarily designed to correct refractive errors, hydrophilic CL are currently seen as promising therapeutic devices. The first generation of CL was made of poly (methyl methacrylate) (PMMA), a non-foldable

rigid material with poor oxygen permeability and wettability properties [137]. These lenses did not provide a comfortable user experience and were replaced for rigid gas-permeable contact lenses (RGP) and soft contact lenses (sCL) [137]. Besides higher flexibility, sCL have better hydrophilic and bioavailability properties and may be composed of hydrogel or silicone-hydrogel [137,138]. Therapeutic agents can be included within CL matrix by three main processes: (i) soaking; (ii) molecular imprinting; and (iii) direct incorporation of drug-loaded nanocarriers [126]. The first method is the most affordable and consists in soaking the lens in an enriched pharmaceutical solution [138]. Despite its simplicity, the soaking method is not efficient and the incorporated molecules are released within 1–3 h [137]. Molecular imprinting is more difficult and involves polymeric modification to imprint in it certain morphological changes that will increase the affinity towards therapeutic molecules and, ultimately, improve the loading

capacity of CL [138]. Throughout this process, functional monomers are used as molds of therapeutic agents [116,138]. Despite its great potential, the complex synthesis process behind molecular imprinting makes it a non-affordable and time-consuming option [137]. In order to surpass these constraints, the incorporation of therapeutic-loaded nanocarriers within the polymeric matrix of CL demonstrated promising results and higher adherence [126]. In fact, similarly to hydrogels, therapeutic-loaded nanocarriers are retained within the lenses for higher periods and, consequently, achieve a more sustained release over time [116]. In a recent work, eluting CL loaded with a dexamethasone (DEX)-polymer film (CL-DEX) were developed to prevent inflammation in the posterior segment of the eye [139]. The findings indicated that, as compared to eye drops, CL promoted higher DEX concentrations ($\approx 200\times$) in the retina of rabbits and lowered DEX systemic exposure [139]. Furthermore, CL-DEX blocked retinal vascular leakage induced by intravitreal injection of VEGF [139]. Finally, *in vivo* biocompatibility studies in rabbits assured CL-DEX safety after a four-week repeated dose [139].

4. Theranostic devices designed for ocular diseases

Theranostic is a cornerstone of nanomedicine that focuses efforts on developing strategies that combine, simultaneously, diagnostic and therapeutic tools [140]. Hence, theranostic devices may function as drug delivery platforms while acting as bioimaging agents [141,142]. Offering the capacity to provide a diagnosis before to, after, or during therapy presents the prospect for tailored medicine, representing a significant advancement in clinical practice [143]. Undoubtedly, there has been a substantial rise in the investigation and advancement of theranostic methodologies, with the majority of systems depending on the integration of inorganic and organic constituents [142]. Nevertheless, the potentialities of theranostic in ARRD and its topical application are still poorly exploited. Here, we highlight the study of Keum *et al.* [144] focused on the development of wireless theranostic CL for the diagnosis and treatment of diabetic retinopathy (Fig. 7-I). This device provides real-time monitoring of glucose tear levels in the of rabbit eyes and subsequent therapeutic delivery of genistein [144]. It was observed that the therapeutic effect of genistein delivered through CL to the retina was comparable to Avastin delivered via intravitreal injection in diabetic retinopathy [144]. As so, smart CL proved to be feasible theranostic tools for continuous diabetic diagnosis and treatment [144]. In a different study, a theranostic smart CL capable of both monitoring IOP and delivering on-demand therapeutic treatment for glaucoma management was developed (Fig. 7-III) [145]. The CL is constituted with a highly sensitive IOP sensor based on gold hollow nanowires, a flexible drug delivery system (DDS), wireless power and communication systems, and an application-specific integrated circuit (ASIC) chip [145]. The *in vivo* results in glaucoma-induced rabbits demonstrated that the smart CL's flexible DDS allowed timolol delivery and was effective in controlling IOP to the normal range values and preventing glaucoma progression [145]. Topical strategies for other diseases (e.g., infectious, or inflammatory) affecting the anterior eye region, have already been reported. For example, CL with a hybrid surface were used as theranostic devices [146]. Surface functionalization was attained using anti-interleukin-1 α (IL-1 α) antibodies as biomarkers for detection of IL-1 α [146]. During corneal herpes simplex virus (HSV-1) infection, IL-1 α is up-regulated and inflammation can be detected through the capture of IL-1 α [146]. Furthermore, poly(sodium 4-styrenesulfonate) (PSS) was the innermost coating layer of CL, acting as an antiviral agent [146]. PSS demonstrated effective antiviral activity against HSV-1, probably by inhibiting virus adsorption onto cell surface receptors, thus preventing viral entry [146].

Tang and co-workers [147], developed silicon nanoparticles (SiNPs) to be used as theranostic agents for imaging and treatment of eye neovascularization (Fig. 7-II). Specifically, SiNPs were decorated with cyclo-(Arg-Gly-Asp-D-Tyr-Cys) (c-(RGDyC)) integrins that feature high binding affinity to human retinal microvascular endothelial cells

(HRMECs) tube formation [147]. As a result, SiNPs-RGD complex was able to detect angiogenic endothelial cells *in vitro* while showing potent antiangiogenic effect [147]. Salem *et al.* [148] used liposomes containing flucytosine capped with gold nanoparticles as a dual-function system for monitorization and treatment. Flucytosine-loaded liposomes were topically applied to treat fungal intraocular endophthalmitis while gold nanoparticles acted as contrasting agents that enabled the tracking of the antifungal agent [148]. It was reported that the developed formulation permeates into intraocular tissues and, consequently, deliver significant amounts of flucytosine to unhealthy eyes [148]. The findings demonstrated a direct relationship between the average uptake of gold nanoparticles and the proportion of eyes that had healed, so validating the effectiveness of the method [148]. A novel composite delivery system based on carbon dots (CD_{C-HP}) and thermo-sensitive hydrogels was developed for the topical eye administration of diclofenac [149]. Hyaluronic acid and carboxymethyl chitosan were used in the synthesis of CD using a one-step hydrothermal technique. The resulting CD was then integrated in a thermo-sensitive *in situ* gel [149]. The CD_{C-HP}-Gel composite provided enhanced pharmacokinetics and extended diclofenac release, demonstrating potential as a drug delivery system [149]. Furthermore, the fluorescence properties of CD allowed the tracking of diclofenac throughout intraocular tissues, demonstrating their efficiency as bioimaging agents [149].

5. Conclusions

The pursuit of non-invasive innovative therapies for posterior eye disorders is a priority and topical approaches are still the most appealing among patients. The idea of treating intraocular conditions with eye drops, hydrogels, or CL is as attractive as difficult. Indeed, following topical ophthalmic administration, therapeutic agents must face long and challenging paths (either through the corneal or conjunctival route) to reach posterior intraocular tissues and the retina. The well-defined anatomic structure of the eye includes several dynamic and static barriers that limit therapeutic delivery along the ocular compartments. As a result, drug bioavailability in the posterior portion of the eye is generally low. Nanomedicine is significantly contributing to overcoming these constraints, and the enhancement of topical treatments by the use of therapeutic loaded nanocarriers is a very promising strategy.

The importance of the rational design of nanomedicines is one of the core messages of this review. Nanomedicines designed rationally based on their intended target, avoid unnecessary errors, and promote higher success rates. Therefore, we emphasize that nanomedicines should be carefully conceived considering the barriers they will encounter in each ophthalmic route. A compilation of experimental data from scientific literature and the physical-chemical characteristics of eye barriers is used to provide a collection of nanocarriers attributes tailored to effectively target posterior tissues. Both in the corneal and conjunctival route, it is determinant to: (i) use deformable soft-matter-based nanocarriers; (ii) use reduced size nanocarriers (≤ 300 nm); and (iii) balance the surface charge in accordance with to the barrier. The balance of the surface charge is particularly relevant because while cationic nanocarriers are promising in the initial stage, to interact and diffuse through the pre-corneal and corneal layers, further ahead they are not adequate and may be hindered in other structures like the vitreous humor. To fight back these dualities, a good option would be developing nanocarriers with mucoadhesive properties to increase interaction with the mucoaqueous layer at the eye surface. In these conditions, the development of neutrally or negatively charged nanocarriers would be advantageous, promoting their diffusion across the eye. Furthermore, besides deformability and size requirements, researchers can also use targeting strategies to enhance nanocarrier penetration into posterior tissues. The presence of several molecular transporter families and cell receptors at the conjunctiva, cornea, or RPE cells' surface is an opportunity to mediate therapeutics distribution toward the target. In addition, permeation enhancers may be included within the nanocarrier

composition to increase ocular penetration. In addition, if therapeutic loaded nanocarriers are rapidly eliminated from the surface of the eye, it is feasible to include the formulations in other topical ophthalmic vehicles such as hydrogels or contact lenses. Overall, these strategies enable the balance between mucoadhesion and penetration, enhancing the therapeutic effect.

Another interesting outlook of nanomedicine in the ophthalmic field is theranostics. Nanomedicines struggle to reach inner eye layers. Hence, it seems unquestionable that the possibility of real-time monitoring would provide critical information to healthcare professionals, either to clarify if the treatment is progressing appropriately or if the clinical situation needs to be reassessed. Theranostics can be a step in this direction, providing treatment and monitoring in the same device.

CRediT authorship contribution statement

Maria João Faria: Conceptualization, Visualization, Writing – original draft, Writing – review and editing. **José M. González-Méijome:** Writing – review and editing. **M. Elisabete C.D. Real Oliveira:** Supervision, Writing – review and editing. **Gonzalo Carracedo:** Supervision, Writing – review and editing. **Marlene Lúcio:** Conceptualization, Visualization, Supervision, Funding acquisition, Writing – review and editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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