Fungal infection of the cornea (mycotic or fungal keratitis, keratomycosis) is a serious disease that can lead to loss of vision if not diagnosed and treated promptly and effectively. The pharmacological approach of management of fungal keratitis involves administration of antifungal agents. However, owing to the physiologic constraints of the eye, only a few drugs define sufficient bioavailability. The need for more potent antifungals with increased activity, shorter treatment durations and fewer adverse effects simultaneously stimulates the drive for the development of new antifungal agents with a broader spectrum and improved pharmacokinetic profile, and the development of advanced novel formulations for drug delivery that could increase drug bioavailability while reducing the adverse effects. In this article, the efforts and scientific potential of these two avenues are discussed. First, the classical and novel antifungal drugs are presented. Second, the classical formulations are compared with the advanced novel nanomedicines, and their potential clinical applications are discussed.

**Keywords**: antifungal • fungal keratitis • liposomes • microparticles • nanolipid carriers • nanoparticles • solid lipid nanoparticles

Fungal infection of the cornea (mycotic or fungal keratitis, keratomycosis) was described for the first time in 1879 in Germany, in a patient who had a corneal ulcer caused by *Aspergillus* spp. Until 1951, only 63 cases were reported in the literature [1], but nowadays fungal keratitis has spread worldwide, with a continuous increase in the number of cases. The distribution pattern varies widely with geographic location and season, factors that determine the prevalence of etiological agents. The overall incidence tends to be higher in tropical and subtropical regions, with *Fusarium* (20–83.6%), *Aspergillus* (16.5–75%) and *Candida* (1–63%) being the most frequent fungi causing keratitis worldwide [2]. While *Fusarium* and *Aspergillus* are the most common fungi isolated from patients in the tropics, *Candida albicans* is the most common pathogen of mycotic keratitis in temperate regions [1,3]. Other pathogens are isolated to a minor extent, and include *Penicillium* (incidence: 0.1–10%), *Curvularia* (incidence: 2.64–15.7%), *Alternaria* (incidence: 0.3–5%) and *Rhizopus* (incidence: 0.06–1%) [4–11]. A study conducted in north China reported that fungal keratitis represented approximately 62% of all cases of severe infective keratitis among the inpatients from January 1999 to December 2004 [12]. Such high incidence is also reported in other places such as India (44%) [13,14], Brazil [15], Australia [16], Thailand (38%) [17], south Florida (35%) [18], Nepal (17%) [19], Saudi Arabia [20] and Ghana (37.6%) [21]. However, in temperate climates, such as Britain and the northern USA, the incidence of fungal keratitis is comparatively low [9–11].

Mycotic keratitis is a serious disease that can lead to loss of vision if not diagnosed and treated promptly and effectively [1]. Regardless of the cause of keratitis, migration of inflammatory cells into the cornea can result in a disruption of the critical condition that maintains transparency, leading to corneal opacification or complete blindness [22]. However, the high morbidity of this condition is not only related to the migration of inflammatory cells, but also to the physical damage caused by the presence of fungal organisms, secondary damage from fungal toxins and enzymes [23], frequently delayed diagnosis, and poor response to available therapeutic options [24].

Fungi cannot penetrate the intact healthy corneal epithelium and do not enter the cornea from episcleral limbal vessels. Hence, trauma is related
to most fungal keratitis cases reported in developing countries [5,35,25], especially among agricultural or outdoor workers [26,27], exposed to corneal trauma with plant or soil matter [4,28], which could either introduce the fungus directly into a corneal epithelial defect or, alternatively, cause a defect to become infected following trauma. Morbidity in these cases can be aggravated by malnutrition [29] and lack of access to healthcare [30].

Another risk factor for fungal keratitis in industrialized countries is contact lens wear [31]. One hypothesis suggests that microscopic defects are introduced by lens wear that enhance microorganism adherence to the otherwise nonadherent corneal epithelium [32,33]. Candida is the principal cause of keratitis associated with therapeutic contact lenses, although cases by filamentous fungi have been reported [34,35]. Recently, there have been epidemic increases in Fusarium keratitis associated with particular contact lens solutions in several parts of the world [36–39].

Less frequently reported risk factors include prolonged use of topical corticosteroids [9,10] and antibiotics, systemic diseases such as diabetes mellitus [40], immunosuppressive diseases [11], prolonged chemo- or immunosuppressive therapy [41], previous eye surgery [42] and chronic eye surface diseases [11].

The diagnosis is difficult since the symptoms are usually non-specific: they include tearing, pain, photophobia, a decrease in vision and redness [43]. Another problem is that features of keratitis caused by yeasts may resemble bacterial keratitis, misleading the diagnosis. In addition, since many of the filamentous fungi grow slowly, the disease often remains unrecognized and untreated for days or weeks until growth is visually detected [32]. In advanced suppurative cases, ulcerative lesions or granular infiltrations in the corneal epithelium may be seen [29]. Pathologic specimens of filamentous fungal keratitis demonstrate hyphae following the tissue planes of the cornea, lying parallel to the corneal collagen lamellae [32]. Neovascularisation may occur as a result of inflammation, which may lead to severe scarring of the cornea. Associated signs indicating the severity of inflammation include the presence of hypopyon and ciliary injection. It is important to determine the etiologic agent of the corneal ulcer. Diagnosis is usually achieved by scraping material from the base of the ulcer and culturing the material on solid and liquid media [24].

**Pharmacological treatments**

The pharmacological approach to the management of fungal keratitis involves the administration of antifungal agents. However, owing to the physiologic constraints of the eye, only a few drugs present adequate bioavailability [44]. Although surgical options (e.g., therapeutic keratoplasty) have a high incidence of infection recurrence [45], in most cases surgery may be recommended [46]. In extremely severe cases, enucleation or evisceration is needed [47,48].

Until the 1940s, relatively few agents were available for the treatment of systemic fungal infections. Nystatin was the first polyeon antifungal to be identified in the late 1940s; however, its use has been discontinued owing to corneal toxicity and poor ocular penetration. By the late 1950s, the broader spectrum, more effective amphotericin B represented a major advance in the treatment of fungal infections [49,50]. However, its clinical use is associated with numerous adverse effects [51]. The search for newer systemic antifungals led to the discovery of the azoles in the 1960s, with the release of ketoconazole in the early 1980s followed by fluconazole and itraconazole in the early 1990s [52]. These agents were available in oral formulations and demonstrated a relatively improved safety profile compared with that of amphotericin B. Nevertheless, they still present a less than optimal pharmacokinetic profile and, in some cases, a narrow spectrum of activity.

The need for more potent antifungals with increased activity against resistant pathogens, shorter treatment durations and fewer adverse effects stimulates both the drive for the development of new antifungal agents with broader spectrum and better pharmacokinetic profile, and the development of advanced novel formulations for drug delivery that could increase drug bioavailability while reducing the adverse effects.

In this article, the efforts and potential of these two avenues are discussed. First, the classical and novel antifungal drugs are presented. The classical formulations are then compared with the advanced novel formulations proposed, and the potential of these are discussed.

**Classical antifungal drugs**

**Polyenes**

Polyenes exert their antifungal effect by binding to the ergosterol in the fungal cell membrane, blocking fungal growth or altering membrane permeability [53]. Amphotericin B can also induce oxidative damage, which may contribute to its fungicidal action [54]. The extent of damage to fungal membranes is dose related; however, beyond a certain concentration, human cells may be affected, which accounts for the polyenes’ toxic effects. Natamycin and amphotericin B are the two antifungal agents of this class in current use for the treatment of ophthalmic mycoses.

**Natamycin**

Natamycin, a tetraene polye, has long been considered the mainstay of treatment for filamentous fungal keratitis [55,56]. As natamycin is poorly soluble in water it is presented as a 5% topical ophthalmic suspension. The initial dosage is normally one drop every hour. Therapy is generally continued for 14–21 days or until there is resolution of active fungal keratitis [301]. It is reported to have a broad spectrum of activity against various fungi, including species of Fusarium, Aspergillus, Candida and Penicillium [5,57,58], although its main limitation is its poor penetration after topical application. This has been attributed to the tissue binding, since 97% of the drug that enters the cornea quickly becomes biologically inactivated [59]. Therapeutic concentrations can still be achieved in the aqueous humor with intense topical administration after removal of the corneal epithelium [59].

**Amphotericin B**

Amphotericin B is a macrolid polye with two special physicochemical properties: amphiphilic behavior owing to the apolar and polar sides of the lactone ring and amphoteric behavior owing
to the presence of ionizable carboxyl and amine groups (Table 1). As a consequence of its amphiphilic and zwitterionic nature and the asymmetrical distribution of hydrophobic and hydrophilic groups, amphotericin B is poorly soluble in all aqueous solvents and in many organic solvents [60].

The primary advantages of amphotericin B include its fungicidal activity against most clinically relevant pathogens [58] and the low occurrence of resistance [61]. It has been widely administered by intravenous, topical, intracameral and intravitreal routes for therapy of ocular infections [56,62–64]. The intravenous administration is the treatment of choice for invasive fungal infections, but this route may cause poor corneal bioavailability and severe nephrotoxicity [65]. Similarly to nata-mycin, the corneal penetration of amphotericin B is reduced in the presence of an intact corneal epithelium [59]. The topical regime often includes administration every 30 min for the first 24 h and every hour for the second 24 h, before being slowly tapered according to the clinical response [50]. Subconjunctival injection has been reported to lead to severe toxic effects, and is no longer recommended. Intracameral injections of amphotericin B may be an effective adjunctive treatment for fungal keratitis unresponsive to conventional antifungal therapy [66], although cataract may occur [67]. A case reported the use of intrastromal corneal injections combined with intravitreal injection of amphotericin B that led to the eradication of the corneal fungal plaques and the intraocular infection [68]. Intravitreal administration, although commonly used [69], has been reported to cause retinal necrosis and detachment if the injection is not made slowly and exactly in the center of the vitreous, as far as possible from the retina [2].

Azoles

The azoles, discovered in the late 1960s, are totally synthetic. They are inhibitors of a cytochrome P450 fungal enzyme involved in the conversion of lanosterol into ergosterol, an essential sterol in fungal cell membranes. The decrease in ergosterol synthesis leads to increased permeability of the fungal cell membrane, alteration of membrane enzymes, inhibition of growth and death of the fungal cell [70].

The azoles are classified as imidazoles or triazoles, on the basis of whether they have two or three nitrogens in the five-membered azole ring. The imidazoles include clotrimazole, isoconazole, econazole, miconazole and ketoconazole, the last two mostly being used in the treatment of ocular fungal infections. The triazoles include fluconazole and itraconazole.

Imidazoles

Miconazole is usually reserved as a second-line drug in the management of fungal keratitis. Very low miconazole levels are obtained in the cornea after intravenous injection, but following the subconjunctival injection higher levels can be noted in corneas with debridement of corneal epithelium. Similarly, after topical administration the penetration is almost ten times higher in debrided corneas [71]. Topical, subconjunctival and intravenous administrations of miconazole have been reported in the 1980s to result in successful outcomes [72–75]. Nonetheless, corneal toxicity has been reported, manifesting itself as a row of pinpoint vesicular elevations in the corneal epithelium associated with surrounding superficial punctate keratitis [74,76]. With the later discovery of other antifungal agents with better pharmacokinetic properties, its use has declined. Notwithstanding, miconazole 1% is one of the most common topical antifungal drugs employed in veterinary cases of fungal keratitis [77–79].

Ketoconazole has pharmacological properties similar to that of miconazole; however, it is absorbable from the gastrointestinal tract and less toxic [80]. It is currently available as oral preparation worldwide. The adult dose of ketoconazole is normally 200–400 mg/day, which can be increased to 800 mg/day. However, gynecomastia, oligospermia and decreased libido have been reported in 5–15% of patients who have been taking 400 mg/day for a long period [80]. Recent in vitro susceptibility studies have shown that the majority of the ocular fungal isolates, including Aspergillus, Candida and some Fusarium species, were sensitive to ketoconazole [81,82]. The oral preparation is often used concomitantly to other topical antifungal agents [83–85], although it can also be administered topically without significant corneal toxicity signs [86]. However, the drawback is its poor water solubility [87]. Case reports can be found in the literature of patients with laboratory-proven fungal corneal infections that were successfully treated with topical ketoconazole. The clinical signs of corneal infection normally disappear after 3–7 weeks of therapy [86].

Triazoles

Flucconazole is a bistriazole antifungal compound with improved physical and pharmacokinetic properties. Fluconazole is a stable, nontoxic, water-soluble, low-molecular-weight (306.2 Da) compound that can be administered by several routes, such as topical [88–92], subconjunctival [93], intravitreal [94] and systemic [95,96]. The subconjunctival regime consists of fluconazole 2% up to 1.0 ml twice daily for at least 5 days [93].

Abbasoglu et al. achieved a fluconazole aqueous humor peak concentration in humans upon single- and multiple-drop applications of a 0.2% solution of 3.5 ± 0.64 and 7.13 ± 0.79 µg/ml, respectively, after 15 min [97]. Antifungal susceptibility tests have reported that among the most common etiological agents in fungal keratitis, Fusarium is the most resistant genera to fluconazole, exhibiting an in vitro MIC of 32–64 µg/ml [98]. Some studies reported lower MIC values for Alternaria alternata (12 µg/ml), Aspergillus (8 µg/ml), Candida (0.2–0.8 µg/ml), Penicillium (4 µg/ml), Curvularia (6–64 µg/ml) and Rhizopus (4–32 µg/ml) [99–102]. Based on this data, topically applied fluconazole may only be effective for the treatment of less resistant fungi. It is possible that higher aqueous humor concentrations, which would cover the MIC for most pathological agents, could be obtained with multiple-dose administration.

Itraconazole, a dioxolane triazole, is very hydrophobic with a relatively higher molecular weight (705.6 Da). It is well absorbed orally, although more than 90% binds to protein in serum [103]. The major drawback of using itraconazole by the oral route for therapy of ocular
Table 1. Physicochemical properties, advantages and disadvantages of the classical antifungal compounds used for the treatment of ocular fungal infections.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Structure</th>
<th>pKa</th>
<th>MW</th>
<th>Log P</th>
<th>Advantage/disadvantage</th>
</tr>
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<tbody>
<tr>
<td>Polyenes</td>
<td></td>
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<tr>
<td>Natamycin</td>
<td><img src="image" alt="Natamycin structure" /></td>
<td>665.7</td>
<td>-3.7</td>
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<td>Broad spectrum of activity</td>
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<td></td>
<td></td>
<td></td>
<td>Poor intact corneal penetration after topical application</td>
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<td></td>
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<td>Low bioavailability</td>
</tr>
<tr>
<td>Amphotericin-B</td>
<td><img src="image" alt="Amphotericin-B structure" /></td>
<td>5.7</td>
<td>924.1</td>
<td>-2.8</td>
<td>Fungicidal activity against most clinically relevant pathogens</td>
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<td></td>
<td></td>
<td>10.0</td>
<td></td>
<td></td>
<td>Nephrotoxicity</td>
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<td></td>
<td>Retinal necrosis and detachment following intravitreal administration</td>
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<tr>
<td>Azoles</td>
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</tr>
<tr>
<td>Miconazole</td>
<td><img src="image" alt="Miconazole structure" /></td>
<td>6.5</td>
<td>16.1</td>
<td>6.2</td>
<td>Subconjunctival administration generally well tolerated</td>
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<td></td>
<td>Corneal toxicity</td>
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<td>Poor intact corneal penetration</td>
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Compiled from [302,303].
Table 1. Physicochemical properties, advantages and disadvantages of the classical antifungal compounds used for the treatment of ocular fungal infections.

<table>
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<tr>
<td><strong>Azoles (cont.)</strong></td>
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<tr>
<td>Ketoconazole</td>
<td><img src="image" alt="" /></td>
<td>2.9</td>
<td>531.4</td>
<td>4.3</td>
<td>The oral preparation is often used concomitantly to other topical antifungal agents with good outcome. Poor water solubility. Requires acid pH for absorption</td>
</tr>
<tr>
<td>Fluconazole</td>
<td><img src="image" alt="" /></td>
<td>2.0</td>
<td>306.2</td>
<td>0.5</td>
<td>Excellent safety profile. Stable, water-soluble. Good intraocular penetration. Poor <em>in vitro</em> activity vs most strains of <em>Aspergillus</em> and <em>Fusarium</em> spp.</td>
</tr>
<tr>
<td>Itraconazole</td>
<td><img src="image" alt="" /></td>
<td>3.7</td>
<td>705.6</td>
<td>5.6</td>
<td>Excellent safety profile. Hydrophobic, high molecular weight: poor bioavailability. Narrow spectrum coverage.</td>
</tr>
<tr>
<td><strong>New azoles</strong></td>
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<tr>
<td>Voriconazole</td>
<td><img src="image" alt="" /></td>
<td>4.9</td>
<td>349.3</td>
<td>1.8</td>
<td>Broader spectrum of activity. Greater efficacy. Reversible disturbance of vision and skin rashes as common side effects. Rapid vitreal clearance.</td>
</tr>
</tbody>
</table>

Compiled from [302,303].
Table 1. Physicochemical properties, advantages and disadvantages of the classical antifungal compounds used for the treatment of ocular fungal infections.

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<tr>
<td>Echinocandins</td>
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<tr>
<td>Caspofungin</td>
<td></td>
<td>9.1</td>
<td>1093.3</td>
<td>-2.8</td>
<td>Fungicidal activity against fluconazole-resistant fungi strains</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>High-molecular-weight rends poor intact corneal topical penetration</td>
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<tr>
<td>Micafungin</td>
<td></td>
<td>9.1</td>
<td>1270.3</td>
<td>-0.4</td>
<td>Water soluble</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Excellent <em>in vitro</em> activity against both <em>Candida</em> and <em>Aspergillus</em></td>
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<td>High-molecular-weight rends poor intact corneal topical penetration</td>
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</table>

Compiled from [302, 303].
fungal infections is its poor penetration into the cornea, aqueous humor and vitreous compared with fluconazole and ketoconazole. However, oral itraconazole was found to be effective in a case of fungal keratitis of the eye caused by *Pichia anomala* when used in combination with topical amphotericin B and natamycin [64], and in a case of fungal keratitis caused by *Scedosporium apiospermum* [104].

Topical itraconazole also proved to be useful for treating infections caused by *Aspergillus* or *Curvularia* spp. [105,106]. Topical itraconazole has also been reported as effective in treating animal models of *Fusarium* keratitis [107], even though its spectrum coverage is narrow against these species [57,82].

Allylamines

Allylamines prevent fungal ergosterol biosynthesis via specific and selective inhibition of fungal squalene epoxidase, thereby interfering with the integrity of fungal cell membrane [108]. Allylamines are less frequently used in the treatment of ocular fungal infections compared with polyenes and azoles. Antifungal agents belonging to this class include amorolfin, butenafine, naftifine and terbinafine, with terbinafine being the most commonly used compound [109].

**Novel antifungal drugs**

Novel antifungal drugs were developed with the aim of solving classical antifungal therapy problems such as severe toxicity (polyenes), narrow antifungal spectrum (especially against filamentous fungi), rapid development of resistance (most azoles), and fungistatic rather than fungicidal effects at the achieved ocular concentrations. Some of these problems are not yet completely solved, but the advances made are presented.

Newer azoles

The newest triazole agents, including ravuconazole, isavuconazole, posaconazole and voriconazole, are synthetic derivatives of fluconazole but have a significantly broader spectrum of activity [57]. Voriconazole is the better studied compound, and up until now there are no records of the clinical efficacy of the other new azole agents against fungal keratitis, with the exception of a few reports describing the use of posaconazole [110–112].

**Voriconazole**

Voriconazole is a new antifungal drug derived from fluconazole by the addition of a methyl group to the propyl backbone and by the substitution of a triazole moiety with a fluoropyrimidine group [113]. The molecular alterations conferred to voriconazole a broader spectrum of activity and greater efficacy than its parent compound, fluconazole. However, voriconazole presents more side effects and drug interactions. The most common side effect is a reversible disturbance of vision (photopsia), which may include blurred vision, altered color discrimination and photophobia. These symptoms are related to changes in electroretinogram tracings, which revert to normal when treatment with the drug is stopped; no permanent damage to the retina has been noted. Skin rashes are the second most common adverse effect and elevations in hepatic enzyme levels may also occur [114].

The mechanism of action is the same as the otherazole agents, but voriconazole also inhibits the 24-methylene dihydrostanasterol demethylase in certain yeast and filamentous fungi [113,114]. The greater efficacy can be confirmed by in vitro susceptibility tests. In general, the MIC of voriconazole for *C. albicans* is 1–2 log lower than the MIC of fluconazole [115]. It also appears to be very effective in the management of ocular infections caused by many filamentous fungi [40], especially in the management of *Aspergillus* ocular infections, as compared with other antifungals [82]. Numerous case reports indicate that voriconazole treatment has been successful where natamycin, amphotericin B or fluconazole have failed, even in cases of drug-resistant fungal keratitis and endophthalmitis [116–122].

Voriconazole is well absorbed following oral administration, with a bioavailability of 90%. A study by Hariprasad et al. demonstrated that orally administered voriconazole achieves therapeutic aqueous and vitreous levels in the noninflamed human eye [123]. After two doses, the mean plasma concentration of voriconazole was 2.13 µg/ml, which resulted in voriconazole concentrations of 0.81 µg/ml in the vitreous and 1.13 µg/ml in the aqueous. The activity spectrum appeared to appropriately encompass the most frequently encountered mycotic species involved in the various causes of fungal endophthalmitis. A similar result was described in a case report of an eye with *Scedosporium apiospermum* keratitis that went on to corneal transplant. It was found that aqueous voriconazole levels following 12 days of oral treatment in the aqueous humor was 1.8 µg/ml, almost seven times higher than the MIC for that specific strain [124].

Topical therapy may also be used in conjunction with oral therapy to increase the amount of drug in the anterior chamber [125]. The topical administration of voriconazole 1% solution every 2 h for 1 day in noninflamed human eyes prior to planned vitrectomy surgery resulted in a mean concentration of 0.6 µg/ml of the drug in the aqueous and 0.15 µg/ml in the vitreous, demonstrating that the drug penetrates well beyond the cornea when applied topically [126]. These results are in accordance with other studies that applied voriconazole topical solution. Recently, a prospective open-label trial involving ten participants that received topically administered 1% voriconazole solution hourly for four doses or four times a day for 3 days, obtained voriconazole concentrations ranging from 0.1 to 1.1 µg/ml in the vitreous humor [127]. In a similar study, 13 human subjects scheduled for elective anterior segment eye surgery received hourly 2% voriconazole eye drops at 4 h presurgery. Significantly, the voriconazole concentration in the aqueous humor of the eye was similar to that reported for the 1% voriconazole solution, suggestive of concentration-independent absorption through an intact infection-free cornea [128]. This is consistent with observations in a recent animal study, where the voriconazole level in the corneas of horses with fungal keratitis did not change when the administered voriconazole eye drop concentration was changed from 1 to 3% [129]. In addition, in the study conducted by Lau et al. it was also observed that no accumulation of voriconazole in the vitreous humour could be detected with a four-times-a-day dosing regimen, suggesting that voriconazole is cleared very rapidly from the posterior...
chamber [127]. This hypothesis is also consistent with results by Shen et al., where the concentration of intravitreal voriconazole at various time points was reported to exhibit exponential decay with a half-life of 2.5 h after single intravitreal injections in a rabbit model [130]. This suggests that in severe cases of fungal keratitis, where pathogens have already spread into the eye or there is a risk of fungal endophthalmitis, considerably higher concentrations of voriconazole or a slow-release formulation would be necessary to sustain therapeutic drug levels in the posterior chamber.

Echinocandins
Echinocandins are lipopeptides that have been synthetically modified from the fermentation broths of various fungi, and have recently emerged as valuable antifungal agents.

They possess a unique mechanism of action, inhibiting β-(1,3)-D-glucan synthase, an enzyme that is necessary for the synthesis of essential components of the cell wall of several fungi. The depletion of these components results in an abnormally weak cell wall unable to withstand osmotic stress [131]. The echinocandins display fungistatic activity against Aspergillus spp. and fungicidal activity against most Candida spp., including strains that are fluconazole resistant. Overall, resistance to echinocandins is still rare and all agents are well tolerated, with similar adverse effect profiles and few drug–drug interactions [132].

Three echinocandins have been approved by the US FDA, namely caspofungin, micafungin and anidulafungin, but up until now there is no record of anidulafungin applied for the treatment of keratitis.

Caspofungin
Caspofungin was the first approved member of the class; it has the most available data and the most indications of the echinocandins [122,133].

Several studies have recently compared the efficacy of topical caspofungin with that of topical amphotericin B. When using an animal model of C. albicans keratitis the authors observed comparable results for 0.5% caspofungin and 0.15% amphotericin B [134]. Similar results were also found for 1% caspofungin and 0.15% amphotericin B topical solutions in an animal model of Fusarium solani keratitis [135].

Since caspofungin has a high molecular weight of 1093.5 Da, the topical administration without corneal epithelium abrasion resulted in no detectable amounts of the drug in the aqueous humor. However, after corneal epithelial abrasion, therapeutic drug levels that cover the MIC of most fungi could be reached [136].

Micafungin
Micafungin is a water-soluble echinocandin with excellent in vitro activity against Candida, Aspergillus and some fungi resistant to other antifungal agents [137,138].

Hiraoka et al. evaluated the efficacy of subconjunctival injection of 0.1% micafungin in the treatment of experimental C. albicans keratitis and observed complete healing of the corneal lesions in six out of eight eyes treated [139]. The remaining two eyes where the drug was not effective presented deeper corneal lesions. Although corneal penetration of micafungin has not been studied yet, the penetration into the deep corneal stroma through an intact epithelial layer seems limited because of its high molecular weight (1292.26 Da). There has been one case report of the clinical application of topical micafungin eyedrops in the treatment of refractory yeast-related corneal ulcers with a satisfactory outcome [140]. Moreover, topical instillation of micafungin solution had no apparent toxicity to the cornea [141].

Classical formulations
The eye is characterized by physiological barriers that limit drug entrance from the blood circulation to its inner structures. These are the blood–aqueous and the blood–retinal barriers [142]. As a consequence, systemic or oral drug therapy requires large drug dosages to reach the site of action in proper amounts, which may cause significant systemic side effects [143]. Intravitreal, periocular and subconjunctival injections could minimize systemic exposure of the drug, but the use of these systems is followed by a series of disadvantages. The intravitreally injected drug is rapidly eliminated by the eye’s natural circulatory process and therefore frequent injections may be required. Likewise, large doses are often needed, giving rise to toxicological problems. Besides, there are also relevant side effects, such as pain, discomfort, increased intraocular pressure, intraocular bleeding, increased chances for infection and the risk of retinal detachment. The major complication for intravitreal injection is endophthalmitis, which can result in severe vision loss [144–146]. In addition, ocular injections are not well accepted by patients. The topical administration is the most convenient route for the management of ocular fungal infections, especially for infection affecting the cornea and anterior chamber structures. Therefore, although sometimes not the most efficient, the topical route is the first choice for starting the administration of drugs on the treatment of ocular fungal infections. The classical formulations applied include topical solutions or suspensions in the form of eye drops or ointments in the form of night creams. More recently, lipid complexes of amphotericin B have also been applied.

Topical eye drops
In several cases, intensive topical antifungal therapy involves the use of multiple antifungal eye drops in very short administration intervals (e.g., half an hour) [147]. Protection mechanisms of the human eye such as lachrymal secretion and blinking reflex cause rapid drainage of the topically applied eye drops [148]. The short preconoreal residence time allied with cornea impermeability results in low bioavailability, and frequent dosing is usually needed to compensate for the rapid preconoreal drug loss.

Water-soluble drugs can be administered in the form of solutions and relatively insoluble drug substances in an aqueous vehicle as a form of suspensions. In this case, the vehicle must contain suitable suspending and dispersing agents to allow good drug dispersibility, maintaining the uniformity of drug dosage. Controlled flocculation of suspensions can be accomplished by adding electrolytes, ionic or nonionic surfactants, or even water-soluble polymers [149]. Owing to the particles’ tendency to be retained in the cul-de-sac, the contact time and duration of action of a suspension exceed
those of a solution [150]. The retention may increase with particle size; however, it is recommended that particles should not exceed 10 µm so that they do not cause discomfort.

Several antifungal drugs have been tested in the form of topical eye drops. These drugs may include natamycin [83], amphotericin B [62,107,154], miconazole [74,152], ketoconazole [86], fluconazole [88–92], itraconazole [107], voriconazole [107,125], caspofungin [134,135] and micafungin [140].

The contact time with the target ocular tissue may depend on the physicochemical properties of the drug and the body’s clearance mechanisms, but may also be highly influenced by the vehicle chosen for drug delivery. Even for the newer antifungal compounds, it has been observed that corneal penetration is insufficient. A recent study concluded that to achieve a sustained high level of caspofungin as an effective antifungal therapy for corneal keratitis, the drug should be administered topically every 30 min after removal of the corneal epithelium [136]. However, developing a sustained-release ocular preparation would overcome the requirement for a frequent dosing.

**Formulations with enhanced solubility**

The most important drawback to the formulation of most common antifungal agents is their scarce solubility in water. Such are the cases of amphotericin B (solubility: 0.001 mg/ml; pKa: 5.7) [60], miconazole (solubility: ≤0.00103 mg/ml; pKa: 6.5), ketoconazole (solubility: 0.007 mg/ml; pKa: 6.5) and itraconazole (solubility: 1.8 mg/ml; pKa: 3.7) [153]. Several attempts have been made to obtain drug formulations suitable for intravenous and topical ophthalmic administration with adequate drug concentrations.

Cyclodextrins have been used to increase ketoconazole aqueous solubility [154]. When hydroxypropyl β-cyclodextrin was used, it produced more than a twofold bioavailability increase after topical instillation in rabbit corneas when compared with the classical ketoconazole suspension [87]. The solubilities of voriconazole, ketoconazole and clotrimazole were also significantly improved with this cyclodextrin in aqueous media [155].

The solubilizer effect of acetate, phosphate and gluconate solutions, along with ethanol, glycerol, macrogol 400, propylene glycol, and surfactants such as polysorbate 20, 60, 80 and sodium taurocholate, were studied in binary or ternary combinations.

Ternary combinations were capable of solubilizing more than 30 mg/ml miconazole and more than 135 mg/ml of ketoconazole [155]. Nevertheless, for the ocular administration of these solutions further tolerability studies must be performed.

Another example is the colloidal dispersion of amphotericin B with sodium deoxycholate (Fungizone®; Bristol- Myers Squibb Co., NJ, USA), which became available in 1958 for the treatment of fungal infections [60]. However, the topical application of such a formulation is known to induce corneal lesions [26,156].

More recent studies have focused on the development of more biocompatible micelles. Micelles composed of a block copolymer poly(2-ethyl-2-oxazoline)-block-poly(aspartic acid) containing amphotericin B (Fungizone) were able to increase drug solubility and efficiency with lower cytotoxicity [157].

**Ointments**

Enhanced ocular retention of oily vehicles has been reported for more than 30 years [158], being attributed to their interaction with the superficial oily layer of the tear film. As a consequence, initial attempts to overcome the poor bioavailability of topicaly instilled drugs typically involved the use of ointments.

Ointments ensured superior drug bioavailability by increasing contact time with the eye, minimizing dilution by tears and resisting nasolachrymal drainage. However, these vehicles have the major drawback of being uncomfortable and causing blurred vision. Consequently, they are mainly used for either administration overnight or for treatment on the outside and edges of eyelids [159]. A series of antifungal drugs have already been formulated in ointments, such as natamycin [152], amphotericin B [160], miconazole [161] and itraconazole [83,84], although in most cases a combined therapy is used.

**Lipid complexes**

To increase the therapeutic index of amphotericin B, lipid complexes were developed. In the commercial drugs Abelcet® (The Liposome Company, NJ, USA) and Amphocil® (Sequoia Pharmaceuticals, Inc., CA, USA), amphotericin B has been formulated with two phospholipids in a 1:1 drug to lipid molar ratio. Amphotec® (Sequoia Pharmaceuticals, Inc., CA, USA) is an amphotericin B formulation with cholesterol sulfate in equimolar concentrations. Amphotec particles resemble discs and have a similar antifungal efficacy to Fungizone but with lower cytotoxic and hemolytic effects. The reduction of renal toxicity has been attributed to the strong affinity of amphotericin B to the cholesterol moieties, which reduces the amount of free amphotericin B in the circulation [60]. A case of Fusarium solani keratitis that progressed to fungal endophthalmitis was successfully treated systemically with the amphotericin B lipid complex Abelcet [162].

**Advanced novel formulations**

The clinical efficacy of an antifungal agent in ophthalmic mycoses depends, to a great extent, on the concentration achieved in the target ocular tissue [163]. Unfortunately, in several cases, topical treatment with classical formulations is not effective enough.

The ability of a drug to penetrate the eye is primarily dependent on its physicochemical properties, such as molecular weight, pKa (which determines the nonionized/ionized proportion of the molecule at a certain pH) and log P, which provides information about its lipophilicity.

With respect to drug delivery, the cornea can be divided into three layers, namely the outer epithelium (lipophilic in nature), the stroma (hydrophilic in nature) and the inner endothelium (also lipophilic) [164,165]. In the human eye, the epithelium contains five to seven layers of cells, each connected by tight junctions, which provide a large barrier that is permeable only to small lipophilic molecules. Because the cornea has hydrophilic as well as lipophilic tissues, it provides an effective bifunctional barrier for the absorption of both lipophilic and hydrophilic compounds. In this way, the overall absorption of moderately lipophilic compounds across the cornea is favored (log P 2–3) [166]. Regardless of the...
administration route, most of the antifungal drugs available do not possess the required physicochemical properties to be absorbed and reach or enter target tissues (Tables 1 & 2).

A promising strategy to overcome these problems involves the development of suitable drug-carrier systems. The in vivo fate of the drug is no longer dependent on the properties of the drug but on the carrier, which should maximize precorneal drug absorption, minimize precorneal drug loss and allow a controlled and localized release of the active drug, while maintaining the simplicity and convenience of the dosage form.

Since only a limited percentage of the administered drug reaches the target tissue, patient compliance is an important aspect to consider when developing an ophthalmic delivery system. As such, attention should be paid to the facility of administration and to the sensorial feeling after the administration, since discomfort (e.g., burning sensation) could induce tear production, followed by drug dilution and drainage through nasolachrymal duct.

Other important aspects to be considered are the retention time, drug-loading capacity and drug protection from metabolic degradation. In fact, if the drug-carrier system is able to prolong the retention while loading a sufficient amount of drug in a protected manner, the interval between administrations can be lengthened. For instance, in the case of intravitreal injections, the reduction in the number of injections would also reduce the potential side effects. Apart from these, all the factors that would influence the overall costs should also be considered, such as the possibility of scaling up production, sterilizing, and the physical and chemical storage stability of the product.

Novel colloidal delivery systems such as polymeric nano- and microparticles, liposomes, solid lipid nanoparticles, and nanostructured lipid carriers, are currently being studied in attempt to fulfill all these requirements.

Polymeric micro- & nanoparticles

A controlled-release strategy is to encapsulate the drug in polymeric microparticles (1–1000 μm) or nanoparticles (1–999 nm). These systems consist of various biocompatible polymeric matrices in which the drug can be adsorbed, entrapped or covalently attached [167]. Biodegradable and biocompatible synthetic polymers such as poly(ε-caprolactone-glycolide) (PLGA) and polyalkylycyanoacrylates are preferred for nanoparticle production. Nonetheless, use of polysaccharides (e.g., curdlan) and macromolecules (e.g., chitosan, albumin and gelatin) has been very well described in the literature [167–169].

Nanoparticulate technologies in general offer interesting benefits such as solubilization of hydrophobic drugs, bioavailability improvement, modification of pharmacokinetic parameters, and protection of drug molecules from physical, chemical and/or biological degradation [168]. Increased residence time of drugs and maintenance of their therapeutic concentrations for longer time intervals could reduce the number of subconjunctival and intravitreal injections required in some treatments, while allowing higher doses without toxicity from initial concentration. A drawback is that intravitreal injections of particulate systems may cause vitreal clouding [170]. However, microparticles tend to sink to the lower part of the vitreal cavity, whereas nanoparticles are more likely to cause clouding in the vitreous [169]. It is also suggested that nanoparticles increase the residence time owing to their bioadhesive nature, a property that would be especially useful for topical delivery. Different polymers can be used to coat nanoparticles and improve adhesion. Studies have shown, for example, that the bioavailability of encapsulated indomethacin doubled when poly(ε-caprolactone) nanoparticles were coated with chitosan [171]. In addition, nanoparticles formed of PLGA and poly(ethylene glycol) (PEG) as a core material and mucoadhesion promoter, respectively, showed prolonged residence time in rabbit eyes [172]. In this way, the ideal size and composition of a polymeric colloidal system would depend on the target. For instance, microparticles can be more effective than nanoparticles for intravitreal administration, but if they are larger than 10 μm they could cause an uncomfortable ‘sand-like’ feeling after topical administration [172,173]. In addition, depending on the drug, higher encapsulation efficiency can be obtained in microparticles than nanoparticles.

The encapsulation of antifungal agents in nanoparticulate carriers has been used with the objective of modifying the pharmacokinetics of drugs, resulting in more efficient treatments with fewer side effects. Although there are no records to date of applying these systems for the treatment of ophthalmic fungal infections, they have been studied for the treatment of similar infections in other organs with promising results.

Several recently published works describe the production of nanoparticles containing amphotericin B aiming to control drug delivery and reduce toxicity [174–177]. For example, amphotericin B

<table>
<thead>
<tr>
<th>Table 2. General in vitro MIC₅₀ values for the different antifungals against the most common pathogens in fungal keratitis.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antifungals</td>
</tr>
<tr>
<td>Natamycin</td>
</tr>
<tr>
<td>Amphotericin B</td>
</tr>
<tr>
<td>Miconazole</td>
</tr>
<tr>
<td>Ketoconazole</td>
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<tr>
<td>Fluconazole</td>
</tr>
<tr>
<td>Itraconazole</td>
</tr>
<tr>
<td>Voriconazole</td>
</tr>
<tr>
<td>Caspofungin</td>
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<tr>
<td>Micafungin</td>
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</tbody>
</table>

It is important to note that the results of in vitro antifungal susceptibility tests may not necessarily predict clinical outcome in keratitis. Host factors, stage of infection, patient compliance to the therapeutic regime and drug levels are all important factors that may influence the clinical response.

Fungi used were not isolated from cases of keratitis.
Table 3. Advantages and disadvantages of advanced novel delivery systems and incorporated antifungal drugs.

<table>
<thead>
<tr>
<th>Advanced novel formulations</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Incorporated antifungal agents</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymeric nanoparticles</td>
<td>May be biocompatible and biodegradable</td>
<td>Burst effect</td>
<td>Amphotericin B</td>
<td>[174–180]</td>
</tr>
<tr>
<td></td>
<td>Able to entrap both hydrophilic and hydrophobic drugs</td>
<td>Limited drug loading</td>
<td>Voriconazole</td>
<td>[181]</td>
</tr>
<tr>
<td></td>
<td>Controlled release</td>
<td>May cause vitreous clouding</td>
<td>Fluconazole</td>
<td>[182]</td>
</tr>
<tr>
<td></td>
<td>Protect drug from metabolic degradation</td>
<td>High cost</td>
<td>Itraconazole</td>
<td>[184]</td>
</tr>
<tr>
<td></td>
<td>Prolonged residence time – bioadhesive properties</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymeric microparticles</td>
<td>Can be prepared by spray drying – large-scale production</td>
<td>Burst effect</td>
<td>Fluconazole</td>
<td>[183]</td>
</tr>
<tr>
<td></td>
<td>May be biocompatible and biodegradable</td>
<td>May cause uncomfortable sensation if ≥10 μm</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Able to entrap both hydrophilic and hydrophobic drugs</td>
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<td></td>
<td>Controlled release</td>
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<td>Protect drug from metabolic degradation</td>
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<tr>
<td></td>
<td>Prolonged residence time – bioadhesive properties</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liposomes</td>
<td>Biocompatible and biodegradable</td>
<td>Poor stability</td>
<td>Amphotericin B</td>
<td>[194–196]</td>
</tr>
<tr>
<td></td>
<td>Able to entrap both hydrophilic and hydrophobic drugs</td>
<td>Difficult to prepare and sterilize</td>
<td>Fluconazole</td>
<td>[191–193]</td>
</tr>
<tr>
<td></td>
<td>Controlled release</td>
<td>High cost</td>
<td></td>
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<tr>
<td></td>
<td>Protect drug from metabolic degradation</td>
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<tr>
<td></td>
<td>Prolonged residence time – precorneal and in vitreous</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SLNs</td>
<td>Easy preparation – large-scale production</td>
<td>Limited drug loading</td>
<td>Clotrimazole</td>
<td>[209–211]</td>
</tr>
<tr>
<td></td>
<td>Easy sterilization</td>
<td>Ketocoazole</td>
<td>[212]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Improved ocular bioavailability</td>
<td>Itraconazole</td>
<td>[214]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prolonged precorneal residence time</td>
<td>Miconazole</td>
<td>[215]</td>
<td></td>
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<tr>
<td></td>
<td>Controlled release</td>
<td>Econazole</td>
<td>[216]</td>
<td></td>
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<tr>
<td>NLCs</td>
<td>Easy preparation – large-scale production</td>
<td>Hydrophilic drugs can show burst effects</td>
<td>Clotrimazole</td>
<td>[209,210]</td>
</tr>
<tr>
<td></td>
<td>Easy sterilization</td>
<td>Ketoconazole</td>
<td>[212]</td>
<td></td>
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<td></td>
<td>Drug loading of lipophilic and possibly hydrophilic drugs</td>
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<td></td>
<td>Improved ocular bioavailability</td>
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<tr>
<td></td>
<td>Prolonged precorneal residence time</td>
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<td></td>
<td>Controlled release</td>
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</table>

NLC: Nanostructured lipid carrier; SLN: Solid lipid nanoparticle.

entrapped into PLGA nanoparticles was shown to improve the oral bioavailability and minimize the adverse effects observed in classical systemic amphotericin B therapy [178]. Nonetheless, nanoparticles have also been used for targeting drug delivery. Amphotericin B-loaded PLA-β-PEG nanoparticles coated with polysorbate 80 have been efficiently produced for brain targeting [179]. Since these systems have been shown to efficiently cross the blood–brain barrier, they represent a promising tool for crossing the retinal–blood barrier and increasing intraocular bioavailability after systemic administration. Further studies should be carried out in this area. In addition, intraperitoneal administration of amphotericin B nanoparticles based on PLGA and dimercaptosuccinic acid in mice showed antifungal efficacy, fewer undesirable effects and a favorable extended dosing interval [180].

Poly(D,L-lactide-co-glycolide) nanoparticles loaded with voriconazole were prepared by the emulsion–solvent evaporation technique. The mean particle size was 132.8 nm when using sodium hexametaphosphate to avoid particle agglomeration. Both in vitro and in vivo studies in mice showed greater antifungal efficacy of drug-loaded nanoparticles by contrast with the drug alone [181]. Nano- or microparticle production has been described for other antifungal agents, such as fluconazole [182,183] and itraconazole [184].

**Liposomes**

Liposomes are biocompatible and biodegradable phospholipid vesicles formed by one or several lipid bilayers. In each bilayer, the nonpolar fatty acid tails are placed in the interior whereas the polar heads are turned outside, containing an aqueous phase both inside and between the bilayers. Owing to their amphiphilic character, liposomes are able to entrap both hydrophilic and hydrophobic compounds in the aqueous compartments or within the lipid bilayers, respectively [185,186]. Liposomes can provide controlled release of incorporated drugs as the spherical lipid shield formed by bilayer membranes provides a permeability barrier to drug release. In this way, the drug is protected from degradation and clearance, and toxicity resultant from high peak concentration is avoided. This property can be especially useful for posterior segment applications [187]. Similarly to polymeric nano- and microparticles, liposomes can minimize some of the adverse side effects encountered by the intraocular administration routes, increasing therapeutic effectiveness [188–190].

Gupta et al., studying the pharmacokinetics of plain and liposome-encapsulated fluconazole after intravitreal injection in rabbit eyes, observed a rapid vitreal clearance and a short half-life (3.08 h) for plain fluconazole, whereas liposome-entrapped fluconazole showed an extended half-life (23.40 h) [191]. The constant
terminal elimination of the liposome-loaded drug from the vitreous was seven times less than the plain drug [191]. However, the same authors later discouraged the use of fluconazole as a sole therapy for endophthalmitis. They reported inferior outcomes for liposome-entrapped fluconazole in a candidal endophthalmitis rabbit model, probably owing to heterogenous distribution throughout the vitreous cavity and initial low drug concentration [192].

Liposomal formulations containing fluconazole for ophthalmic controlled release were also prepared using the reverse-phase evaporation technique [193]. Soybean phosphatidylycholine and cholesterol in specific weight ratios were used, and selected formulations tested for their in vivo ocular antifungal effect. Conversely, the authors of this work reported that, after in vivo administration in a model of Candida keratitis, fluconazole liposomal formulations achieved complete healing in a shorter time than plain fluconazole solution. In addition, the frequency of instillation could be reduced [193].

A reduction in ocular toxicity of subconjunctival injection of liposomal amphotericin B has also been reported. Comparisons were made with conventional amphotericin B deoxycholate formulation in a rabbit model. The study reported that subconjunctival injection of amphotericin B deoxycholate formulation or deoxycholate alone induced severe corneal and conjunctival edema with necrosis and infiltration of inflammatory cells; whereas the liposomal formulation induced only mild inflammation near the injection site. The authors also observed satisfactory concentrations in corneal stroma after the liposomal formulation injections [194]. In fact, a liposomal formulation named AmBisome® (Vestar, Inc., CA, USA) containing amphotericin B is commercially available. The formulation is supplied lyophilized as a powder and must be reconstituted in water directly before use, producing liposomes with a mean diameter of 60–70 nm [60]. Because of its hydrophobicity, amphotericin B binds predominantly to the lipid bilayer rather than being placed in the small hydrophilic core of the liposome. The liposomal material consists of hydrogenated soy phosphatidylcholine and distearoylphosphatidylglycerol. Moreover, the negative charge of the distearoylphosphatidylglycerol can interact with the positive amino group of the amphotericin B, forming an ionic complex in the bilayers [60]. In addition, a broad antifungal activity spectrum has been defined by the liposomal formulation [195]. In a recent study, the corneal availability following systemic administration of parenteral amphotericin B lipid complex or liposomal amphotericin B was compared with that of amphotericin B deoxycholate in a rabbit model [196]. The authors reported that no drug could be detected in the corneas of the non-inflamed eyes, but in a uveitis-induced model the penetration into the cornea was significantly higher after systemic administration of liposomes, followed by lipid complexes and conventional amphotericin B deoxycholate [196].

It needs to be considered, however, that the type of vesicles formed and the formulations constituents may interfere with the final toxicity and antifungal activity of the drug. It has been observed that small unilamellar vesicles [197–199], multilamellar vesicles [200–202] or large multilamellar vesicles [201] containing amphotericin B perform differently [198]. Similarly, fluconazole showed different MIC values in different vesicle types [198,203]. Inhibition of the antifungal activity of miconazole and ketoconazole by phospholipids has also been reported. Such an effect seems to be dependent on the phospholipid concentration [198]. Moreover, sterols present in the formulation may interfere with the fungicidal activity of liposomal amphotericin B. It has been observed that ergosterol- and cholesterol-containing liposomes were less effective against C. albicans compared with the sterol-free liposomes [204].

Significant progress has been made in demonstrating the advantages of liposome-mediated drug delivery in ophthalmology. In some cases, liposomes have shown to improve efficacy, reduce toxicity, prolong activity and provide site-specific delivery. Despite

<table>
<thead>
<tr>
<th>Table 4. Aspects to be considered on choosing an ophthalmic delivery system and the performance of advanced novel delivery systems.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aspects to consider</strong></td>
</tr>
<tr>
<td>Facility of administration</td>
</tr>
<tr>
<td>Sensorial feeling after administration† (blurred vision, burning sensation, lacrimation)</td>
</tr>
<tr>
<td>Drug loading capacity</td>
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<tr>
<td>Possibility of drug targeting</td>
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<tr>
<td>Precoanal retention time</td>
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<tr>
<td>In vitro residence time</td>
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<tr>
<td>Controlled drug release</td>
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<tr>
<td>Avoidance of burst effect</td>
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<tr>
<td>Avoidance of toxicity</td>
</tr>
<tr>
<td>Scaling up of production</td>
</tr>
<tr>
<td>Easy to sterilize</td>
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<tr>
<td>Storage stability</td>
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</tbody>
</table>

It is important to consider the form in which formulations are dispensed. The overall storage stability tends to be significantly higher if the formulations are dispensed in lyophilized form.

†The scale indicates the absence of such events, + + + being indicative of the lowest probability of the formulation to cause undesirable sensorial feeling after administration.

‡Not reported.

- Poor; +: Good; + +: Very good; + + +: Excellent; NLC: Nanostructured lipid carrier; SLN: Solid lipid nanoparticle.
these reasons, which make liposomes a potentially useful system for ocular delivery, until now there have been very few attempts to apply them for the treatment of ophthalmic fungal infections. Problems usually encountered were the short shelf life, limited drug-loading capacity, use of aggressive conditions for preparation and sterilization issues [165]. Temperatures required for autoclaving can cause irreversible damage to vesicles while filtration reduces the vesicle to an average of 200 nm, limiting its use to small vesicles.

Solid lipid nanoparticles & nanostructured lipid carriers
Solid lipid nanoparticles (SLNs) are the first generation of nanoparticles composed of lipids that are solid at room and body temperatures, stabilized with an emulsifying layer in an aqueous dispersion. They offer the possibility of a controlled drug delivery, since drug mobility in a solid lipid is lower compared with an oily phase. Other advantages of such carriers include the use of physiological compounds in the composition, the fast and effective production process, including the possibility of large-scale production, the avoidance of organic solvents in the production procedures, and the possibility of producing high concentrated lipid suspensions [205]. The main disadvantage, however, is the low drug-loading capacity [206], which is mainly related to the possibility of drug expulsion during storage [207].

Nanostructured lipid carriers (NLCs) are another type of lipid nanoparticle being developed to overcome some limitations of SLNs. NLCs are prepared not only from solid lipids but from a blend of a solid lipid with a certain amount of oil, to maintain a melting point above 40°C. Mixing very different molecules, such as long-chain glycerides of the solid lipid with short-chain glycerides of the liquid lipid, creates crystals with many imperfections [208]. Apart from localizing the drug between fatty acid chains or lipid lamellae, these imperfections provide a location for the additional loading of drug molecules. These drug molecules can then be incorporated in the particle matrix in a molecularly dispersed form, or be arranged in amorphous clusters. There is also more flexibility for modulation of drug release, increasing the drug loading and preventing its leakage.

Lipid nanoparticles (SLNs and NLCs) are interesting systems for the ocular delivery of drugs. Similar to emulsions, they are composed of accepted excipients, and can be produced on a large industrial scale using an established and low-cost homogenization process. In addition, SLNs and NLCs show the advantages of a solid matrix similar to polymeric nanoparticles, having the ability to protect chemically labile drugs and to modulate release (from very fast to extremely prolonged release). Surface modifications can be used to prolong preocular residence time. Similarly to liposomes, several SLNs and NLCs have been successfully prepared for the incorporation of antifungal drugs but aimed for different administration routes, such as transdermal drug delivery.

Clotrimazole-loaded SLNs and NLCs have been prepared by hot high-pressure homogenization with entrapment efficiency higher than 50%. After 3 months of storage at different temperatures the mean diameters of SLNs and NLCs remained below 1 μm [209]. The entrapment efficiency and the drug-release profile were dependent on the concentration and the lipid mixture employed. NLCs showed higher entrapment efficiency owing to their liquid parts. In agreement with these results, NLCs also depicted a faster release rate in comparison to SLNs with the same lipid concentration. Incorporated clotrimazole in triplamitine-based SLNs and NLCs stabilized with tyloxapol were also obtained. The particles displayed a spherical shape and a narrow size distribution with a mean diameter smaller than 200 nm [210]. The SLN containing clotrimazole displayed a prolonged release character [211].

Lipid particles containing ketoconazole were also obtained using the hot high-pressure homogenization technique, using Compritol® (Compritol 888 ATO, Gattefossé, Weil am Rhein, Germany) as the solid lipid and the natural antioxidant α-tocopherol as the liquid lipid compound for the preparation of NLCs. The authors verified that the SLN matrix was not able to protect the chemically labile ketoconazole against degradation under light exposure. By contrast, the NLCs were able to stabilize the drug, but the aqueous NLC dispersion showed size increase during storage. Possible solutions would be light-protected packaging for the SLNs or NLCs physically stabilized in a gel formulation [212]. In accordance, another study revealed that after a shelf life of 2 years, more than 95% of clotrimazole and less than 30% of ketoconazole incorporated in SLNs and NLCs were detected in the developed formulations. Still, these values were shown to be higher than those obtained with reference emulsions of similar composition and droplet sizes [213].

Other antifungal agents that were successfully incorporated in SLNs include itraconazole [214], miconazole [215] and econazole [216].

Therefore, it is expected that in the near future lipid nanoparticle will become available for the treatment of ophthalmic fungal infections. Despite the drug-loading difficulties, several compounds commonly used in the treatment of ocular diseases have been incorporated into lipid nanoparticles, such as tobramycin [217], gatifloxacin [218], cyclosporine [219] and timolol maleate [220]. Lipid nanoparticles have shown sustained release and enhancement of drug bioavailability in all such cases [217].

Expert commentary
For the treatment of ocular fungal infections, one should keep in mind that there are no ideal antifungal agents or administration regimens. As such, the pharmacological treatment should be chosen considering disease-specific conditions, possible side effects, and the drug’s ability to reach the site of infection and achieve therapeutic concentrations.

Few significant advances have been reached in treating ophthalmic fungal infections. The major problem encountered is the poor water solubility of most of the drugs. Larger molecule sizes (>500 Da) also restrict their intrinsic permeability. Although some formulations with enhanced drug solubility can be easily prepared using cyclodextrins, polymers or suitable surfactants, these solutions may suffer from the drawback of having low residence time at the ocular surface and being
rapidly drained. Owing to short residence time and corneal impermeability to most compounds, the topical treatment is often not effective.

Polymeric nano- and microparticles could therefore be a suitable alternative. Despite not yet being applied for the treatment of fungal keratitis, promising results have been shown for other targets. It is believed that polymeric particles containing antifungal agents could be used to increase drug availability, reduce toxicity and prolong interval of administration. Similarly, liposomes and SLNs offer sustained drug delivery with low toxicity. However, the former represents a challenge when considering large-scale production, whereas the latter has a lower drug-loading capacity. NLCs have emerged as a novel delivery system that could incorporate the advantages of those lipid-based delivery systems and overcome their limitations. In the last few years, NLC formulations have been successfully prepared for the incorporation of antifungal drugs but have not yet been fully employed in the treatment of ocular diseases. It is expected that in the next few years more studies will be performed using polymeric particles and lipid-based systems for the ocular route, resulting in more efficient therapeutic options.

**Five-year view**

It is expected that in the near future more knowledge will be available on the corneal permeation profile of novel antifungal agents. From that point it is also expected that novel nanomedicines would be applied for the ocular delivery of antifungal agents, leading to higher bioavailability and fewer adverse effects.

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**Key issues**

- Fungal keratitis occurs throughout the world, but the overall incidence tends to be higher in tropical and subtropical regions. The most frequent fungi causing keratitis worldwide are *Fusarium* (incidence 20–83.6%), *Aspergillus* (incidence 16.5–75%) and *Candida* (incidence 1–63%).
- Fungal keratitis risk factors include trauma, contact lens wear, prolonged use of topical corticosteroids, immunosuppressive diseases, previous eye surgery and chronic eye surface diseases.
- The pharmacological approach of management of fungal keratitis involves the administration of antifungal agents. However, owing to the physiologic constraints of the eye, only a few drugs present adequate bioavailability.
- Classical antifungal drugs act mainly in the fungal cell membrane. The two most commonly used classes are the polyenes and the azoles. The first includes nystatin and amphotericin B, while the second includes miconazole, ketoconazole, fluconazole and itraconazole.
- Novel drugs have been developed with the aim of solving classical antifungal therapy problems. The novel azole voriconazole is more potent but leads to some adverse effects. The new class echinocandins possesses a broad spectrum but the compounds belonging to this class will probably have low corneal penetration owing to their high molecular weights.
- Nanoparticulated systems containing antifungal drugs could be used to prolong drug delivery and reduce toxicity.
- Liposomes containing antifungal drugs may be useful for intraocular administration. They can minimize some of the adverse side effects encountered by these administration routes and prolong drug residence time, increasing therapeutic effectiveness when no other options are available.
- Antifungal agents have successfully been incorporated into solid lipid nanoparticles and nanostructured lipid carriers, but have not yet been fully employed in the treatment of ocular diseases.

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• Amphotericin B entrapped into poly(lactide-co-glycolide) nanoparticles was shown to improve the oral bioavailability and minimize the adverse effects observed in classical systemic amphotericin B therapy.


• Amphotericin B/poly(lactic acid)-β-poly(ethylene glycol) nanoparticles coated with polysorbate 80 have been efficiently produced for brain targeting.


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