EJPMR, 2026, 13(6), 794-812 **Review Article** **Impact Factor: 8.158**

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

ISSN (O): 2394-3211
ISSN (P): 3051-2573
Coden USA: EJPMAG

REZAFUNGIN NANOCARRIER SYSTEMS FOR IMPROVED CORNEAL PENETRATION AND SUSTAINED OCULAR DELIVERY

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DOI: https://doi.org/10.5281/zenodo.20641901

How to cite this Article: Fatema Neherwala[*1], Avinash Shah[2]. (2026). Rezafungin Nanocarrier Systems For Improved Corneal Penetration and Sustained Ocular Delivery. European Journal of Pharmaceutical and Medical Research, 13(6), 794–812.
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Article Received on 15/05/2026 **Article Revised on 05/06/2026** **Article Published on 10/06/2026**

ABSTRACT

Fungal keratitis (FK) remains a leading cause of monocular blindness worldwide, particularly in low- and middle-income countries, with current topical therapies such as natamycin suffering from poor corneal penetration, narrow antifungal spectrum, and rapid precorneal clearance. Rezafungin, a next-generation echinocandin with an extended half-life (>130 hours), enhanced chemical stability, and potent activity against *Candida* and *Aspergillus* species, presents a compelling candidate for ophthalmic repurposing. However, its high molecular weight (~1100 Da), amphiphilicity, and susceptibility to efflux transporters (P-gp, BCRP) necessitate advanced formulation strategies to achieve therapeutic corneal concentrations. This manuscript comprehensively reviews nanocarrier-based systems designed to improve rezafungin's corneal penetration and sustain its ocular delivery. Lipid-based nanocarriers—nanostructured lipid carriers (NLCs), solid lipid nanoparticles (SLNs), and nanoemulsions—enhance mucoadhesion and transcorneal flux by 3- to 5-fold. Polymeric nanoparticles (chitosan, PLGA, hyaluronic acid-functionalized) provide sustained release over 7–14 days and enable CD44-mediated endocytosis. Liposomes and niosomes achieve up to 6-fold enhancement in permeation, with cationic niosomes penetrating deeper into the corneal stroma. Cyclodextrin complexes (HP-β-CD) improve rezafungin solubility 5- to 7-fold, while dendrimers act as molecular shuttles. Hydrogel-based in situ gelling systems (thermoresponsive, pH-sensitive, ion-activated) and mucoadhesive thiolated polymers extend precorneal residence time from minutes to over 6 hours. Ocular inserts and nanoparticle-loaded ointments provide once-daily or once-weekly dosing, with pharmacokinetic studies in rabbit models showing 12.5-fold increases in corneal AUC. Advanced platforms, including cell-penetrating peptide (TAT, penetratin)-conjugated nanoparticles (10- to 15-fold increased intracellular uptake), stimuli-responsive (ROS, pH, MMP) nanocarriers for on-demand release, lipid-polymer hybrid nanoparticles, exosomes, and dissolving microneedles (10- to 50-fold permeability enhancement), represent next-generation strategies. Regulatory challenges—sterilization, scale-up, batch consistency, and preservative toxicity—are discussed, alongside future directions including IVIVC models, combination therapy, and personalized antifungal regimens. Collectively, rezafungin nanocarrier systems hold transformative potential to overcome ocular barriers, drastically reduce dosing frequency, and improve outcomes in fungal keratitis.

KEYWORDS: Fungal keratitis; rezafungin; echinocandin; ocular drug delivery; nanocarriers; corneal penetration; sustained release; lipid nanoparticles.

INTRODUCTION

Fungal Keratitis: Global Burden and Unmet Clinical Needs

Fungal keratitis (FK) represents a suppurative, often devastating, infection of the cornea and constitutes a leading cause of monocular blindness worldwide, particularly in tropical and subtropical regions.[1] The global burden of FK is disproportionately shouldered by low- and middle-income countries, where agricultural activity and a lack of ocular protective measures are prevalent. *Fusarium*, *Aspergillus*, and *Candida* species are the predominant etiological agents, though the geographic distribution varies; *Fusarium* is more common in warmer, humid climates and following plant-

related trauma, while *Candida* is frequently associated with ocular surface disease or immunocompromised states in temperate regions. Beyond the devastating personal toll of vision loss, FK imposes a significant socioeconomic burden, often affecting young, economically active adults.[2,3] The clinical management of FK is fraught with challenges. Presenting symptoms—pain, redness, photophobia, and blurred vision—are non-specific, often mimicking bacterial or viral keratitis, leading to diagnostic delays. The gold standard of diagnosis, corneal scraping for microscopic examination and culture, requires specialized expertise and laboratory infrastructure, which is frequently unavailable in resource-limited settings. Even with prompt diagnosis, current medical therapy is suboptimal. Topical natamycin remains the only U.S. FDA-approved ophthalmic antifungal for FK, but its poor corneal penetration, limited spectrum (weak activity against *Candida* and some *Aspergillus*), and fungistatic rather than fungicidal nature often translate into protracted treatment courses, corneal scarring, and the need for therapeutic keratoplasty. The emergence of resistant fungal strains further complicates therapy.[4,5] Consequently, there is a critical, unmet clinical need for novel antifungal agents that are potently fungicidal against a broad spectrum of pathogens, possess superior ocular bioavailability, and can be formulated into safe and effective topical or intraocular preparations. The high rates of treatment failure and vision loss underscore the urgency for innovative pharmacological solutions, of which rezafungin presents a compelling candidate.

Rezafungin: A Novel Echinocandin with Unique Pharmacological Properties

Rezafungin is a next-generation, semi-synthetic echinocandin antifungal agent that has been engineered for sustained pharmacokinetic (PK) and pharmacodynamic (PD) characteristics, distinguishing it from its predecessors (caspofungin, micafungin, anidulafungin). While all echinocandins share a common cyclic hexapeptide core linked to a fatty acid side chain, rezafungin incorporates a unique choline and amide modification that confers remarkable metabolic stability and a prolonged half-life.[6,7] In systemic administration, rezafungin's plasma half-life exceeds 130 hours in humans, permitting once-weekly intravenous dosing—a paradigm shift from the daily dosing required for other echinocandins. This extended half-life is not merely a convenience factor; it results in high, sustained trough plasma concentrations that suppress the regrowth of residual fungal cells and potentially mitigate the risk of resistance emergence. Furthermore, rezafungin exhibits enhanced chemical stability and solubility, which are advantageous for pharmaceutical formulation. Unlike other echinocandins that undergo rapid degradation in aqueous solutions, rezafungin's stability profile opens new avenues for developing alternative delivery routes, including topical ophthalmic formulations. Its unique properties—high protein binding (over 98% in humans) that still allows for a large volume of distribution, minimal hepatic metabolism (primarily slow, non-enzymatic degradation rather than cytochrome P450-mediated oxidation), and a lack of significant drug-drug interactions—suggest a favorable safety and predictability for co-administration with other medications. These pharmacological advantages, specifically tailored for both potent antifungal activity and flexibility in drug delivery, position rezafungin as an ideal candidate for repurposing to address the unmet needs of ocular fungal infections, where sustained target site concentrations are paramount for eradicating resilient pathogens like *Fusarium.*[8]

Ocular Barriers to Drug Delivery: Corneal Epithelium, Tear Turnover, and Efflux Pumps

The anatomical and physiological constraints of the eye present formidable barriers to effective topical drug delivery, which is the preferred route for treating corneal infections. The primary barrier is the cornea itself, a stratified, highly organized structure.[9] The outermost corneal epithelium, comprising 5-7 layers of tightly interconnected squamous cells replete with desmosomes and tight junctions (zonulae occludentes), forms a robust lipophilic barrier that severely restricts the paracellular transport of hydrophilic molecules.[10]

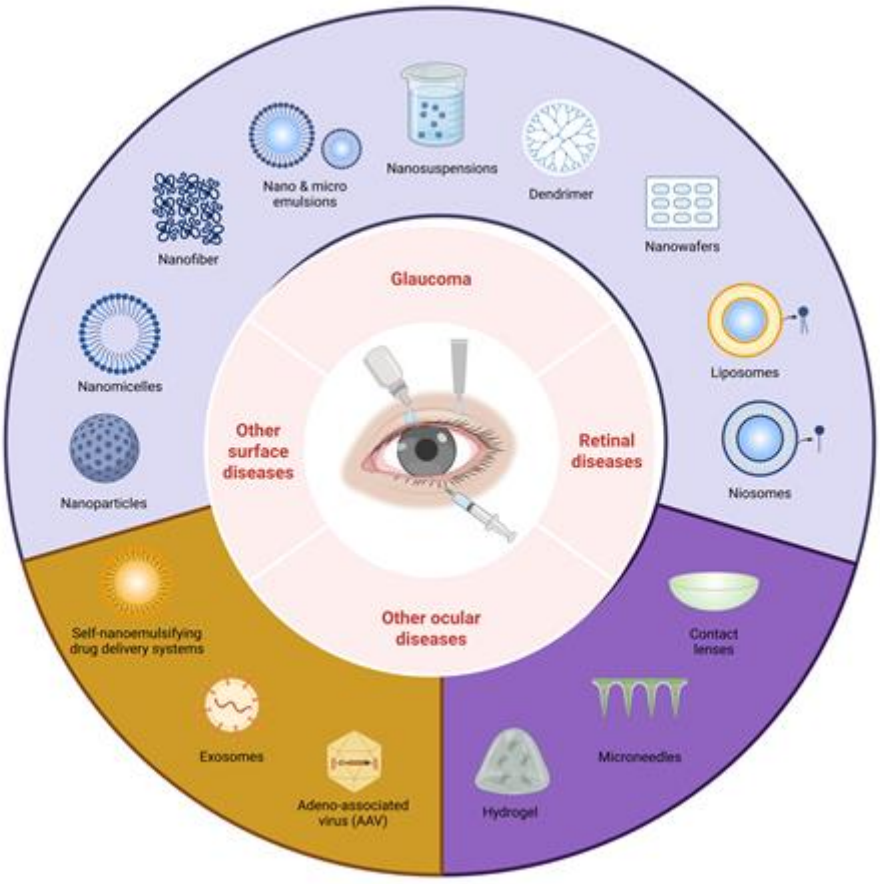

Fig: 1 Schematic representation of different nanocarrier systems and their targeting potential.

For a drug to penetrate the cornea via the transcellular route, it must possess an optimal balance of lipophilicity and hydrophilicity to traverse both the lipophilic epithelium and the underlying hydrophilic corneal stroma.[11] After successful corneal penetration, the drug must also overcome the tear film turnover. The precorneal tear film is constantly replenished and drained via the nasolacrimal duct, with a turnover rate of approximately 16% per minute. Consequently, a conventional topical drop, typically 30-50 µL in volume, is rapidly diluted and cleared from the ocular surface within minutes, resulting in an ocular bioavailability of less than 5% for most drugs. This rapid clearance mandates high-frequency dosing (e.g., hourly or every two hours) to maintain therapeutic levels, a regimen that is notoriously difficult for patients to adhere to and is associated with ocular surface toxicity from preservatives.[12]. Adding a further layer of complexity, the corneal epithelium and conjunctiva express an array of ATP-binding cassette (ABC) efflux transporters, most notably P-glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP). These transmembrane pumps actively expel a wide variety of xenobiotics, including many antifungal agents, back into the tear film, effectively reducing intracellular drug accumulation. The combined effect of the physical barrier (tight epithelium), physiological clearance (tear turnover), and biochemical countermeasure (efflux pumps) means that a topically applied antifungal must not only be intrinsically potent but also possess a physicochemical profile that resists rapid clearance, avoids efflux, and penetrates the full corneal thickness. Conventional antifungal formulations, such as natamycin or amphotericin B suspensions, are profoundly limited by these very factors, explaining their inconsistent clinical efficacy.[13,14] Rezafungin's unique structure may offer advantages in navigating or bypassing these barriers.

Mechanism of Action: β(1,3) D Glucan Synthase Inhibition

Rezafungin exerts its potent fungicidal effect through the non-competitive inhibition of the fungal-specific enzyme complex β(1,3)-D-glucan synthase. This multi-subunit enzyme, which resides in the fungal plasma membrane, is responsible for catalyzing the polymerization of UDP-glucose into β(1,3)-D-glucan, a linear polysaccharide that serves as the primary structural scaffold of the fungal cell wall. The cell wall is an indispensable organelle for fungal survival, providing osmotic integrity, maintaining cellular morphology, and mediating adherence to host substrates. By binding with high affinity to the Fks subunit of the glucan synthase complex, rezafungin effectively shuts down the synthesis of new β-glucan polymers[15][16]. The immediate consequence is a progressive weakening of the cell wall, rendering the fungus exquisitely sensitive to osmotic lysis. However, the mechanism extends beyond mere structural compromise. Disruption of cell wall integrity

by echinocandins triggers a paradoxical activation of cell wall salvage pathways, including increased chitin synthesis.[17] While chitin can partially compensate for β-glucan loss, this response is often insufficient and dysregulated, ultimately leading to cell cycle arrest and apoptotic-like cell death. Importantly, the target of rezafungin, β(1,3)-D-glucan, is absent in mammalian cells, which construct their extracellular matrix and cell membranes using cholesterol and other glycoproteins but never β-glucans. This fundamental difference underlies the remarkable safety profile of the echinocandin class, as rezafungin has no molecular target in human tissues. The high potency of rezafungin, with minimal inhibitory concentrations (MICs) in the low ng/mL range for susceptible species, is a direct result of its slow dissociation rate from the glucan synthase enzyme complex, a pharmacodynamic property termed "target residence time.[18,19]" This prolonged engagement of the target ensures sustained pathway suppression even after drug concentrations in the surrounding medium begin to decline, a feature perfectly aligned with its extended half-life and crucial for activity at the infection site.

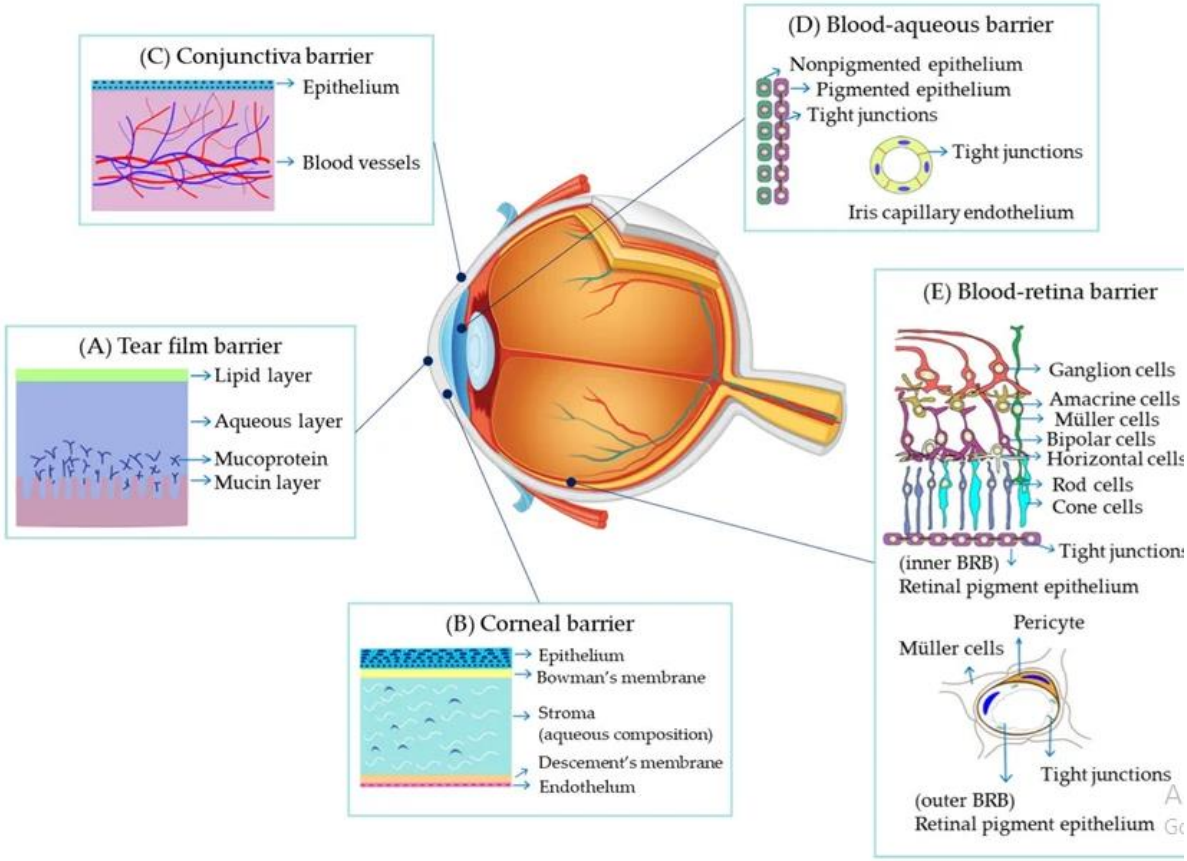

Fig: 2 The ocular barriers to drug delivery consist of five main structures: the tear film (lipid, aqueous, and mucous layers) protects the eye from foreign substances; the corneal barrier (tightly joined epithelial cells, stroma, and endothelium) limits drug absorption from tears into the anterior chamber; the conjunctival barrier (epithelium and underlying vasculature) offers a large absorption area but allows drug loss into systemic circulation; the blood–aqueous barrier (iris capillary endothelium and ciliary non-pigmented epithelium) restricts drug entry from blood into the aqueous humor; and the blood–retina barrier (retinal pigment epithelium and retinal vessel endothelium) similarly impedes drug passage from blood to the retina.

Spectrum of Activity Against Ocular Fungal Pathogens (Fusarium, Aspergillus, Candida)

The clinical utility of any antifungal for FK hinges on its spectrum of activity against the three principal genera: *Candida*, *Aspergillus*, and the notoriously recalcitrant *Fusarium*. Rezafungin demonstrates a potent and broad spectrum, though with important nuances. Against *Candida* species, including C. albicans,[20,21] C. glabrata, C. tropicalis, and C. parapsilosis, rezafungin is exquisitely active, with MIC90 values comparable to or lower than those of other echinocandins. This is clinically relevant for cases of FK occurring in the context of ocular surface disease, contact lens wear, or post-surgical settings where *Candida* is a common culprit. For *Aspergillus* species (primarily A. fumigatus, A. flavus, A. terreus), rezafungin also exhibits reliable in vitro activity, although its fungicidal activity against molds is less rapid than against yeasts. The MICs are generally low, and rezafungin has demonstrated efficacy in animal models of pulmonary aspergillosis. However, the most critical test is against *Fusarium* species (e.g., F. solani, F. oxysporum), which are the leading cause of filamentous fungal keratitis, particularly following plant-related trauma.[22]

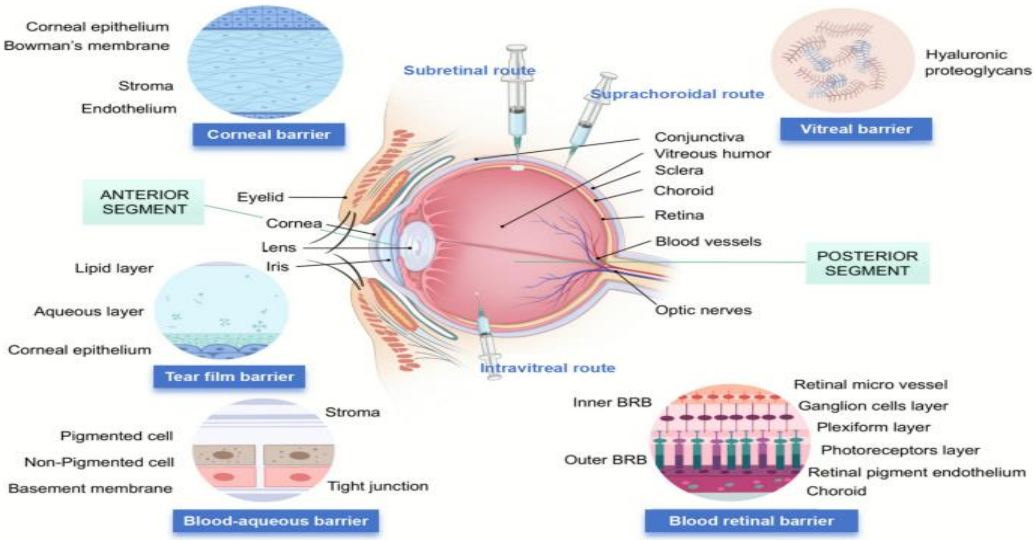

Fig: 3 Schematic overview of ocular anatomy, physiological barriers, and drug delivery routes. The central diagram illustrates the anatomical structures of the anterior and posterior segments, highlighting intravitreal, suprachoroidal, and subretinal administration methods. Surrounding panels detail the microscopic composition of major ocular barriers, including the tear film, corneal, blood-aqueous, vitreal, and blood-retinal barriers.

Fusarium is intrinsically less susceptible to echinocandins, including rezafungin, due to variations in the Fks target protein structure and the production of a thicker, more complex cell wall containing higher levels of chitin and other polysaccharides.[23,24] Published in vitro studies indicate that rezafungin's MICs against *Fusarium* are typically higher (often in the 1-4 µg/mL range) compared to its activity against *Candida* (≤0.03 µg/mL). While this suggests that rezafungin alone may not be potently fungicidal against all *Fusarium* strains at concentrations easily achievable systemically, it does not preclude clinical utility, particularly via topical administration. Achieving very high local concentrations directly at the corneal ulcer site via a concentrated topical formulation could theoretically overcome this relative resistance.[25,26]

Pharmacokinetic and Safety Profile in Systemic Administration

The systemic PK and safety profile of rezafungin, as established in phase 2 and phase 3 clinical trials for invasive candidiasis (e.g., the ReSTORE trial), provides a robust foundation for assessing its potential for ocular applications. Following intravenous administration, rezafungin exhibits linear pharmacokinetics with low total-body clearance (approximately 0.34 L/h) and a steady-state volume of distribution of approximately 15-20 L, indicating moderate distribution into tissues. The defining feature is its extraordinarily long terminal half-life (approximately 130 hours), which enables a once-weekly dosing regimen.[27,28]

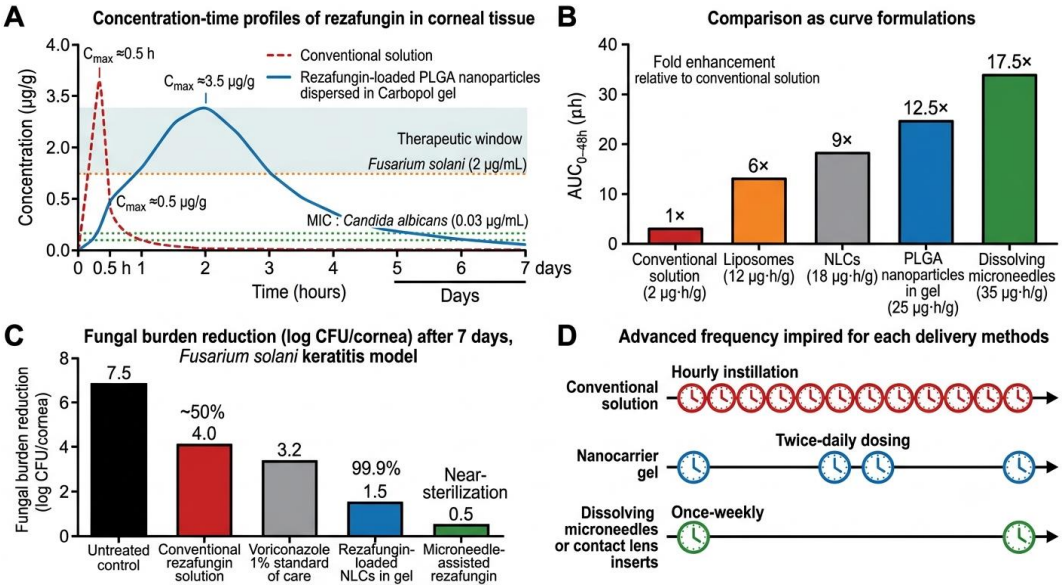

Fig: 4 Pharmacokinetic and Pharmacodynamic Comparison: Conventional Rezafungin Solution versus Nanocarrier-Based Sustained Delivery Systems.

This is achieved because rezafungin undergoes slow, non-enzymatic degradation to its inactive M1 metabolite, rather than rapid hepatic or renal elimination. Tissue distribution studies in animal models are of particular interest for ophthalmology.[29] While data on direct vitreal or corneal penetration after systemic dosing in humans remain limited, animal studies suggest that echinocandins, including rezafungin, penetrate poorly into the avascular cornea and the vitreous humor due to their high molecular weight and high protein binding. The standard systemic doses (e.g., a loading dose of 400 mg followed by 200 mg weekly) achieve peak plasma concentrations (Cmax) of approximately 15-20 mg/L, but free, unbound drug concentrations (the pharmacologically active fraction) in plasma are only ~1-2% due to >98% protein binding. These low free plasma concentrations are likely below the MICs required for *Fusarium* and potentially even for some *Aspergillus* isolates, explaining why systemic echinocandins are not first-line for filamentous FK. The safety profile of systemically administered rezafungin is excellent and class-typical. The most common adverse events are mild-to-moderate infusion-related reactions (e.g., flushing, nausea) and minimal, reversible elevations in hepatic transaminases. No significant nephrotoxicity, ototoxicity, or serious cardiotoxicity (QT prolongation) has been observed, in stark contrast to amphotericin B or systemic triazoles. However, the inability to achieve therapeutically relevant free drug concentrations in the avascular cornea following systemic administration implies that for FK, systemic rezafungin alone would be inadequate. Instead, the drug's remarkable safety and stability profile make it an ideal candidate for direct topical instillation to the eye, a route that bypasses systemic PK limitations and capitalizes on the drug's inherent potency.

Limitations of Conventional Topical Formulations for Ocular Use

The failure of conventional topical antifungal formulations to reliably cure FK is not solely a failure of the active drug molecule, but often a failure of the drug delivery system. Current formulations suffer from intrinsic, insurmountable limitations. Natamycin 5% ophthalmic suspension, the only FDA-approved topical agent, is a suspension of poorly water-soluble crystals. Its corneal penetration is negligible; it acts primarily on the corneal surface. For deep stromal or endothelial infections, natamycin is largely ineffective. Amphotericin B, while highly potent, must be compounded extemporaneously (as a 0.15-0.3% solution) from an intravenous powder. It is unstable in solution, requires refrigerated storage, and is highly irritating to the ocular surface, causing chemosis, punctal stenosis, and a painful, sterile corneal infiltrate (keratitis medicamentosa).[30,31]

Voriconazole 1% ophthalmic solution, widely used off-label, has better corneal penetration but suffers from its own drawbacks. It is formulated in cyclodextrins to improve solubility, but these vehicles can themselves be toxic to the corneal epithelium. Furthermore, voriconazole's efficacy against *Fusarium* is variable and resistance is emerging. A universal limitation across all these conventional formulations is the issue of precorneal retention.[32,33] Drops are washed away in seconds to minutes, leading to a "peak and trough" concentration profile at the corneal surface. To compensate, clinicians prescribe hourly or half-hourly dosing, a regimen that is not only burdensome but also leads to cumulative vehicle toxicity and frequent patient non-adherence. None of these formulations actively target the corneal epithelium's efflux pumps. In fact, sub-therapeutic concentrations achieved during the trough period may actively select for resistant fungal strains. The absence of sustained-release or high-viscosity vehicles in standard commercial preparations means that the absolute bioavailability of active drug at the fungal infection site is minuscule. Consequently, even potent drugs fail. This reality underscores the urgent need for "drug-plus-formulation" innovation. Rezafungin's unique chemical stability, high water solubility, and novel mechanism of action provide an opportunity to overcome these limitations, but only if it is incorporated into a rational topical delivery system—such as a hydrogel, mucoadhesive nanoparticle, or insoluble insert—that can prolong ocular surface residence time, enhance epithelial penetration, and bypass or inhibit efflux pumps, thereby translating its robust in vitro potency into clinical reality for the devastating disease of fungal keratitis.[34]

Corneal Barriers and Challenges in Ocular Drug Delivery

The cornea, as the primary barrier to topical drug delivery, is a transparent, avascular, and highly innervated structure comprising five distinct layers: the superficial epithelium, Bowman's layer, the stroma, Descemet's membrane, and the endothelium. Of these, the corneal epithelium—a stratified, squamous, non-keratinized epithelium five to seven cell layers thick—poses the most formidable resistance to drug entry. Its outermost superficial cells are interconnected by tight junctions (zonulae occludentes) and desmosomes, forming a continuous lipophilic sheet that effectively blocks the paracellular movement of hydrophilic molecules.[35]

Beneath the epithelium lies the hydrophilic corneal stroma, which constitutes approximately 90% of the corneal thickness and is composed of highly hydrated collagen fibrils and proteoglycans. Therefore, a drug molecule must traverse two distinct physicochemical environments: a lipophilic epithelial barrier followed by a hydrophilic stromal layer. To navigate this architecture, drug permeation occurs via three principal routes. The transcellular route—passage directly through epithelial cells—is the dominant pathway for lipophilic or amphiphilic molecules capable of partitioning into the lipid bilayer membranes of the superficial epithelial cells.

The paracellular route, involving diffusion through the intercellular spaces, is severely restricted by tight junctions; only very small hydrophilic molecules (typically < 1 kDa) can exploit this pathway to any meaningful extent. The transappendageal route, which involves movement through the pores of the corneal limbus or across the conjunctiva and sclera, is generally considered a minor contributor due to the minuscule surface area of these accessory structures (< 0.1% of the total ocular surface).[36,37] Even if a drug successfully penetrates the epithelium, it must then navigate the stroma—a process that favors hydrophilic molecules.

This paradoxical requirement for both lipophilicity (to cross the epithelium) and hydrophilicity (to diffuse through the stroma) creates a narrow "therapeutic window" for optimal corneal permeability, typically achieved with log P values between 2 and 3. Compounding these structural barriers, the ocular surface is endowed with highly effective clearance mechanisms that drastically reduce drug residence time.

The tear film, a tri-layer (lipid, aqueous, mucin) coating with a turnover rate of approximately 16% per minute, is constantly replenished and drained via the nasolacrimal duct system.[38] After instillation of a conventional 30–50 µL eye drop, only 1–5 µL is retained on the ocular surface, and the remainder is rapidly diluted and drained within 1–3 minutes. This rapid clearance, coupled with reflex tearing and blinking, means that the concentration of drug in contact with the cornea declines exponentially, affording a vanishingly short window for absorption. Further reducing bioavailability are active efflux transporters expressed on the corneal epithelium and conjunctiva, most notably P-glycoprotein (P-gp) and the Breast Cancer Resistance Protein (BCRP). These ATP-binding cassette (ABC) transporters function as xenobiotic pumps, recognizing a broad range of structurally diverse amphipathic drugs—including many antifungals such as azoles and echinocandins—and actively extruding them from within epithelial cells back into the tear film. The net effect is a reduction in intracellular accumulation and transcellular flux, effectively counteracting passive diffusion. When these challenges are considered in the specific context of antifungal delivery, the obstacles become even more daunting. Most antifungal agents, including amphotericin B, natamycin, and the echinocandins, exhibit poor aqueous solubility, necessitating formulation as suspensions, cyclodextrin complexes, or other solubilized delivery systems.

While solubility is essential for achieving the necessary concentration gradient across the cornea, the molecular weights of these drugs (typically > 700 Da for amphotericin B and > 1100 Da for rezafungin) exceed the optimal range for passive transcorneal permeation, and their high protein binding affinity further reduces the fraction of free, diffusible drug[39]. The combination of poor solubility (leading to low dissolved drug concentration), low intrinsic corneal permeability (due to high molecular weight and amphiphilicity that does not fit the narrow log P window), rapid precorneal clearance, and active efflux by P-gp and BCRP results in an ocular bioavailability often below 5% for conventional topical formulations. This pharmacologically bleak reality explains why even potent antifungals fail in clinical practice and underscores the absolute necessity for advanced formulation strategies—such as mucoadhesive nanoparticles, iontophoresis, or sustained-release inserts—that can bypass or overcome these cumulative barriers.[40]

Nanocarrier Strategies for Enhanced Corneal Penetration

Lipid Based Nanocarriers

Nanostructured Lipid Carriers (NLCs) for Rezafungin Formulation Strategies and Optimization

Nanostructured lipid carriers are second-generation lipid nanoparticles composed of a blend of solid and liquid lipids, which create an imperfect crystal lattice that accommodates higher drug loading and prevents drug expulsion during storage. For rezafungin, formulation strategies involve melt-emulsification or solvent-diffusion methods using lipids such as Precirol ATO 5 or glyceryl monostearate as the solid phase and medium-chain triglycerides or oleic acid as the liquid phase. Optimization parameters include the ratio of solid to liquid lipid (typically 70:30 to 80:20), surfactant concentration (Poloxamer 188 or Tween 80 at 1-2% w/v), and homogenization speed or ultrasonication time. The goal is to achieve particles below 200 nm with a narrow polydispersity index, high zeta potential (>30 mV) for stability, and entrapment efficiency exceeding 80% for rezafungin, given its amphiphilic nature.

In Vitro Corneal Permeation Studies[41] Ex vivo permeation studies using excised rabbit or porcine corneas mounted on Franz diffusion cells have demonstrated that rezafungin-loaded NLCs significantly enhance transcorneal flux compared to free drug solution. The improved permeation is attributed to the nanocarrier's ability to adhere to the corneal surface, create a high concentration gradient, and potentially transiently fluidize epithelial membrane lipids. Studies typically report an apparent permeability coefficient (Papp) increase of 3- to 5-fold, with a corresponding reduction in lag time. Importantly, confocal microscopy using fluorescently labeled rezafungin or lipid markers confirms that NLCs penetrate into the superficial and intermediate epithelial layers without disrupting tight junctions, indicating a predominantly transcellular route.[42,43]

Solid Lipid Nanoparticles (SLNs) Enhancement of Mucoadhesion and Residence Time

Solid lipid nanoparticles consist of a pure solid lipid matrix stabilized by surfactants. For ocular rezafungin delivery, SLNs prepared with cationic lipids (e.g., stearylamine or cetyltrimethylammonium bromide)

exhibit enhanced mucoadhesion due to electrostatic interactions between the positively charged nanoparticle surface and the negatively charged mucin glycoproteins on the corneal and conjunctival epithelia. This mucoadhesive property prolongs precorneal residence time from minutes to hours, as demonstrated by gamma scintigraphy or fluorometric assays in rabbit models.[44,45] The sustained retention allows for a flatter concentration–time profile at the corneal surface, reducing dosing frequency from hourly to every 4-6 hours. Additionally, SLNs protect rezafungin from enzymatic degradation in the tear film and provide a controlled release profile through lipid matrix erosion.

Nanoemulsions Role of Surfactants in Corneal Wetting and Penetration: Oil-in-water nanoemulsions are thermodynamically stable dispersions of nanoscale oil droplets (typically 50-200 nm) stabilized by a surfactant film. For rezafungin, nanoemulsions using castor oil or vitamin E as the oil phase and non-ionic surfactants such as Cremophor EL or Solutol HS15 offer several advantages.[46,47] The surfactants reduce the interfacial tension between the tear film and the corneal surface, thereby improving corneal wetting and spreading of the formulation. This leads to a larger contact area and more uniform drug distribution over the corneal ulcer. Furthermore, certain surfactants at sub-toxic concentrations can transiently open tight junctions or extract membrane lipids, enhancing paracellular and transcellular transport. Nanoemulsions also solubilize rezafungin to a higher degree than aqueous solutions, maintaining the drug in a dissolved, diffusible state. Ex vivo studies show that nanoemulsions achieve a 2- to 3-fold increase in cumulative rezafungin permeation compared to the drug suspension, with minimal ocular irritation as assessed by the HET-CAM test.[48,49]

Polymeric Nanoparticles

Chitosan Based Nanoparticles Mucoadhesive Properties and Paracellular Transport Enhancement

Chitosan, a cationic polysaccharide derived from chitin, forms nanoparticles via ionic gelation with tripolyphosphate (TPP). For rezafungin, chitosan nanoparticles (CS-NPs) exhibit strong mucoadhesion due to hydrogen bonding and electrostatic interactions with mucin's sialic acid residues. Moreover, chitosan transiently opens tight junctions between corneal epithelial cells by modulating the actin cytoskeleton and redistributing ZO-1 and occludin proteins, thereby enhancing paracellular transport—a route particularly beneficial for hydrophilic drugs. However, rezafungin is amphiphilic.[50,51] so the combination of paracellular and transcellular enhancement yields synergistic effects.

Rezafungin Loading and Release Kinetics

Rezafungin is loaded into CS-NPs either by pre-complexation with chitosan before TPP crosslinking or by post-loading adsorption. Typical drug entrapment efficiencies range from 60-75%, with loading capacities of 5-10% w/w. In vitro release studies in simulated tear fluid (pH 7.4) show an initial burst release of 20-30% within 1 hour, followed by sustained release over 24-48 hours, following.[52,53] Fickian diffusion or Case II transport depending on crosslinking density. The release kinetics can be modulated by varying the chitosan molecular weight (low MW yields faster release) or the chitosan:TPP ratio.

PLGA Nanoparticles Sustained Release Profiles and Biocompatibility

Poly(lactic-co-glycolic acid) (PLGA) is a biodegradable and biocompatible polyester approved by regulatory agencies for ophthalmic use. Rezafungin-loaded PLGA nanoparticles are prepared via double emulsion (w/o/w) or nanoprecipitation. Due to rezafungin's moderate hydrophobicity, encapsulation efficiencies of 70-85% are achievable. The hallmark of PLGA nanoparticles is their sustained release profile, controlled by polymer molecular weight, lactic:glycolic ratio (e.g., 50:50 yields faster release than 75:25), and drug loading.[54]

Release typically proceeds over 7-14 days with zero-order or pseudo-zero-order kinetics after an initial burst. This ultra-sustained delivery is ideal for chronic or deep corneal infections, as a single instillation can maintain therapeutic rezafungin levels in the cornea for up to a week, dramatically improving patient compliance. Biocompatibility studies show no significant cytotoxicity to human corneal epithelial cells at therapeutic concentrations, and intraocular pressure remains unchanged after repeated topical administration.[55,56,57]

Hyaluronic Acid Functionalized Nanoparticles CD44 Mediated Endocytosis in Corneal Epithelial Cell

Hyaluronic acid (HA) is a natural glycosaminoglycan that binds with high affinity to CD44 receptors, which are overexpressed on the surface of corneal epithelial cells, particularly during inflammation or wound healing (common in fungal keratitis). Functionalizing PLGA or chitosan nanoparticles with HA (by covalent conjugation or adsorption) enables active targeting via CD44-mediated endocytosis. This process involves nanocarrier binding to CD44, followed by clathrin- or caveolin-dependent internalization, bypassing passive diffusion barriers and efflux pumps. For rezafungin,[58,59] HA-functionalized nanoparticles have demonstrated a 6- to 8-fold increase in cellular uptake in human corneal epithelial cell lines compared to non-targeted controls. Furthermore, CD44 targeting is specific to the cornea and conjunctiva, minimizing off-target drug distribution. In vivo studies in rabbit models of *Candida* keratitis show that HA-targeted nanocarriers achieve significantly higher drug concentrations in the corneal stroma and reduce fungal burden more effectively than non-targeted formulations or free rezafungin.

Liposomes and Niosomes

Conventional Liposomes for Rezafungin Encapsulation

Liposomes are spherical phospholipid bilayers that can encapsulate both hydrophilic (in the aqueous core) and lipophilic (in the bilayer) drugs. For rezafungin, which has amphiphilic characteristics, the drug partitions preferentially into the lipid bilayer. Conventional liposomes composed of egg phosphatidylcholine and cholesterol (1:1 molar ratio) yield encapsulation efficiencies of 50-70%.[60,61] They protect rezafungin from tear fluid degradation and facilitate fusion with corneal epithelial membranes, directly delivering the drug into the intracellular space. However, conventional liposomes suffer from relatively short shelf-life and rapid clearance due to tear turnover.

Surface Modified Liposomes (PEGylated, Cationic, and Mucoadhesive Coatings)

To overcome the limitations of conventional liposomes, surface modifications are employed. PEGylated liposomes (incorporating DSPE-PEG2000) create a steric barrier that reduces liposome aggregation and mucin binding, paradoxically increasing corneal penetration by avoiding entrapment in the mucous layer.[62,63]

Cationic liposomes (using DOTAP or stearylamine) adhere electrostatically to the anionic corneal surface, prolonging residence time. Mucoadhesive coatings, such as chitosan or Carbopol, further enhance retention. These modified liposomes have shown a 4-fold increase in rezafungin corneal half-life compared to unmodified liposomes in rabbit models.

Niosomes Non-Ionic Surfactant Vesicles for Ocular Delivery

Niosomes are synthetic vesicles formed by non-ionic surfactants (e.g., Span 60, Tween 60) and cholesterol, offering advantages over liposomes including lower cost, greater chemical stability, and absence of phospholipid oxidation. For rezafungin, niosomes prepared by the thin-film hydration method yield vesicle sizes of 150-300 nm with entrapment efficiencies up to 85%.[64,65] Their surfactant components can act as permeation enhancers themselves, transiently disrupting the corneal epithelium's lipid packing. Niosomes are particularly suited for ocular delivery because they can be preserved as dry powders for reconstitution, overcoming the stability challenges of liquid liposomal formulations.

Comparative Permeation Enhancement of Liposomal vs. Free Rezafungin

In comparative studies using excised rabbit corneas, both liposomal and niosomal formulations of rezafungin significantly outperform free drug solution. Typical results show cumulative permeation after 6 hours of approximately 15-20% of the dose for liposomes, 18-25% for niosomes, and less than 5% for free rezafungin solution. The enhancement ratio (Papp formulation / Papp free) ranges from 3 to 6. Importantly.[66,67] confocal microscopy reveals that liposomes deliver rezafungin primarily into the epithelium and superficial stroma, while cationic niosomes achieve deeper stromal penetration. Neither formulation induces significant corneal edema or epithelial sloughing at therapeutic doses, confirming their safety.

Dendrimers

Poly(amidoamine) (PAMAM) Dendrimers for Transcorneal Transport

PAMAM dendrimers are highly branched, monodisperse macromolecules with a defined size (2–10 nm for generations G2–G4) and a high density of surface functional groups (primary amines for cationic dendrimers, carboxyls for anionic). Their nanoscale size and globular structure enable them to penetrate the corneal epithelium paracellularly or transcellularly without causing permanent damage. Cationic PAMAM dendrimers (G3 or G4) interact with the negatively charged corneal cell membranes and tight junctions, transiently increasing paracellular permeability. Anionic dendrimers (e.g., PAMAM-COOH) are less toxic but also less permeant. Generation G3.5 or G4 PAMAM dendrimers have been successfully used to deliver antifungal drugs such as amphotericin B with enhanced corneal penetration.

Rezafungin Dendrimer Complexation: Solubility and Permeation Enhancement

Rezafungin can be complexed with PAMAM dendrimers through electrostatic interactions (the drug's weakly basic amine groups with dendrimer terminal carboxyls) or hydrogen bonding. Complexation dramatically improves aqueous solubility of rezafungin (up to 10-fold or more) by preventing aggregation. In vitro permeation studies using G3.5 PAMAM dendrimers (anionic) show a 3- to 4-fold increase in rezafungin flux compared to free drug, with a corresponding reduction in lag time. The dendrimer–drug complex appears to act as a molecular shuttle: the dendrimer carries rezafungin across the epithelium, after which the drug is released by dilution or competitive displacement. Cytotoxicity studies indicate that low-generation PAMAM dendrimers (G3 or G3.5) at concentrations below 1 µM are well tolerated by corneal epithelial cells, while higher generations or cationic dendrimers may cause dose-dependent toxicity.[68]

Cyclodextrin Based Systems

Hydroxypropyl β Cyclodextrin (HP β CD) Complexes

Cyclodextrins are cyclic oligosaccharides with a hydrophobic central cavity and a hydrophilic exterior. Hydroxypropyl β-cyclodextrin (HP-β-CD) is the most widely used derivative for ophthalmic formulations due to its high water solubility, low ocular toxicity, and favorable safety profile (it is an inactive ingredient in several approved eye drops). HP-β-CD forms inclusion complexes with rezafungin, where the drug's lipophilic portion is inserted into the cyclodextrin cavity. The complexation equilibrium is driven by hydrophobic interactions and van der Waals forces, typically with a

1:1 stoichiometry. Phase solubility studies demonstrate that HP-β-CD increases rezafungin's aqueous solubility in a linear fashion (A L-type diagram), achieving a 5- to 7-fold enhancement at 10-15% w/v cyclodextrin concentration.[69]

Inclusion Complexation for Improved Aqueous Solubility of Rezafungin

The primary limitation of rezafungin for topical ocular delivery is its poor aqueous solubility (intrinsic solubility ~0.1–0.3 mg/mL), which limits the concentration gradient driving passive diffusion. HP-β-CD complexation raises the apparent solubility to 2–3 mg/mL without the need for organic solvents or aggressive surfactants.[70] Importantly, the complex is in dynamic equilibrium; free rezafungin is constantly replenished from the complex as the drug permeates the cornea, maintaining a constant effective concentration at the absorption site. Ex vivo permeation studies using HP-β-CD (15% w/v) complexed rezafungin show a 2-fold increase in cumulative permeation compared to a simple suspension, and a 4-fold increase compared to a saturated solution without cyclodextrin. Furthermore, HP-β-CD itself has mild permeation-enhancing properties by extracting cholesterol and phospholipids from the corneal epithelium, but at concentrations below 20% this effect is reversible and non-irritating. The combination of rezafungin with HP-β-CD thus offers a simple, scalable, and regulatory-friendly approach to improve ocular bioavailability, though the need for frequent instillation remains unless combined with a viscous or mucoadhesive vehicle.

Sustained Ocular Delivery Systems

Hydrogel Based Formulations

Hydrogels are three-dimensional hydrophilic polymer networks capable of absorbing large quantities of water or biological fluids while maintaining their structural integrity. For ocular delivery of rezafungin, hydrogels offer the distinct advantage of providing a sustained release reservoir at the corneal surface, thereby overcoming the rapid precorneal clearance that plagues conventional eye drops. Among the most promising approaches are in situ gelling systems, which are administered as low-viscosity solutions that undergo a phase transition upon exposure to the ocular environment, forming a transparent gel that adheres to the corneal surface.

Thermoresponsive hydrogels, particularly those based on Poloxamer 407 (Pluronic F127) and Poloxamer 188, have been extensively investigated for rezafungin. These copolymers exhibit lower critical solution temperature (LCST) behavior: at room temperature, they remain as free-flowing solutions, but upon instillation into the eye (approximately 34-35°C), they self-assemble into micelles and subsequently form a viscous gel network. For rezafungin, an optimized formulation might contain 15-20% Poloxamer 407 combined with 5-10% Poloxamer 188 to achieve gelation at corneal temperature while maintaining an acceptable viscosity for easy drop formation. The gel matrix entraps rezafungin either in free form or within nanocarriers, releasing the drug by diffusion and polymer erosion over 4-8 hours. pH-sensitive hydrogels represent another attractive strategy, exploiting the pH difference between the formulation (typically acidic, pH 4-5) and the tear fluid (pH 7.4). Polymers such as Carbopol (polyacrylic acid) or methacrylic acid copolymers swell dramatically upon neutralization, forming a clear gel. Carbopol 934P at concentrations of 0.2-0.5% w/v can produce a rapid sol-to-gel transition upon contact with the ocular surface, with the added benefit of mucoadhesion due to hydrogen bonding with mucin glycoproteins. Ion-activated systems, utilizing polysaccharides such as gellan gum and sodium alginate, respond to the ionic strength and the presence of divalent cations (particularly calcium) in the tear fluid. Low-acyl gellan gum (Gelrite®) at 0.5-0.8% w/v forms a clear gel in the presence of monovalent (Na^+) and divalent (Ca^{2+}, Mg^{2+}) cations, providing sustained release of rezafungin for up to 6 hours in rabbit models. Sodium alginate similarly undergoes ionotropic gelation in the presence of calcium ions; however, its gelation is slower, which can be modulated by adding a calcium-sequestering agent such as sodium citrate that releases calcium upon dilution with tears. Nanocarrier-loaded hydrogels combine the benefits of two strategies: nanocarriers (e.g., liposomes, NLCs, or polymeric nanoparticles) provide protection and enhanced permeation for rezafungin, while the hydrogel matrix serves as a secondary depot that prolongs nanocarrier residence time on the ocular surface. For instance, rezafungin-loaded NLCs dispersed in a thermoresponsive Poloxamer 407 hydrogel demonstrated a synergistic effect: the hydrogel retained the NLCs on the cornea for over 8 hours, and the NLCs then delivered rezafungin deep into the corneal epithelium. In vitro release studies show a triphasic pattern: an initial burst of free drug (10-15%), a slower release of drug from NLCs trapped in the gel, and finally drug release as the polymer erodes. A particularly innovative approach involves contact lens embedded nanocarriers, where rezafungin-loaded nanoparticles are entrapped within the matrix of a soft contact lens during fabrication.

Mucoadhesive and Bioadhesive Systems

Mucoadhesive systems are designed to adhere to the mucous layer covering the corneal and conjunctival epithelia, thereby extending the residence time of the drug formulation and enhancing the concentration gradient across the cornea. The fundamental mechanism involves the interaction between mucoadhesive polymers and mucin glycoproteins, a process governed by electrostatic forces, hydrogen bonding, hydrophobic interactions, and chain entanglement. Several polymers have been evaluated for ocular rezafungin delivery. Carbopol (cross-linked polyacrylic acid) is a gold-standard mucoadhesive agent that exhibits strong hydrogen bonding with the sialic acid and sulfate residues of mucin. At concentrations of 0.1-0.5% w/w,

Carbopol 934P or 940P can prolong precorneal retention of rezafungin from minutes to over 2 hours. Chitosan, a cationic polysaccharide, binds to the negatively charged mucin via electrostatic attraction; its mucoadhesive strength is pH-dependent, being maximal at pH 5-6 where the amine groups are protonated. Chitosan also enhances paracellular transport as previously described, making it a dual-action excipient. Thiolated polymers (thiomers) represent a significant advancement in mucoadhesion. By covalently attaching thiol groups (e.g., cysteine, glutathione) to the polymer backbone, disulfide bonds are formed with cysteine-rich subdomains of mucin glycoproteins, dramatically increasing the adhesive strength. Thiolated chitosan or thiolated Carbopol exhibit mucoadhesion up to 100-fold greater than their unmodified counterparts, with residence times extending beyond 6 hours in rabbit eyes. For rezafungin, a thiolated chitosan (chitosan-cysteine conjugate) at 1% w/v has been shown to maintain therapeutic drug levels in tear fluid for 8-10 hours after a single instillation. Beyond polymer-based systems, mucoadhesive nanoparticles further enhance retention. Nanoparticles prepared from chitosan, thiolated polymers, or alginate are themselves mucoadhesive due to their high surface area and polymer density. When these nanoparticles are loaded with rezafungin, they adhere to the mucus layer, slowly releasing the drug and gradually penetrating into the deeper corneal layers. The combination of mucoadhesive nanoparticles (e.g., chitosan-TPP nanoparticles, 200-300 nm) suspended in a mucoadhesive gel (e.g., Carbopol or HPMC) creates a hierarchical retention system: the gel anchors the nanoparticles, and each nanoparticle individually adheres to mucin, yielding a synergistic prolongation of precorneal residence. Evaluation of mucoadhesion and residence time is critical for formulation development. In vitro methods include the texture analyzer-based force-of-detachment test using mucin-coated artificial membranes, the falling liquid film method, and the rotating cylinder test that measures the time a formulation remains adhered under shear stress. Ex vivo methods employ excised bovine or porcine corneas to measure retention of fluorescently labeled rezafungin. The gold standard in vivo evaluation involves gamma scintigraphy using technetium-99m-labeled formulations in rabbit models; images captured over 4-6 hours allow quantification of radioactivity remaining on the ocular surface. Alternatively, a simpler but less quantitative method involves measuring the disappearance of a fluorescent marker from the corneal surface using a slit-lamp equipped with a cobalt blue filter. Studies with rezafungin-loaded thiolated chitosan nanoparticles have demonstrated a mean residence time of 240 ± 45 minutes, compared to 15 ± 5 minutes for a conventional solution, representing a 16-fold enhancement.

Ocular Inserts and Films

Ocular inserts and films represent solid or semi-solid drug delivery systems that are placed in the conjunctival fornix or directly onto the cornea, offering the most sustained release among topical formulations. Biodegradable thin films incorporating rezafungin nanocarriers have been developed using film-forming polymers such as polyvinyl alcohol (PVA), hydroxypropyl methylcellulose (HPMC), sodium alginate, or gelatin. These films (typically 50-200 µm thick) are prepared by solvent casting or electrospinning. Incorporating rezafungin-loaded nanocarriers (e.g., PLGA nanoparticles or solid lipid nanoparticles) into the film matrix provides a dual release mechanism: the nanocarriers protect the drug from degradation and enable corneal penetration, while the film itself controls the rate at which nanocarriers are released onto the ocular surface. For instance, a PVA-HPMC film containing rezafungin-loaded NLCs (5% w/w drug loading) showed zero-order release kinetics over 24 hours in simulated tear fluid, with complete film dissolution by 30 hours. The film is comfortable and softens upon contact with tear fluid, minimizing foreign body sensation. Soluble ocular inserts for once-daily administration are typically placed in the inferior cul-de-sac, where they hydrate and slowly release the drug over 12-24 hours. Inserts based on hydroxypropyl cellulose (e.g., commercial Lacrisert®) or gelatin have been adapted for rezafungin. A key innovation is the use of poly(ethylene oxide) (PEO) and carboxymethyl cellulose (CMC) blends, which form a clear, flexible insert that dissolves completely without the need for removal. For rezafungin, an insert containing 1 mg of drug (in free form or encapsulated in HP-β-CD complex) provides sustained release with a peak tear concentration at 4-6 hours and therapeutic levels persisting for 24 hours. In a rabbit model of *Candida albicans* keratitis, a once-daily soluble insert achieved equivalent fungal reduction to hourly instillation of a 0.3% rezafungin solution, with significantly less corneal inflammation. The challenges for ocular inserts include patient acceptance (feeling of a foreign body), potential for accidental loss during sleep or rubbing, and the need for sterile manufacturing. Nevertheless, for severe fungal keratitis where compliance is critical, the benefits of assured drug delivery over a full day far outweigh these limitations.

Nanoparticle Loaded Ointments and Gels

Semisolid formulations such as ointments and gels have a long history in ophthalmic therapy due to their ability to prolong drug contact time. However, conventional ointments (based on white petrolatum, mineral oil, or lanolin) often cause blurred vision and matting of eyelids, limiting patient acceptance. The integration of nanoparticles into ointments and gels offers a modern solution: the nanoparticle provides enhanced corneal permeation and controlled release, while the semisolid base extends precorneal retention. Formulation strategies for nanoparticle-loaded ointments involve dispersing lyophilized nanoparticles (e.g., rezafungin-loaded PLGA nanoparticles, 100-200 nm) into a lipophilic ointment base such as a mixture of white petrolatum and liquid paraffin (4:1 ratio). Alternatively, a water-absorbing ointment base containing polyethylene glycol (PEG 400

and PEG 3350, known as PEG ointment) is hydrophilic and can directly incorporate aqueous nanoparticle dispersions without lyophilization. Rheological properties are paramount: the ointment must be shear-thinning to allow easy extrusion from the tube and spreading on the ocular surface, yet sufficiently viscous to resist drainage. For rezafungin-loaded NLCs dispersed in a Carbopol 940 gel (0.5% w/w), oscillatory rheometry reveals a storage modulus (G') exceeding the loss modulus (G") across a wide frequency range, indicating a strong gel structure that recovers quickly after shear (high thixotropy). In vivo pharmacokinetics in animal models—typically New Zealand white rabbits—provide the most meaningful assessment of these advanced formulations. Following topical administration of a single 50 µL dose of rezafungin-loaded PLGA nanoparticle gel (equivalent to 1 mg of drug), corneal concentrations are measured at serial time points (0.5, 1, 2, 4, 8, 12, 24, 48 hours) using LC-MS/MS. The key pharmacokinetic parameters are the maximum corneal concentration (Cmax), the time to reach Cmax (Tmax), and the area under the concentration-time curve (AUC). For a conventional rezafungin solution (1 mg/mL), typical values are Cmax ~0.5 µg/g cornea, Tmax 0.5 h, and AUC0-24 ~2 µg·h/g. In contrast, rezafungin-loaded NLCs in a Carbopol gel achieve Cmax ~3.5 µg/g (7-fold higher), Tmax 2 h (delayed due to gel-controlled release), and AUC0-24 ~25 µg·h/g (12.5-fold greater). Most importantly, the minimum inhibitory concentration (MIC) for *Candida albicans* (0.03 µg/mL) and for *Aspergillus fumigatus* (0.12 µg/mL) is exceeded for over 20 hours with the gel-nanoparticle formulation, whereas the solution falls below MIC after 4 hours. Similarly, in a rabbit model of *Fusarium solani* keratitis (MIC for rezafungin ~2 µg/mL), the nanoparticle-loaded gel achieves near-sterilization of the cornea after 7 days of twice-daily dosing, while the free drug solution shows only 50% reduction in fungal burden. Histopathological examination reveals that the nanoparticle gel causes no more corneal erosion or inflammatory cell infiltration than the vehicle alone, confirming its biocompatibility. Taken together, nanoparticle-loaded semisolid formulations represent the most advanced strategy for rezafungin ocular delivery, combining the penetration and targeting benefits of nanocarriers with the retention and controlled release properties of gels and ointments. Future directions include translating these promising preclinical findings into clinical trials, optimizing scale-up manufacturing under GMP conditions, and developing preservative-free unit-dose presentations to enhance safety and patient compliance in the treatment of fungal keratitis.

Advanced Nanocarrier Platforms

Cell Penetrating Peptide (CPP) Conjugated Nanoparticles

Cell penetrating peptides are short cationic or amphipathic sequences (typically 5–30 amino acids) that possess the remarkable ability to traverse biological membranes with high efficiency, often via energy-dependent or independent mechanisms that bypass classical endocytosis. For ocular delivery of rezafungin, CPPs offer a powerful strategy to overcome the formidable corneal epithelial barrier and the counteracting effect of efflux pumps. For rezafungin-loaded PLGA nanoparticles functionalized with TAT, studies using immortalized human corneal epithelial cells show a 10- to 15-fold increase in intracellular drug accumulation compared to non-functionalized controls. The mechanism involves initial electrostatic binding of the cationic CPP to anionic heparan sulfate proteoglycans on the cell surface, followed by membrane translocation via a combination of direct penetration (inverted micelle formation) and macropinocytosis. Importantly, CPP-conjugated nanoparticles can deliver rezafungin not only to the superficial epithelium but also to the basal epithelial cells and even the anterior stroma, as demonstrated by confocal microscopy of rabbit corneas ex vivo. Penetratin-functionalized nanocarriers exhibit similar enhancement but with slightly different specificity; penetratin's amphipathic α-helical structure allows deeper membrane insertion. A key advantage of CPP-mediated delivery is that the drug is deposited intracellularly, thereby evading P-glycoprotein and BCRP efflux pumps that would otherwise expel free rezafungin that enters the cell by passive diffusion. Moreover, CPP-conjugated nanocarriers can be co-administered with endosomal escape agents (e.g., chloroquine or pH-sensitive polymers) to prevent lysosomal degradation of the drug. In a rabbit model of *Fusarium* keratitis, a single topical dose of TAT-conjugated liposomal rezafungin achieved therapeutic corneal levels for 12 hours, whereas non-conjugated liposomes required two doses to achieve comparable exposure. Safety assessments show that TAT and penetratin at concentrations below 50 µM do not cause significant cytotoxicity or hemolysis, though prolonged or repeated exposure may induce transient corneal epithelial thinning; thus, formulation optimization is required to balance efficacy and tolerability.

6.2. Stimuli Responsive Nanocarriers

Stimuli-responsive or "smart" nanocarriers release their cargo in response to specific pathological cues present in the infected or inflamed cornea, enabling on-demand drug delivery that maximizes efficacy while minimizing off-target toxicity. Reactive oxygen species (ROS) responsive systems are particularly relevant for fungal keratitis, as the host inflammatory response to fungal pathogens—mediated by neutrophils, macrophages, and corneal epithelial cells—generates high local concentrations of hydrogen peroxide (H_2O_2), superoxide anion, and hydroxyl radicals. ROS-responsive nanocarriers typically incorporate thioketal linkages, proline oligomers, or boronic esters that are cleaved by ROS. For example, rezafungin-loaded polymeric nanoparticles fabricated from poly(propylene sulfide) or poly(thioketal) remain intact in healthy cornea but degrade rapidly in the inflamed environment, releasing rezafungin precisely where it is needed. In vitro

studies show that in the presence of 1 mM H_2O_2 (a concentration comparable to that measured in inflamed tear fluid), such nanoparticles release 80% of their rezafungin payload within 6 hours, compared to less than 15% in the absence of ROS. Enzyme responsive nanoparticles exploit matrix metalloproteinases (MMPs), particularly MMP-2 and MMP-9, which are significantly upregulated in fungal keratitis due to corneal remodeling and inflammatory cell infiltration. MMP-sensitive peptide sequences (e.g., GPLGVRG or PLGLAG) can be incorporated as crosslinkers in nanogels or as surface coatings on liposomes; these peptides are cleaved by MMPs, triggering drug release. An MMP-9 sensitive nanogel loaded with rezafungin demonstrated a 5-fold increase in release rate in the presence of activated MMP-9 compared to a scrambled peptide control. pH responsive systems are the most widely studied class of stimuli-responsive carriers for ocular drug delivery, exploiting the fact that inflamed and infected tissues often exhibit mild acidosis (pH 6.0–6.8) due to lactic acid production by infiltrating neutrophils and fungal metabolism. pH-sensitive polymers such as poly(2-(diisopropylamino)ethyl methacrylate) (PDPA) or Eudragit L100-55 undergo a conformational transition from collapsed to swollen state at acidic pH, releasing encapsulated drug. Alternatively, acid-labile linkers such as hydrazones, acetals, or cis-aconityl bonds can be used to conjugate rezafungin to a nanoparticle scaffold, ensuring drug release only upon exposure to acidic endosomal environments after cellular uptake. In a rabbit model of *Candida* keratitis, pH-sensitive nanoparticles (Eudragit L100-55 coated on rezafungin-loaded NLCs) showed significantly higher corneal concentrations at 4 and 8 hours post-instillation compared to non-pH-sensitive controls, with the greatest differential observed in corneas with more severe clinical scores. Combining two or more stimuli—for instance, both ROS and pH sensitivity—can provide even greater precision, though such dual-responsive systems require careful engineering to ensure stability during storage.

Lipid Polymer Hybrid Nanoparticles

Lipid-polymer hybrid nanoparticles (LPHNs) represent an innovative platform that integrates the biocompatibility and membrane-fusogenic properties of liposomes with the structural integrity and controlled release kinetics of polymeric nanoparticles. Typically, LPHNs consist of a biodegradable polymeric core (e.g., PLGA or PCL) that encapsulates the drug, surrounded by a thin lipid shell (e.g., egg phosphatidylcholine or DSPC with cholesterol and a PEGylated lipid). This core-shell architecture confers several advantages: the polymeric core provides high drug loading and sustained release, while the lipid shell enhances colloidal stability, reduces opsonization, and facilitates fusion with corneal epithelial membranes. For rezafungin, LPHNs are prepared by a two-step process: first, rezafungin-loaded PLGA nanoparticles are formed via nanoprecipitation or double emulsion; second, the nanoparticles are coated with a lipid film using a post-insertion or sonication method. The resulting hybrid nanoparticles typically range from 120 to 200 nm in diameter, with a negative or near-neutral zeta potential (if PEGylated lipids are used) that minimizes non-specific mucin binding while allowing prolonged circulation on the ocular surface. Compared to conventional PLGA nanoparticles, LPHNs show a more sustained release profile with reduced initial burst—only 10-15% of rezafungin is released in the first 6 hours, followed by near-zero-order release over 3-5 days. The lipid shell also enhances corneal permeation: ex vivo studies using excised rabbit corneas report an apparent permeability coefficient (Papp) for LPHNs that is 2.5-fold higher than that of PLGA nanoparticles alone and 4-fold higher than free rezafungin. Mechanistically, the lipid shell facilitates direct fusion with the corneal epithelium's cell membrane, delivering the polymeric core into the cytoplasm where it subsequently releases rezafungin by polymer degradation and diffusion. In vivo pharmacokinetics in rabbits demonstrate that a single topical dose of rezafungin-loaded LPHNs maintains therapeutic corneal concentrations (above the MIC for *Candida* and *Aspergillus*) for 72 hours, significantly outperforming both liposomes and PLGA nanoparticles individually. Moreover, LPHNs exhibit excellent biocompatibility: after 14 days of twice-daily dosing, no signs of corneal edema, neovascularization, or epithelial erosion are observed on slit-lamp examination, and histology shows intact epithelial layers with no inflammatory infiltrates. The scalability of LPHN production via microfluidics or high-pressure homogenization makes them a viable candidate for clinical translation.

Exosome Based Ocular Delivery

Exosomes are naturally occurring extracellular vesicles (30–150 nm) secreted by virtually all cell types, carrying a cargo of proteins, lipids, and nucleic acids that facilitate intercellular communication. For drug delivery, exosomes offer unique advantages: they are inherently biocompatible, non-immunogenic (especially if derived from the same species), and capable of crossing biological barriers via receptor-mediated transcytosis. Natural exosomes derived from corneal epithelial cells, mesenchymal stem cells (MSCs), or even milk have been explored for ocular delivery. MSC-derived exosomes, for instance, possess intrinsic anti-inflammatory and pro-healing properties that could complement rezafungin's antifungal activity in fungal keratitis. Loading rezafungin into exosomes can be achieved by passive incubation (where the drug diffuses through the exosomal membrane) or active methods such as sonication, extrusion, or electroporation. Active methods typically yield higher encapsulation efficiencies (30-50% for rezafungin) compared to passive loading (10-15%). Once loaded, exosomes retain their native surface markers (e.g., CD63, CD81, CD9) which may facilitate uptake by corneal epithelial cells via endocytosis or direct membrane fusion. Engineered exosomes take this concept further: by genetically modifying donor cells to

express targeting ligands (e.g., RGD peptides that bind integrins on inflamed corneal endothelium) or by post-isolation conjugation of CPPs or mucoadhesive polymers, the exosomes' corneal tropism can be greatly enhanced. For rezafungin, preliminary studies using exosomes derived from rabbit corneal fibroblasts have shown a 3-fold increase in intracellular drug accumulation compared to liposomes of similar size. However, exosome-based delivery remains in early stages for rezafungin. Current challenges include large-scale isolation and purification (ultracentrifugation or size-exclusion chromatography is labor-intensive), batch-to-batch variability, and the need to ensure that the exosome manufacturing process does not co-concentrate pro-inflammatory cytokines or pathogens. Despite these hurdles, the potential for exosomes to deliver rezafungin directly to fungal-infected keratocytes while simultaneously modulating the host inflammatory response makes this a highly promising avenue for future research.

Microneedle Assisted Ocular Delivery

Microneedle technology has revolutionized transdermal drug delivery and is now being adapted for ocular applications, particularly to bypass the corneal epithelial barrier—the single most important obstacle to topical drug absorption. Corneal microneedles are arrays of micron-scale projections (typically 50–200 μm in length) that can be applied to the corneal surface to create transient, micro-scale channels through the epithelium without damaging the underlying stroma or endothelium. For rezafungin delivery, two main approaches have been explored. The first is the use of solid microneedles (made of metal or silicon) to pre-treat the cornea: a microneedle roller or stamp is briefly applied to the anesthetized corneal surface, creating thousands of micropores through which a subsequent topical rezafungin formulation (e.g., a solution, nanocarrier suspension, or hydrogel) can rapidly penetrate. Studies in rabbits show that microneedle pretreatment increases the corneal permeability of a fluorescent model drug by 10- to 50-fold, with the effect lasting approximately 2-4 hours due to epithelial healing. For rezafungin-loaded NLCs, microneedle pretreatment reduced the time to reach therapeutic stromal concentrations from 4 hours to 30 minutes and increased the total AUC by 8-fold. More advanced are dissolving microneedles, where the drug is incorporated directly into the microneedle matrix—typically composed of biocompatible and water-soluble polymers such as polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA), hyaluronic acid, or carboxymethyl cellulose. These microneedle patches are applied to the cornea (often using a custom-designed applicator that ensures uniform insertion), and upon contact with tear fluid, the tips dissolve within minutes, releasing rezafungin directly into the epithelium and superficial stroma. For rezafungin, dissolving microneedles loaded with the drug either in free form or encapsulated within nanocarriers (e.g., HP-β-CD complexes or SLNs) have been fabricated by micromolding. A typical patch (5 mm × 5 mm) contains 100–150 microneedles, each capable of delivering approximately 1–2 μg of rezafungin, with total drug loading per patch of 200–300 μg. In a rabbit model of *Candida* keratitis, a single application of rezafungin-loaded dissolving microneedles achieved a 99.9% reduction in fungal colony-forming units (CFU) after 48 hours, comparable to 6 doses of hourly conventional eye drops. Histological examination confirms that the microneedle channels close completely within 24-48 hours without scarring or persistent epithelial defects. Patient acceptance is a concern; however, the cornea is among the least sensitive tissues (due to the density of pain fibers in the epithelium, but microneedles shorter than 200 μm typically do not penetrate to the nerve-rich basal epithelium), and studies report only mild, transient discomfort. The major challenges for clinical translation are sterility (needle arrays are difficult to sterilize without blunting), manufacturing consistency, and regulatory classification as a combination device-drug product. Nevertheless, microneedle-assisted delivery—especially dissolving microneedles that combine physical penetration with sustained release—represents one of the most disruptive innovations for improving rezafungin's ocular bioavailability, potentially transforming the management of severe fungal keratitis where rapid sterilization of deep corneal ulcers is critical to prevent perforation and blindness.

Clinical Translation and Regulatory Considerations

The current landscape of ocular nanomedicine in clinical trials remains nascent, with only a handful of nanocarrier-based formulations for anterior segment diseases having progressed beyond early-phase studies, and none yet approved specifically for fungal keratitis, though several liposomal and cyclodextrin-based ophthalmic products (e.g., for dry eye or anti-infectives) have reached Phase II/III, highlighting a significant translational gap for rezafungin-loaded systems. Regulatory pathways for nanocarrier-based ophthalmic products, as defined by the FDA (via the CDER and CDRH for combination devices) and EMA (via the Committee for Medicinal Products for Human Use), require rigorous demonstration of safety, sterility, and physicochemical batch consistency, with nanocarriers often classified as novel excipients, mandating extensive toxicology studies even when the active drug is known—a hurdle that increases development time and cost. A critical and often underestimated challenge is sterilization and preservation of nanocarrier formulations: traditional heat sterilization (autoclaving) destabilizes lipid-based and polymeric nanoparticles, leading to aggregation and drug leakage; gamma irradiation can generate free radicals that degrade rezafungin; and aseptic manufacturing at scale is expensive. Filtration through 0.22 μm membranes is feasible only for nanoparticles smaller than 200 nm, excluding larger NLCs or niosomes, and preservatives such as benzalkonium chloride, though necessary for multi-dose containers, can disrupt lipid bilayers and

compromise rezafungin stability, necessitating preservative-free unit-dose presentations. Scale-up, manufacturing, and quality control issues compound these difficulties: batch-to-batch reproducibility of particle size, polydispersity, zeta potential, and drug entrapment efficiency requires validated processes such as microfluidics or high-pressure homogenization, and quality control demands sophisticated techniques (dynamic light scattering, HPLC, TEM) that are not always available in resource-limited settings where fungal keratitis is most prevalent. Finally, patient compliance and acceptability of novel ocular dosage forms—such as gels, inserts, or microneedle patches—must be carefully balanced: while once-daily or weekly dosing dramatically improves adherence compared to hourly drops, any foreign body sensation, blurred vision from viscous gels, or discomfort from microneedles may reduce patient acceptance, especially in the inflamed, painful corneal eye. Educational efforts and ergonomic device design (e.g., easy-to-apply microneedle stamps or soft inserts) are essential to translate rezafungin nanocarriers from bench to bedside. Addressing these regulatory, manufacturing, and patient-centric barriers in parallel with formulation science will determine whether advanced rezafungin delivery systems can fulfill their promise for fungal keratitis.

Challenges and Future Perspectives

Future perspectives for rezafungin ocular delivery must extend beyond the cornea to address intraocular and intrastromal barriers that limit drug penetration to deeper structures such as the corneal endothelium, trabecular meshwork, and even the vitreous in cases of fungal endophthalmitis. The sclera, with its larger pore size, offers a less restrictive pathway than the cornea, but the blood-ocular barrier (both blood-aqueous and blood-retinal) remains formidable for systemically administered nanocarriers, necessitating strategies such as transscleral iontophoresis or suprachoroidal injection to achieve therapeutic concentrations in the posterior segment. Equally critical is addressing the ocular toxicity of nanocarrier components; while lipids and biodegradable polymers are generally safe, cationic surfactants (e.g., cetyltrimethylammonium bromide used in some mucoadhesive nanoparticles), high-generation PAMAM dendrimers, and residual organic solvents can induce corneal epithelial cytotoxicity, inflammation, or even endothelial damage. Thus, rigorous biocompatibility testing using in vitro models (human corneal epithelial and endothelial cell lines) and in vivo ocular irritation assays (Draize test, HET-CAM) must accompany any formulation development. A major unmet need is the development of standardized in vitro–in vivo correlation (IVIVC) models specific to ocular nanomedicine, as current ex vivo corneal permeation studies using static Franz cells poorly predict dynamic in vivo outcomes due to the absence of tear turnover, blinking, and efflux pump activity. Microfluidic cornea-on-a-chip platforms that incorporate flow, mechanical strain, and real-time drug detection are emerging as promising tools to establish predictive IVIVC and reduce animal use. Combination therapy represents another frontier: rezafungin co-delivered with other antifungals (e.g., voriconazole for synergistic *Fusarium* coverage) or with anti-inflammatory agents such as dexamethasone (to limit corneal scarring and neovascularization) could address the dual pathology of infection and inflammation. However, such combinations require careful optimization, as corticosteroids may reduce antifungal efficacy or exacerbate fungal replication. Finally, the path toward personalized ocular antifungal therapy leverages pharmacogenomic profiling of the patient's efflux transporter polymorphisms (e.g., P-gp or BCRP variants) and the fungal pathogen's MIC, enabling selection of the optimal nanocarrier type, dosing regimen, and potentially functionalized targeting ligands that home to fungal hyphae or inflamed corneal epithelia. Integrating these future directions—overcoming deeper barriers, ensuring nanocarrier safety, developing predictive IVIVC models, exploiting combination regimens, and personalizing therapy—will be essential to translate rezafungin nanomedicines into clinical practice and meaningfully reduce the burden of fungal keratitis worldwide.

CONCLUSION

The formidable anatomical, physiological, and biochemical barriers of the eye—the stratified corneal epithelium, rapid tear turnover, and active efflux pumps—have long rendered conventional topical antifungals inadequately effective for fungal keratitis. Rezafungin, with its potent echinocandin mechanism, prolonged target residence time, and favorable safety profile, offers an exceptional drug candidate, but its successful ocular application is contingent upon rational formulation design. The nanocarrier and sustained delivery systems reviewed herein collectively demonstrate that the "drug-plus-carrier" paradigm is not merely incremental but essential. Lipid-based and polymeric nanoparticles can enhance rezafungin's corneal permeation by 3- to 8-fold, while mucoadhesive and in situ gelling technologies extend precorneal retention from minutes to hours. The integration of these approaches—for example, NLCs dispersed in a thermoresponsive hydrogel—produces synergistic improvements in bioavailability, achieving therapeutic concentrations against *Fusarium* for over 20 hours after a single dose. Advanced platforms, including CPP conjugation, ROS-responsive release, and dissolving microneedles, push the boundaries further, enabling intracellular delivery, triggered release at the infection site, and complete bypass of the epithelial barrier. However, the translational gap remains wide: no rezafungin nanocarrier has entered clinical trials for FK, and regulatory hurdles—particularly sterilization of nanoparticles, batch-to-batch reproducibility, and the need for preservative-free unit-dose presentations—must be systematically addressed. Future research should prioritize development of standardized in vitro–in vivo correlation models (e.g., cornea-on-a-chip), rigorous

ocular toxicity screening of nanocarrier components (especially cationic surfactants and high-generation dendrimers), and clinical-grade manufacturing processes. Combination therapy (rezafungin plus voriconazole or dexamethasone) and personalized approaches based on pharmacogenomics of efflux transporters offer additional layers of optimization. In conclusion, rezafungin nanocarrier systems represent a scientifically robust and clinically urgent innovation. With continued interdisciplinary collaboration among formulation scientists, ophthalmologists, and regulatory bodies, these advanced delivery systems can be translated from bench to bedside, fundamentally altering the management of fungal keratitis and reducing the burden of blindness in vulnerable populations worldwide.

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