Fungal keratitis: Advances in molecular mechanisms of fungal invasion, pattern recognition receptor signaling, neutrophil-mediated damage and antifungal resistance (Review)

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Abstract. Fungal keratitis is a potentially blinding corneal infection in which pathogen persistence and host inflammatory injury complicate treatment. The present narrative review integrated fungal invasion, pattern recognition receptor signaling, neutrophil responses and antifungal susceptibility within a framework in order to understand treatment outcomes. PubMed literature was reviewed, with priority given to clinical studies, corneal isolates and experimental keratitis models. Evidence from corneal infection studies were distinguished from mechanisms inferred from non-ocular fungal studies, with attention to differences among pathogens. Dectin-1 and Toll-like receptor signaling associated fungal recognition with inflammatory responses. Neutrophil recruitment, extracellular traps and oxidative responses were examined in association to fungal clearance and tissue injury. Acquired resistance, intrinsic low susceptibility and phenotypic tolerance were distinguished from inadequate corneal drug exposure and other causes of treatment failure. Therapeutic approaches included natural compounds, modulation of host inflammatory and antioxidant responses, biofilm-directed interventions, improved drug delivery and antifungal combinations. Topical manogepix has shown activity in experimental keratitis, whereas a clinical study of its prodrug fosmanogepix evaluated invasive mold disease. Most host-directed and biofilm-targeted approaches remain preclinical. The central therapeutic challenge is to reduce tissue injury while maintaining fungal clearance. Adjunctive immune modulation requires evaluation of treatment timing and intensity alongside effective antifungal therapy. Clinical translation also requires adequate corneal exposure, acceptable ocular toxicity and evidence of benefit over standard antifungal treatment.

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1. Introduction

Fungal keratitis is a potentially blinding corneal infection (1-4). It is particularly prevalent in tropical and subtropical regions, where corneal trauma during agricultural work is a notable risk factor (1,2,4). A global burden analysis published in 2021 estimated ~1.05 million new cases annually, with the highest incidences in Asia and Africa (1).

The corneal epithelium provides a physical barrier, while locally produced antimicrobial peptides contribute to ocular surface defense (5-7). During infection, pattern recognition receptors (PRRs) on corneal epithelial cells and resident immune cells detect fungal components and initiate inflammatory signaling (5,8-11). Cytokines and chemokines then promote the recruitment of neutrophils, monocytes and macrophages to the cornea (8,9,11,12). Experimental studies of *Aspergillus* and *Fusarium* keratitis demonstrate that neutrophils help restrict fungal growth (13,14). However, excessive inflammation and oxidative stress can damage corneal tissue, as shown in experimental models of *Aspergillus* and *Candida* keratitis (12,15). The challenge is to limit corneal injury while preserving effective fungal clearance.

Clinical overlap with bacterial keratitis complicates diagnosis and can delay appropriate antifungal treatment (16-19).

Direct microscopy of corneal scrapings can provide rapid evidence of fungal infection, whereas culture supports organism identification but requires more time (17,20). *In vivo* confocal microscopy and molecular assays offer complementary diagnostic information, although their use depends on available resources and expertise (17,21-23).

Major causes of fungal keratitis include *Fusarium* and *Aspergillus* species, dematiaceous fungi and *Candida* species (2,20,24). Trauma is an important risk factor for filamentous fungal keratitis (2,20). *Candida* keratitis often occurs in eyes with pre-existing ocular surface disease or previous corneal surgery (2,24,25). Additional risk factors include contact lens wear, topical corticosteroid exposure and systemic immunosuppression (2,24,25). Studies of corneal isolates have demonstrated differences in antifungal susceptibility between *Fusarium* and *Aspergillus* (20,26). For example, a study found higher minimum inhibitory concentrations (MIC) of voriconazole and itraconazole for *Fusarium* than for *Aspergillus* isolates (20). Mechanistic findings therefore need to be interpreted in association to the infecting species and the experimental model.

Treatment of fungal keratitis is complicated by variable antifungal susceptibility and difficulties in achieving adequate drug exposure within infected corneal tissue (18,26). Topical natamycin 5% remains the first-line treatment for filamentous fungal keratitis (27,28). Despite treatment, progressive infection can cause corneal perforation and require therapeutic keratoplasty (29-31). Other options include topical voriconazole for selected susceptible filamentous infections and topical amphotericin B for *Candida* keratitis; systemic or targeted local antifungal delivery may be considered for deep or refractory infection (18,31). Clinical treatment failure alone does not establish acquired antifungal resistance (26,31), delayed diagnosis, limited corneal drug penetration, advanced stromal involvement and persistent host inflammatory injury can also contribute to poor treatment response (18,31).

Larger infiltrates and deeper stromal involvement at presentation are associated with poorer outcomes in filamentous fungal keratitis (32,33). Persistent culture positivity during treatment is also associated with a greater need for therapeutic keratoplasty in moderate to severe cases (34). These prognostic studies do not distinguish direct fungal injury from damage caused by host inflammatory responses.

A study of patient tear samples demonstrated activation of the alternative complement pathway in filamentous fungal keratitis (35). In experimental *A. fumigatus* keratitis, Dectin-1 signaling through spleen tyrosine kinase (Syk) contributes to inflammatory responses and fungal clearance (11). Mouse studies have also implicated nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1) in limiting corneal inflammation during *A. fumigatus* infection (36,37). These experimental findings require further evaluation before they can guide adjunctive treatment in patients.

The present narrative review examines the interaction between fungal persistence and host inflammatory injury in fungal keratitis. It associates fungal invasion and PRR signaling to neutrophil responses and oxidative tissue damage (Fig. 1). Acquired resistance, intrinsic susceptibility and tolerance are distinguished from inadequate drug exposure and other causes of treatment failure. Keratitis-specific findings are distinguished from mechanisms inferred from non-ocular fungal infections and compare evidence across fungal species. This synthesis informs the evaluation of antifungal and host-directed therapies, with particular attention to treatment timing and preservation of fungal clearance.

2. Literature search strategy and study selection

PubMed (https://pubmed.ncbi.nlm.nih.gov/) was used to develop the present narrative review. An initial search for fungal keratitis was supplemented by topic-based searches on 21 October 2026. No publication-date restrictions were applied. Disease searches combined medical subject headings with title and abstract terms for fungal keratitis, mycotic keratitis, keratomycosis and fungal corneal infection. These searches were supplemented with terms covering fungal invasion, pattern recognition receptors, neutrophils, oxidative stress, antifungal susceptibility, biofilms and therapeutic interventions. Separate searches addressed selected fungal mechanisms and fosmanogepix without requiring terms for corneal infection. No language, species, study-design or free-full-text filters were applied during supplementary retrieval. ClinicalTrials.gov (https://clinicaltrials.gov/) was also consulted to verify trial status. Publisher websites, including ScienceDirect (https://www.sciencedirect.com/), Wiley Online Library (https://onlinelibrary.wiley.com/) and PLOS (https://journals.plos.org/), were consulted to verify article details and identify additional reports not retrieved through PubMed.

Study selection prioritized primary research involving patients with fungal keratitis, corneal isolates and experimental corneal infection models. Non-ocular fungal studies were included when they helped to explain relevant mechanisms, with their applicability to corneal infection explicitly qualified. Only English-language full-text publications were eligible for inclusion. Eligible publications addressed fungal invasion, host recognition or immune injury, antifungal susceptibility, biofilms or therapeutic interventions, using corneal evidence or mechanistic evidence with an explicitly qualified relevance to corneal infection. Non-English publications, reports available only as abstracts and publications unrelated to these topics were excluded from the evidence synthesis. Our selection considered fungal species, model relevance, experimental controls and microbiological or corneal outcomes. Clinical studies informed the interpretation of treatment effects, while reviews provided background and help identify primary research. Conflicting findings and studies reporting no treatment benefit were also considered. The synthesis distinguished clinical observations, experimental findings and proposed mechanisms.

3. Molecular mechanisms of fungal invasion of the cornea

Fungal adhesion and breach of the corneal epithelial barrier. Disruption of the corneal epithelial barrier facilitates fungal access to underlying tissues (38). Depending on the extent of injury, exposed extracellular matrix components, including fibronectin, laminin and collagen, can provide surfaces for fungal attachment (38,39). Corneal infection models reveal differences between fungal adhesion and subsequent invasion. Among the isolates examined, *F. falciforme* adhered

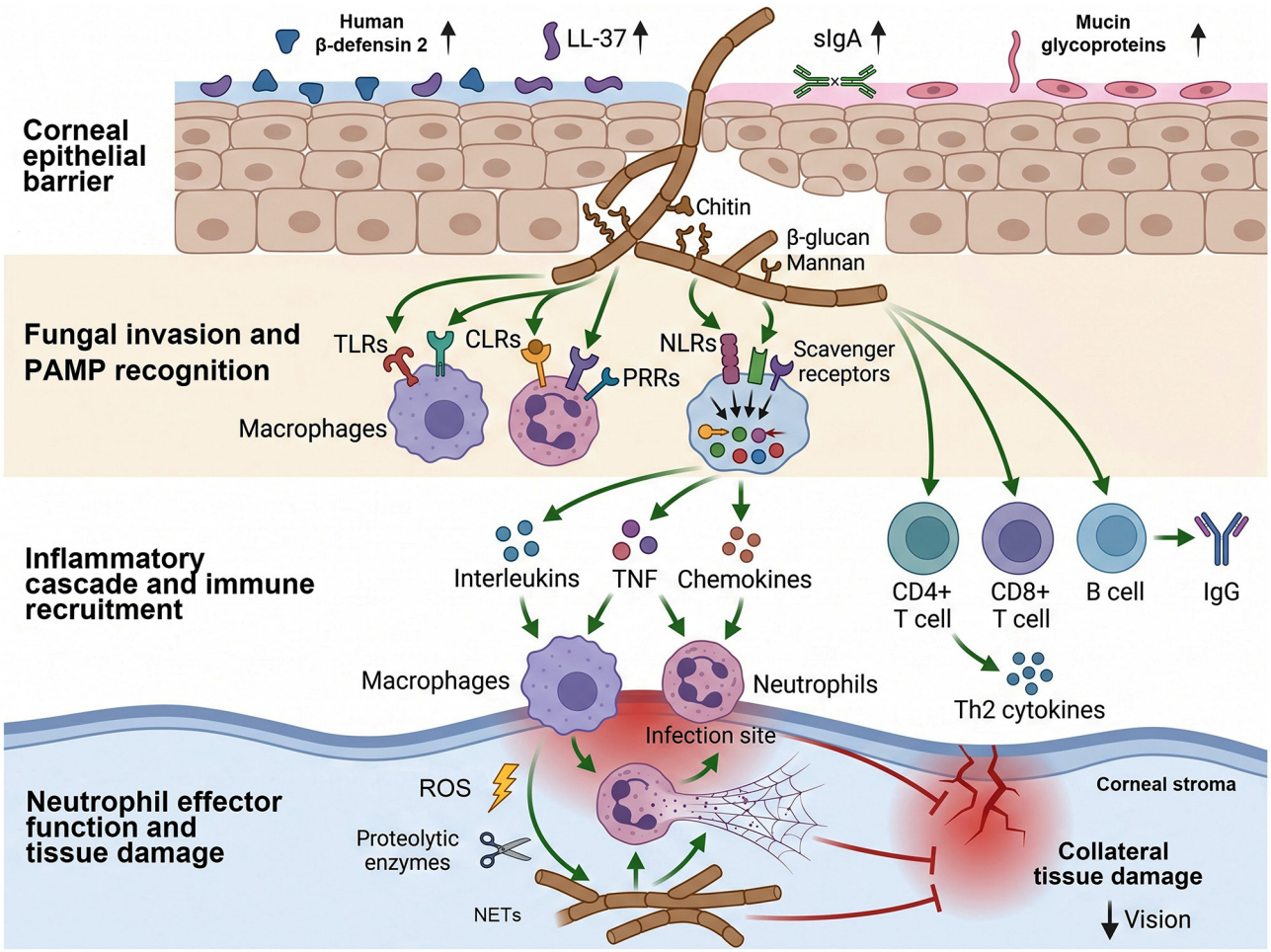


Figure 1. Host defense and inflammatory injury in fungal keratitis. Fungal recognition promotes immune cell recruitment and antifungal responses. Excessive inflammation can contribute to corneal injury. NET and adaptive immune effects depend on the experimental setting. PAMPs, pathogen-associated molecular patterns; PRRs, pattern recognition receptors; TLRs, Toll-like receptors; CLRs, C-type lectin receptors; NLRs, nucleotide-binding oligomerization domain-like receptors; ROS, reactive oxygen species; NETs, neutrophil extracellular traps; TNF, tumor necrosis factor; sIgA, secretory immunoglobulin A; IgG, immunoglobulin G; CD, cluster of differentiation; Th2, type 2 helper T cells; LL-37, human cathelicidin peptide.

more strongly to human corneal epithelial cells than *F. keratoplasticum*, whereas *F. keratoplasticum* showed greater invasion and tissue damage (40). *In vitro* binding studies show that *A. fumigatus* conidia bind to fibronectin, laminin and collagen, supporting conidial attachment to these matrix proteins (41-43). These studies were conducted outside corneal infection models and provide indirect support for a role of matrix binding in corneal adhesion. Following attachment, hyphal growth enables penetration of epithelial layers and extension into the stroma, as observed in reconstructed human corneal models infected with *F. keratoplasticum* (Fig. 2) (40). Corneal isolates of *A. flavus* and *F. solani* produce extracellular serine protease and metalloprotease activities when cultured with collagen (44). However, the serine protease activity detected in culture was not detected in infected rabbit corneas (44). Host cells also contribute to the protease response. In a three-dimensional stromal model, *F. petroliphilum* infection increased matrix metalloproteinase expression in human corneal fibroblasts (45). Fungal adaptation to local conditions also influences invasion. In scarified human corneas studied *ex vivo*, loss of pH-response transcription factor pacC function in *A. nidulans* reduced stromal penetration, linking a pH-responsive regulatory pathway to invasion in this model (46). The relative contributions of fungal enzymes and host proteases to barrier failure remain to be defined in species-specific corneal models (44,45).

Roles of fungal toxins and virulence factors in tissue invasion. Fungal virulence in the cornea involves both tissue invasion and survival during host immune attack (14,45). Proteomic analysis identified abundant alkaline serine protease alkaline protease 1 (Alp1) among proteins secreted by *A. flavus* corneal isolates (47). The isolates were also evaluated in a *Galleria mellonella* infection model, so their virulence phenotypes do not directly establish the role of Alp1 in corneal invasion (47). Screening of keratitis isolates has also detected extracellular enzyme activities and mycotoxin production, including protease and lipase activities, and mycotoxins, including aflatoxins and T-2 toxin, in culture (48). These findings identify candidate virulence factors, but their activity and contribution to tissue injury within infected corneas require direct evaluation. In experiments outside the eye, gliotoxin inhibits phagocytosis by rodent macrophages and human neutrophils (49,50). Its contribution to virulence

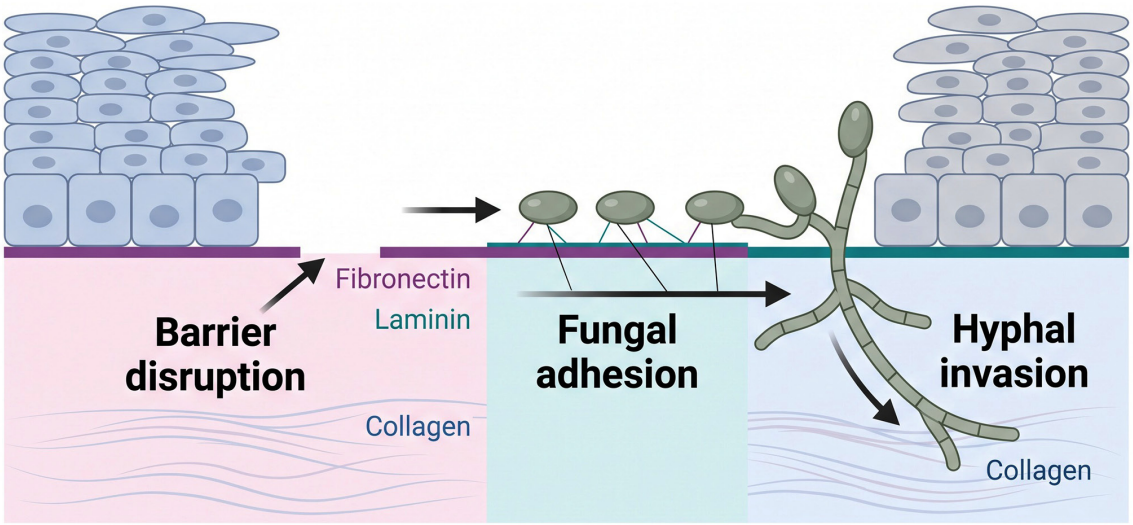


Figure 2. Fungal adhesion and invasion following corneal barrier disruption. Epithelial injury exposes extracellular matrix components that support fungal attachment. Subsequent hyphal growth enables penetration into underlying tissue. The schematic integrates evidence from non-ocular matrix-binding studies and experimental corneal invasion models.

varies with host immune status in mouse models of invasive aspergillosis (51). However, *A. fumigatus* mutants lacking gliotoxin biosynthetic gene P or gliotoxin biosynthetic gene Z showed no notable reduction in fungal burden after intrastromal inoculation in an acute mouse keratitis model (14). Deletion of yeast AP-1 (*yap1*) or combined deletion of the three superoxide dismutase genes reduced fungal survival in the same model (14). Melanin has been detected in fungal hyphae within patient corneal samples, including infections caused by *A. flavus* and *Fonsecaea pedrosoi* (52). These observations establish melanization during corneal infection, but do not demonstrate its contribution to immune evasion or tissue damage. Strain variation also influences disease severity. In a study of *A. flavus* corneal isolates, strains carrying kexin-like endoprotease (*kexB*) mutations caused milder keratitis in mice than wild-type comparators (53). The specific contribution of *kexB* requires confirmation using isogenic mutants and complemented strains. The contribution of individual virulence factors therefore depends on the fungal strain, host immune status and experimental setting (14,51). Functional studies in corneal models are needed to distinguish factors that sustain infection from those merely detected during disease.

Fungal biofilm formation and reduced antifungal susceptibility. Keratitis-associated fungal isolates can form biofilms on experimental surfaces, including contact lenses, and exhibit reduced susceptibility to some antifungal agents (54,55). Mature biofilms formed by filamentous corneal isolates contain networks of hyphae embedded in an extracellular matrix (55). In a clinical *F. solani* isolate, this matrix contained carbohydrates, proteins and extracellular DNA (56). A recent study of corneal *Fusarium* isolates found that mature biofilms were less responsive to antifungal exposure than biofilms treated during adhesion (57). Such findings do not by themselves establish acquired genetic resistance or demonstrate the cause of treatment failure in patients. Mechanistic evidence from non-ocular *C. albicans* biofilm models implicates efflux pumps in reduced fluconazole susceptibility during early biofilm development (58). The same study found lower ergosterol levels during subsequent maturation. These findings do not establish equivalent mechanisms in corneal *Fusarium* biofilms (58). Drug responses also differ among biofilm models. One study of *Fusarium* keratitis outbreak isolates found similar natamycin susceptibility in planktonic and biofilm growth, alongside species-dependent responses to amphotericin B and voriconazole (54). Another study of a single *F. solani* corneal isolate reported reduced natamycin susceptibility after biofilm maturation (56). In *C. albicans*, biofilm matrix inhibited neutrophil extracellular trap formation and protected against neutrophil attack (59). This evidence comes from cell assays and a rat catheter infection model, and its relevance to corneal infection requires direct investigation (59). These observations provide a rationale for investigating biofilm disruption as an adjunct to antifungal therapy (56). Evaluation should include viable fungal burden and corneal safety, alongside changes in biofilm biomass.

4. Role of pattern recognition receptor signaling in fungal keratitis

Dectin-1 receptor and its downstream signaling pathways. Dectin-1 is a C-type lectin receptor that recognizes fungal β-glucans and participates in innate immune responses in experimental fungal keratitis (11,60). In *A. fumigatus*, these ligands become exposed during conidial germination and hyphal growth (11). Resident corneal macrophages and dendritic cells initiate cytokine production and leukocyte recruitment in murine *A. fumigatus* keratitis (11). Studies of rat corneal epithelium and cultured human corneal epithelial cells also support a role for Dectin-1 in inflammatory cytokine responses (60,61). In macrophages exposed to *A. fumigatus*, Dectin-1 promotes Syk phosphorylation and nuclear factor κB (NF-κB) activation (Fig. 3) (11). In infected mouse corneas, Dectin-1 knockdown reduced phosphorylation of c-Jun N-terminal kinase (JNK), a member of the mitogen-activated protein kinase (MAPK) family (62). JNK inhibition also

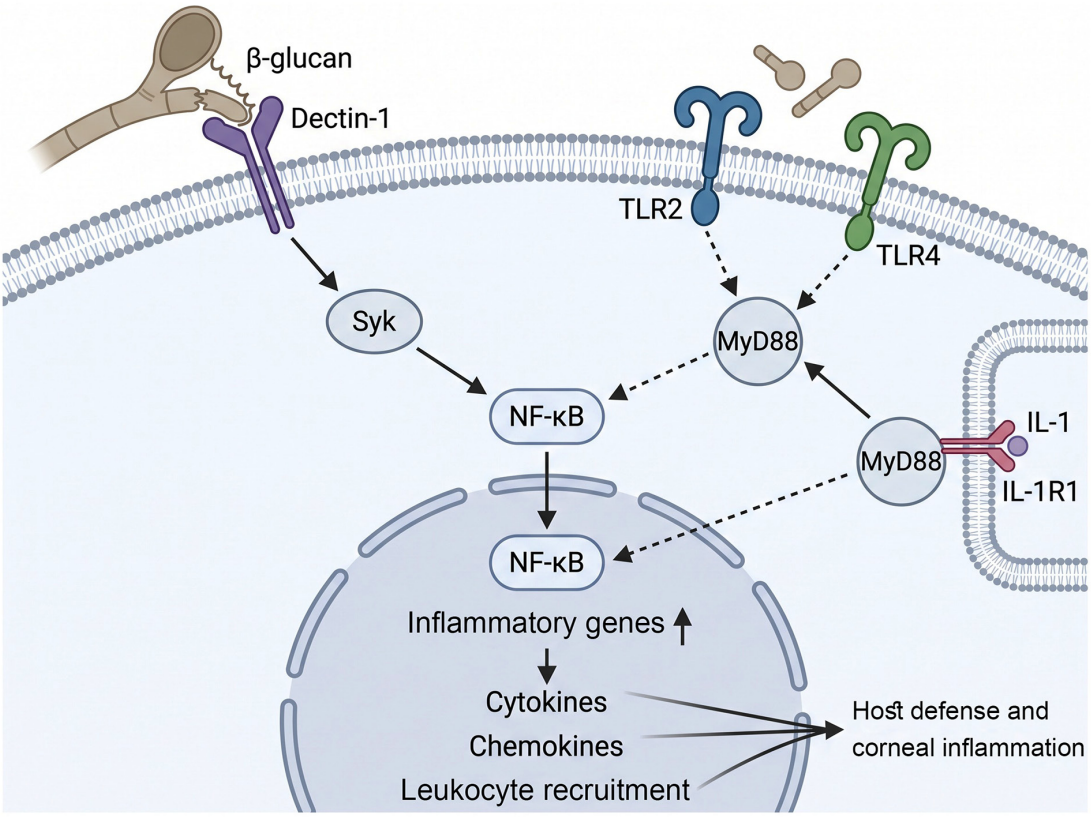


Figure 3. Innate receptor signaling in fungal keratitis. Dectin-1 recognition of β-glucan activates Syk and NF-κB signaling. TLR2, TLR4 and IL-1R1 contribute to responses involving MyD88, inflammatory mediators and leukocyte recruitment. Dashed connections summarize signaling whose contribution varies among infection models. Syk, spleen tyrosine kinase; NF-κB, nuclear factor κB; TLR, Toll-like receptor; IL-1, interleukin-1; IL-1R1, interleukin-1 receptor type 1; MyD88, myeloid differentiation primary response 88.

reduced interleukin (IL)-1β production, supporting its involvement in this inflammatory response (62). Dectin-1-deficient mice showed reduced early cellular infiltration but impaired fungal clearance during infection with a clinical *A. fumigatus* isolate (11). In another mouse model, pretreatment with the Dectin-1 agonist curdlan increased markers of inflammatory macrophage polarization and worsened corneal inflammation (63). Conversely, laminarin treatment initiated before infection reduced corneal inflammation in mouse *A. fumigatus* models (12,63). A Dectin-1-specific nanobody administered after infection also reduced corneal disease scores and the expression of IL-1β and IL-6 (64). Treatment with α-melanocyte-stimulating hormone (α-MSH) reduced corneal fungal burden and inflammation in a mouse *A. fumigatus* model (65). Reduced Dectin-1 expression and JNK and extracellular signal-regulated kinase phosphorylation accompanied these effects (65). These observations do not establish Dectin-1 as a direct molecular target of α-MSH. These findings support further investigation of Dectin-1 modulation as an adjunct to antifungal treatment. Studies should establish when intervention limits corneal injury without compromising fungal clearance. Validation across fungal species and evaluation alongside effective antifungal therapy are needed before clinical translation.

Toll-like receptors (TLRs) in synergistic fungal recognition. TLR2 and TLR4 contribute to inflammatory cytokine responses and antifungal defense in the cornea, although which receptors are required for these responses varies by fungal species and experimental model (Fig. 3) (66-68). Increased expression of both receptors has been detected in corneal specimens from patients with *F. solani* keratitis (66). In cultured human corneal epithelial cells, antibodies against TLR2 or TLR4 reduced cytokine responses to *A. fumigatus* antigens (67). Both TLR2 and Dectin-1 also contribute to epithelial antimicrobial responses. Silencing either receptor reduced the induction of human β-defensin 2 and human cathelicidin peptide in cultured human corneal epithelial cells exposed to heat-inactivated *F. solani* (69). The contribution of myeloid differentiation primary response 88 (MyD88) requires careful interpretation because this adaptor also transduces signals from the IL-1 receptor type 1 (IL-1R1) (11,70). In mouse models of *A. fumigatus* and *Fusarium* keratitis, deletion of either MyD88 or IL-1R1 impaired inflammatory cell recruitment and fungal clearance (11,70). These findings indicate that MyD88-dependent protection cannot be attributed solely to TLR signaling (11,70). Receptor requirements also differed among the fungal species and infection models examined. TLR4 deficiency increased fungal recovery without reducing cellular infiltration in murine *A. fumigatus* keratitis and contact lens-associated *F. oxysporum* keratitis (11,71). TLR2 deficiency did not impair fungal clearance in these models, whereas it impaired control of fungal growth in experimental *C. albicans* keratitis (11,68,71). Therefore, increased receptor expression alone does not establish which TLR is required for protection against a particular pathogen (66,68,71).

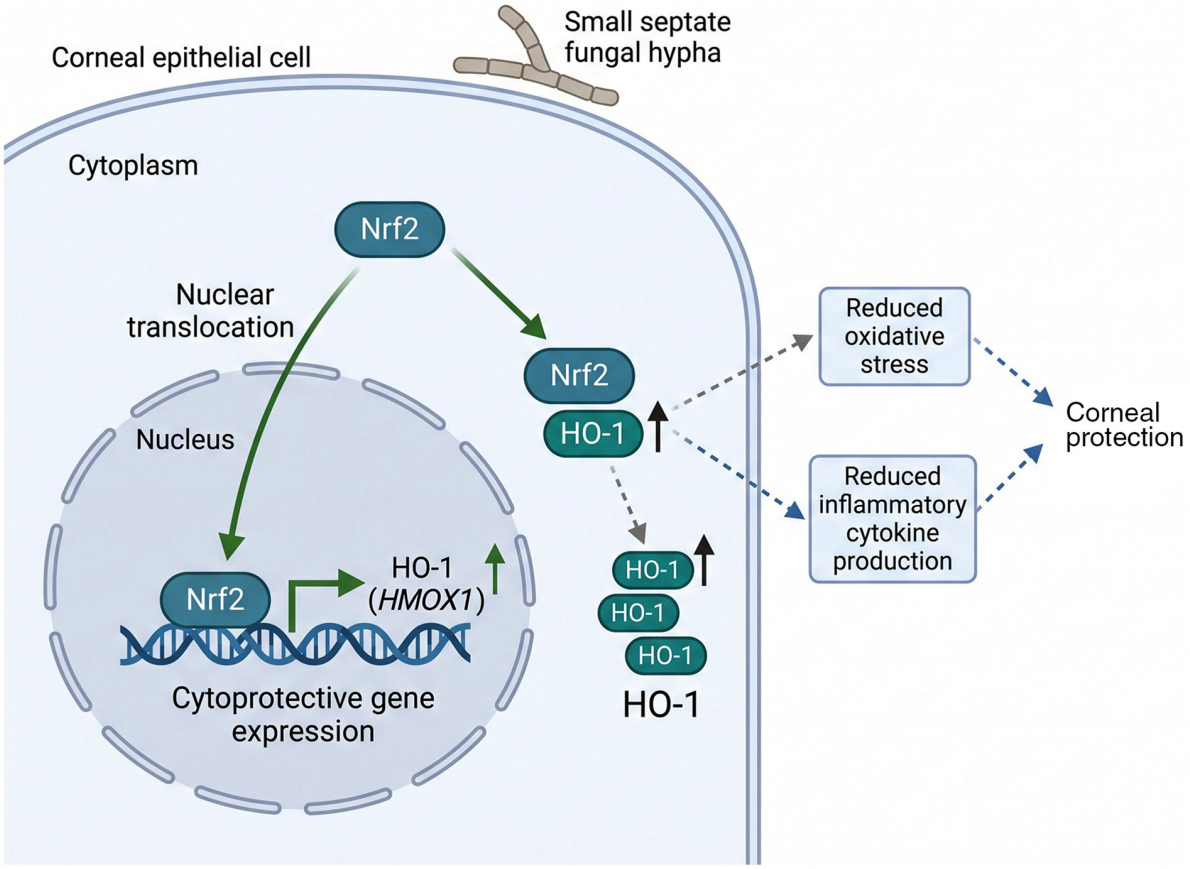


Figure 4. Experimental Nrf2/HO-1-mediated cytoprotection. Nuclear accumulation of Nrf2 promotes cytoprotective gene expression, including *HMOX1*, which encodes HO-1. Dashed arrows summarize downstream antioxidant and anti-inflammatory effects investigated in experimental models. Upward arrows indicate increased expression. Nrf2, nuclear factor erythroid 2-related factor 2; HO-1, heme oxygenase-1.

Pharmacological studies have reported reduced inflammation alongside changes in TLR-associated signaling. Schaftoside reduced neutrophil infiltration and corneal disease severity in murine *A. fumigatus* keratitis, accompanied by lower TLR4 and MyD88 expression (72). Thymol reduced corneal inflammation together with the expression of TLR4, MyD88, NF-κB and IL-1β in a mouse model of *A. fumigatus* keratitis (73). Quercetin showed antifungal activity and reduced TLR4-associated inflammatory responses in cell and mouse models (74). These findings do not establish selective TLR4 inhibition as the sole mechanism of protection. TLR-directed treatment therefore requires assessment of fungal clearance as well as tissue inflammation. Future studies should establish how the timing and extent of immune modulation affect these outcomes when combined with antifungal therapy.

Anti-inflammatory and protective roles of the Nrf2/HO-1 pathway. Studies in experimental *A. fumigatus* keratitis support a protective role for Nrf2/HO-1 signaling in regulating corneal inflammation (36,37,75). Activated Nrf2 accumulates in the nucleus and promotes the expression of cytoprotective genes, including HO-1 (Fig. 4) (36,37). Dimethyl itaconate reduced corneal disease severity, neutrophil infiltration and inflammatory cytokine expression in infected mice (37). In cultured human corneal epithelial cells, pharmacological inhibition of Nrf2 or HO-1 attenuated its suppression of cytokine production (Fig. 4) (37). Complementary evidence comes from dimethyl fumarate. Nrf2 silencing weakened dimethyl fumarate's suppression of caspase-1 and gasdermin D cleavage in fungus-stimulated human corneal epithelial cells, supporting Nrf2 involvement in the regulation of pyroptosis (76). Hydroxytyrosol also reduced inflammatory cytokine production in fungus-stimulated cells, and pharmacological Nrf2 inhibition counteracted this effect (75). A study of farrerol connected Nrf2 activation to autophagy-dependent degradation of Kelch-like ECH-associated protein 1 (77). Farrerol reduced oxidative stress and enhanced macrophage killing of conidia, in addition to reducing inflammation and fungal burden in a mouse keratitis model (77). Perillaldehyde treatment increased Nrf2/HO-1 expression and reduced Dectin-1 expression in experimental *A. fumigatus* infection (36). Rutin likewise increased Nrf2/HO-1 signaling while reducing Dectin-1 expression and Syk phosphorylation (78). These concurrent changes do not by themselves establish a direct inhibitory association between Nrf2/HO-1 and Dectin-1/Syk (36,78). Evidence also extends to experimental *C. albicans* keratitis. In infected mice, thymoquinone reduced corneal inflammation and fungal burden alongside increased Nrf2 and HO-1 expression (79). These findings remain preclinical. Reduced fungal burden cannot be attributed to host Nrf2 activation alone. Perillaldehyde, dimethyl itaconate, hydroxytyrosol and dimethyl fumarate also inhibited fungal growth directly *in vitro* (36,37,75,76). These results support further evaluation of Nrf2/HO-1 modulation as an adjunct

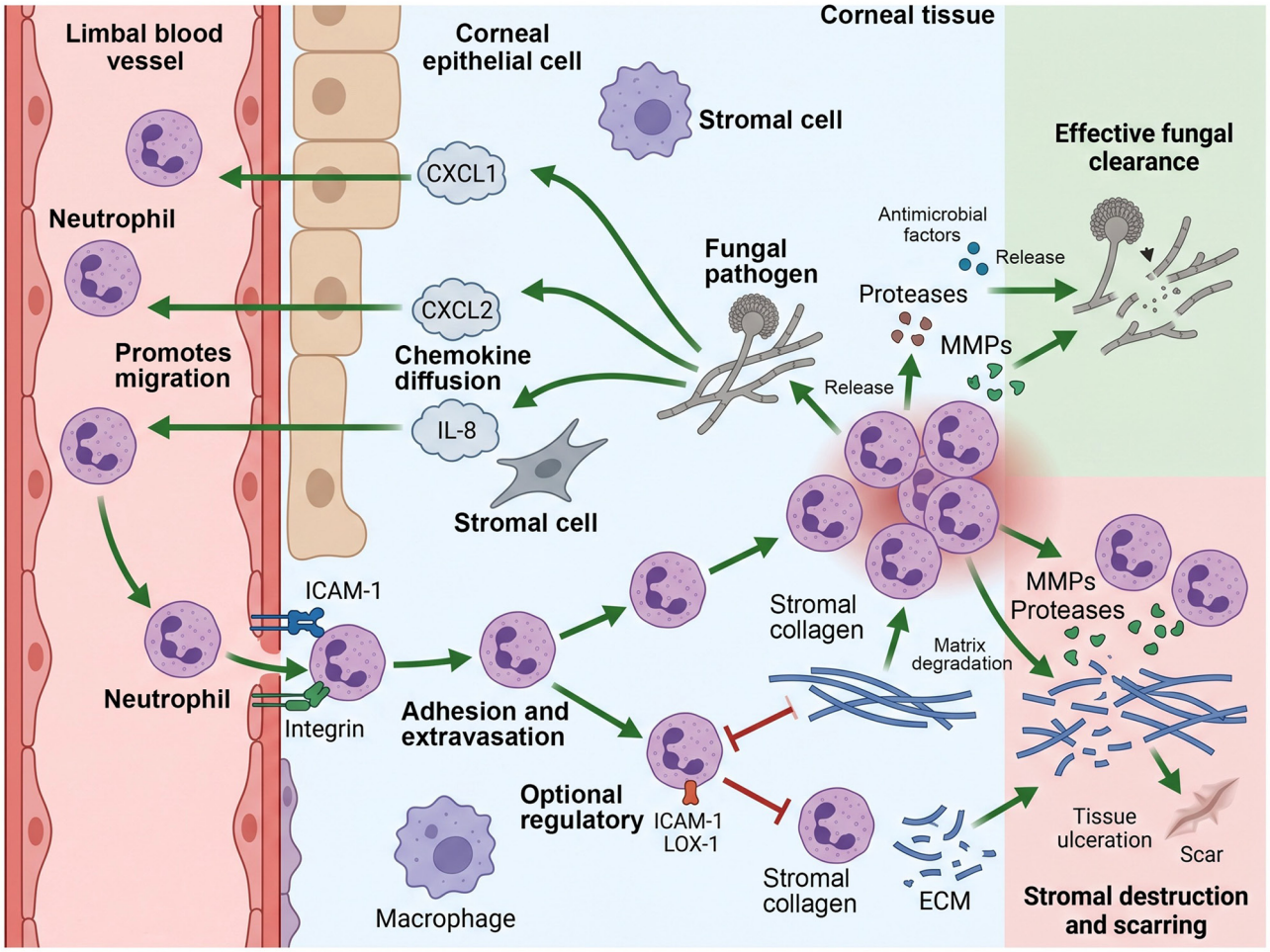


Figure 5. Neutrophil recruitment and stromal injury in fungal keratitis. Chemokines and adhesion molecules recruit neutrophils from limbal vessels. Antifungal responses support fungal control, while excessive proteolysis contributes to stromal injury. Modulation of recruitment remains experimental. CXCL1 and CXCL2, C-X-C motif chemokine ligands 1 and 2; IL-8, interleukin-8; ICAM-1, intercellular adhesion molecule-1; MMPs, matrix metalloproteinases; ECM, extracellular matrix; LOX-1, lectin-like oxidized low-density lipoprotein receptor-1.

to antifungal treatment. Such studies should define effective doses and treatment windows while assessing both corneal injury and viable fungal burden across different pathogens.

5. Neutrophil-mediated corneal tissue damage

Neutrophil recruitment and activation mechanisms. Neutrophils are among the earliest and most abundant innate immune cells recruited to the cornea during fungal keratitis (Fig. 5) (11,80). In mouse models of *Aspergillus* and *Fusarium* keratitis, IL-1R1/MyD88 signaling supports C-X-C motif chemokine ligands (CXCL)1 production and neutrophil recruitment to the infected cornea (11,70). Human corneal ulcers caused by these fungi show increased expression of IL-8, also known as CXCL8, together with predominantly neutrophilic infiltrates (80). Limbal vascular responses also contribute to early recruitment. In murine *F. solani* keratitis, mast cell activation promotes vascular dilation and intercellular adhesion molecule-1 (ICAM-1) expression (81). Stabilizing mast cells with cromolyn reduced early neutrophil infiltration but increased fungal growth and subsequent corneal perforation (81). Once recruited, neutrophils require effective antifungal responses. Experiments using mouse keratitis models and human neutrophils identified CD18-dependent activation of the phagocyte NADPH oxidase as an important mechanism of defense against *Aspergillus* and *Fusarium* hyphae (14). Protease activity provides an association between inflammatory responses and tissue damage. Increased levels of matrix metalloproteinase (MMP)-8 and MMP-9 have been detected in corneal tissue from patients with fungal keratitis (82). Increased expression of these enzymes also occurs in experimental *C. albicans* keratitis, although tissue measurements do not establish neutrophils as their sole source (83). In a primed mouse model of *A. fumigatus* keratitis, the Janus kinase inhibitor ruxolitinib reduced corneal MMP-9 activity but impaired fungal clearance without reducing neutrophil recruitment (84). This finding shows why reduced protease activity alone cannot establish therapeutic benefit. In another mouse model of *A. fumigatus* keratitis, pretreatment with an antibody against lectin-like oxidized low-density lipoprotein receptor-1 reduced ICAM-1 expression, neutrophil infiltration and corneal disease scores (85). These results support further investigation of recruitment pathways but do not establish that their inhibition preserves fungal clearance during an established infection (85). Early neutrophil recruitment is important for fungal containment, but protective and damaging responses

cannot be assigned to strictly separate disease stages. Studies of adjunctive immune modulation should therefore assess the timing and extent of inhibition alongside effective antifungal treatment, with fungal burden and corneal injury evaluated together.

Dual role of neutrophil extracellular traps (NETs). NETs were identified in corneal scrapings from all 14 patients in a small clinical series of fungal keratitis (86). NETs are extracellular web-like structures composed mainly of DNA, histones and granular proteins such as neutrophil elastase and myeloperoxidase (87,88). Higher NET grades were associated with shorter treatment courses in the clinical series, but this observational finding does not establish a causal protective effect (86). In murine *C. albicans* keratitis, dexamethasone reduced NET formation and neutrophil infiltration while increasing fungal burden and disease severity (89). These concurrent effects do not isolate the contribution of NET suppression to the worsening infection (89). In human and mouse neutrophils, *A. fumigatus* hyphal extracts and β-glucan induced NET formation through complement receptor 3 and reactive oxygen species (ROS) (90). Peptidylarginine deiminase 4 (PAD4) was also required for NET formation under these experimental conditions (90). However, infected PAD4-deficient mice showed no notable differences in corneal fungal burden, neutrophil infiltration or opacity compared with wild-type mice (90). PAD4-dependent NET formation was therefore dispensable for infection control in this model (90). The possibility of NET-mediated corneal injury is supported indirectly by experiments in non-ocular tissues. Isolated NETs caused cytotoxicity in cultured lung epithelial and endothelial cells, with histones and myeloperoxidase contributing to this effect (88). These findings provide a rationale for investigating corneal toxicity but do not establish NETs as a cause of stromal scarring in fungal keratitis. A recent study reported improved disease outcomes with vitamin C in murine *F. solani* keratitis, while accompanying neutrophil assays showed no notable change in NET formation (91). This observation further cautions against attributing therapeutic benefit to NET suppression without direct evidence (91). Whether NET modulation can improve outcomes remains an experimental question. Future studies should evaluate such interventions alongside antifungal treatment and compare different treatment stages, using viable fungal burden and corneal healing as joint outcomes.

ROS and oxidative stress-induced damage. ROS generated by activated neutrophils are important mediators of antifungal defense in experimental *Aspergillus* and *Fusarium* keratitis (14). Mice lacking NADPH oxidase 2, the catalytic subunit of the phagocyte NADPH oxidase, showed increased fungal growth in infected corneas (14). Fungal antioxidant systems also influence the effectiveness of this defense. In *A. fumigatus*, Yap1-regulated responses, superoxide dismutases and the thioredoxin pathway protect hyphae against neutrophil-mediated oxidation and support survival during corneal infection (14). In murine *C. albicans* keratitis, increased markers of lipid peroxidation and oxidative DNA damage indicated host oxidative injury (15). Human corneal epithelial cells exposed to heat-killed *C. albicans* also showed increased ROS and p38 MAPK activation (15). Pretreatment with the p38 inhibitor SB203580 reduced cellular ROS and markers of oxidative injury, including malondialdehyde, 4-hydroxynonenal and 8-hydroxy-2'-deoxyguanosine (15). In mice, administration before infection reduced corneal opacity, while fungal culture counts at day 1 were similar to controls (15). This limited assessment does not establish preserved fungal clearance throughout infection (15). Reducing ROS does not necessarily reduce inflammation during active infection. In murine *A. fumigatus* keratitis, diphenyleneiodonium treatment initiated before infection reduced ROS production but increased fungal burden, neutrophil infiltration and disease severity (92). These findings caution against broad suppression of the oxidative response without assessing its effects on fungal control. Nrf2/HO-1 modulation has also been investigated in experimental *A. fumigatus* keratitis, as aforementioned (37). Dimethyl itaconate reduced corneal inflammation and fungal burden when treatment began 24 h after infection (37). However, it also inhibited fungal growth directly *in vitro*, so the reduction in fungal burden cannot be attributed to host antioxidant signaling alone (37). These results support evaluating corneal protection together with preservation of neutrophil oxidative killing (Fig. 6). Future studies of adjunctive treatment should define the dose and timing of redox modulation alongside antifungal therapy, with viable fungal burden and corneal healing assessed across different pathogens.

6. Molecular mechanisms of antifungal resistance

Drug target alterations and drug efflux. Antifungal susceptibility in fungal keratitis varies with the fungal species and antifungal agent (26). An elevated MIC alone does not distinguish intrinsic low susceptibility from acquired resistance (93). Azoles inhibit sterol 14α-demethylase (CYP51), disrupting ergosterol biosynthesis (94). Target amino acid substitutions can reduce azole inhibition, whereas target overexpression can increase the amount of enzyme available (Fig. 7) (94,95). Functional testing of clinically derived *C. albicans* ergosterol pathway gene *(ERG)11* variants demonstrated reduced azole susceptibility but did not establish their contribution to keratitis (95). Direct evidence from corneal infection includes an *A. fumigatus* isolate carrying the Cyp51A G54E substitution. This isolate was resistant to itraconazole but remained susceptible to voriconazole and posaconazole (96). Drug efflux provides an additional route to azole resistance. In *C. albicans*, ATP-binding cassette (ABC) transporters and major facilitator superfamily transporters can reduce intracellular azole accumulation (Fig. 7) (97). Expression of *F. keratoplasticum ABC1* in *Saccharomyces cerevisiae* increased azole resistance, supporting its function as a drug efflux transporter (98). However, this study used a non-ocular clinical isolate and did not test its contribution to keratitis treatment failure (98). In the *F. solani* species complex, CYP51 sequence variation was associated with differences in voriconazole MICs in non-ocular clinical and environmental isolates (93). A separate *in vitro* study showed increased *CYP51A* expression following azole exposure (99). These findings identify candidate contributors to reduced susceptibility, but do not establish acquired resistance during corneal infection. Changes in membrane sterols can also affect polyene susceptibility. In a *C. lusitaniae* laboratory model, disruption of *ERG6* conferred amphotericin

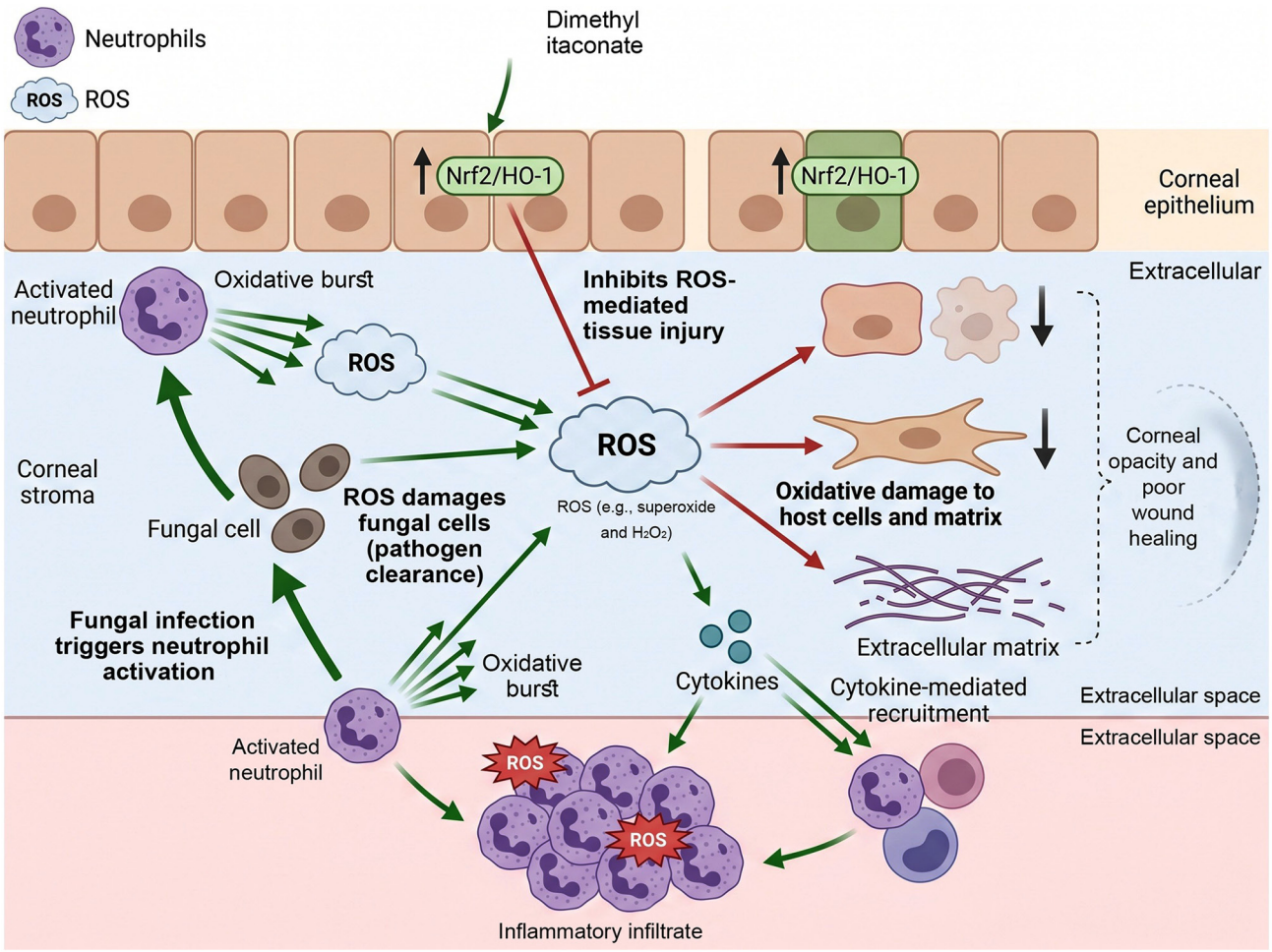


Figure 6. Protective and damaging effects of ROS in fungal keratitis. Neutrophil-derived ROS support fungal control, while excessive oxidative stress contributes to host tissue injury. Dimethyl itaconate is shown as an experimental modulator of Nrf2/HO-1 signaling. ROS, reactive oxygen species; Nrf2, nuclear factor erythroid 2-related factor 2; HO-1, heme oxygenase-1; H_2O_2, hydrogen peroxide.

B resistance (100). Natamycin binds ergosterol and inhibits yeast growth without increasing plasma membrane permeability (101). The clinical relevance of each mechanism requires assessment for the specific pathogen and drug. Susceptibility results should also be interpreted alongside drug exposure at the infection site and clinical response.

Mechanisms of reduced antifungal susceptibility in biofilms. Keratitis-associated fungal isolates can form biofilms with reduced antifungal susceptibility, although this phenotype does not establish acquired genetic resistance (54,55). In non-ocular *C. albicans* biofilm models, the extracellular matrix contains carbohydrates, proteins, lipids and extracellular DNA (102). This matrix sequestered fluconazole and reduced intracellular drug accumulation (Fig. 8) (103). However, matrix protection cannot be reduced to a uniform barrier to drug diffusion. In laboratory *Candida* biofilms, antifungal penetration did not ensure complete killing of embedded cells (104). Cellular mechanisms also vary with biofilm development. In a non-ocular *C. albicans* model, deletion of *Candida* drug resistance (*CDR*)*1*, *CDR2* and multidrug resistance 1 increased fluconazole susceptibility in early biofilms (58). Mature biofilms remained poorly susceptible despite these deletions (58). In non-ocular *A. fumigatus* biofilm models, oxygen depletion during maturation reduced susceptibility to voriconazole and amphotericin B (105). Small populations of *C. albicans* biofilm cells survived high amphotericin B concentrations and were described as persisters (106). A later study found that incomplete drug exposure could produce apparent survivors. Persister detection was also inconsistent across clinical isolates under the revised experimental conditions (107). These studies do not establish persister-mediated treatment failure in fungal keratitis. A study of 14 corneal *Fusarium* isolates linked antifungal-tolerant biofilms to more complex treatment, including combined antifungal therapy or corneal transplantation (57). This small observational series supports clinical relevance but does not establish biofilm formation as an independent cause of treatment failure.

Stress adaptation and antifungal tolerance. Antifungal tolerance is distinct from resistance measured by standard MIC testing (108). In *C. albicans* exposed to fluconazole, tolerance involves slow growth of a subpopulation at concentrations above the MIC (Fig. 8) (108). In a non-ocular study of *C. albicans* biofilms, impairing heat shock protein 90 (Hsp90) function increased susceptibility to fluconazole (109). Hsp90 depletion reduced calcineurin protein levels in planktonic cells but not in biofilms, indicating that its downstream effects

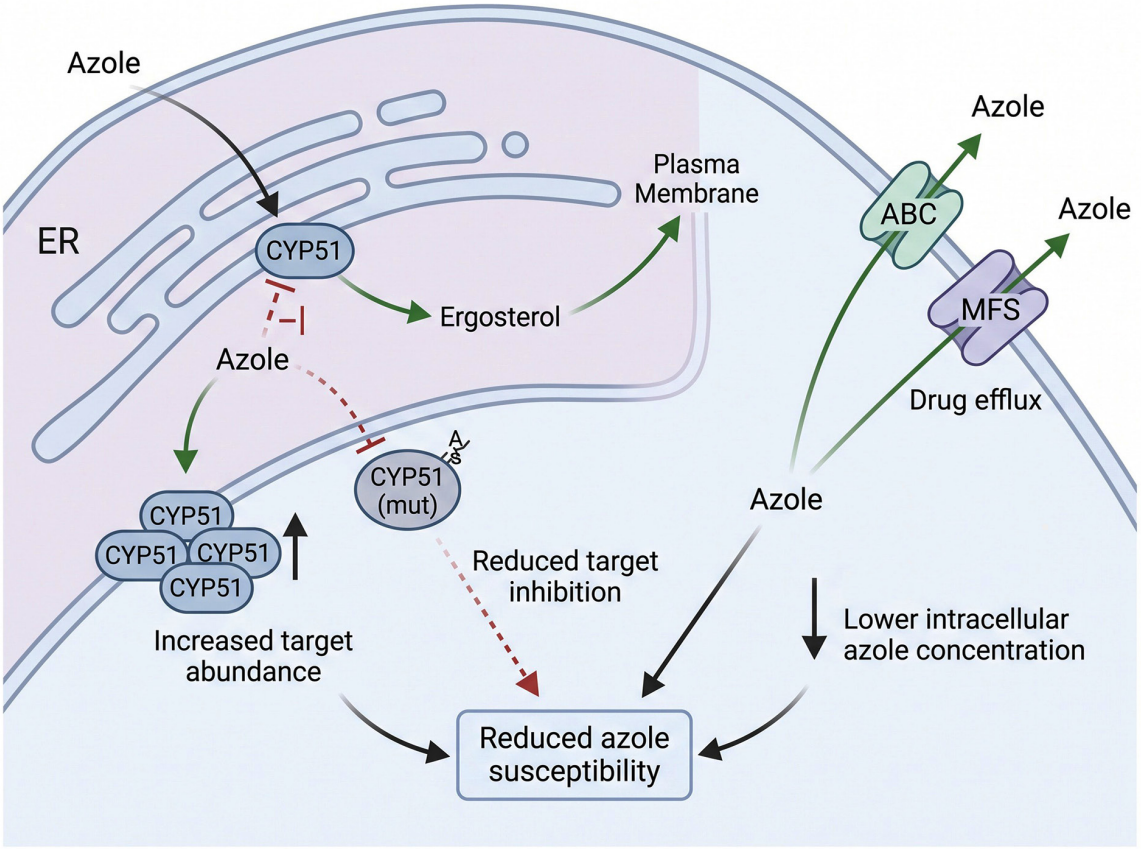


Figure 7. Target alterations and drug efflux reducing azole susceptibility. CYP51 alterations, increased target abundance and drug efflux can reduce azole susceptibility. Their contributions vary with fungal species and drug, with limited validation in keratitis. Arrows indicate biological processes or drug transport, and barred lines indicate inhibition. CYP51, sterol 14α-demethylase; ER, endoplasmic reticulum; ABC, ATP-binding cassette; MFS, major facilitator superfamily; mut, mutant.

depend on the growth state (109). Deletion of the fungal calcineurin regulatory subunit gene calcineurin subunit B1 reduced pathogenicity in an immunosuppressed mouse model of *C. albicans* keratitis (110). Topical fluconazole combined with cyclosporine A accelerated disease resolution compared with either agent alone in the same model (110). Artesunate reduced the expression of genes encoding Hsp90 and calcineurin pathway components in *A. fumigatus* cultures (111). It also reduced fungal burden in mouse keratitis (111). These findings do not establish direct inhibition of either fungal pathway as the cause of the treatment benefit. In non-ocular *C. albicans* experiments, increased chitin synthesis compensated for echinocandin-induced cell wall damage and reduced drug efficacy (112). In experimental *A. fumigatus* keratitis, Yap1, superoxide dismutases and the thioredoxin system supported fungal survival against neutrophil-derived oxidative stress (14). This protection against host killing does not itself demonstrate resistance to antifungal drugs (14). In *C. albicans*, both growth temperature and chromosome copy-number changes affect fluconazole tolerance (113). Tolerance should therefore not be classified solely by reversibility. These findings support further preclinical evaluation of fungal stress-response inhibitors alongside antifungal therapy (14,110). Such studies should establish effective corneal exposure and determine whether improved fungal clearance can be achieved without compromising host defense or tissue repair. Table I summarizes factors affecting antifungal susceptibility and treatment response. Evidence from keratitis is distinguished from findings in non-ocular models.

7. Novel therapeutic strategies

Natural compounds with dual antifungal and anti-inflammatory effects. Several natural compounds have shown antifungal and anti-inflammatory effects in experimental fungal keratitis (36,114,115). Isorhamnetin, a plant-derived flavonoid, inhibited *A. fumigatus* growth and altered hyphal membrane integrity *in vitro* (114). In infected mice, it reduced corneal fungal burden, neutrophil recruitment and inflammatory mediator expression (114). Kaempferol, another natural flavonoid, also reduced fungal burden and inflammatory cell recruitment in *A. fumigatus*-infected mouse corneas (115). Perillaldehyde, a monoterpenoid found in *Perilla frutescens*, inhibited *A. fumigatus* growth *in vitro* and reduced corneal fungal burden and disease severity in mice (36). Rutin, a flavonoid glycoside, similarly inhibited fungal growth and disrupted fungal structures *in vitro*, with lower fungal burden and inflammation in experimental *A. fumigatus* keratitis (78). Thymoquinone, a quinone constituent of *Nigella sativa* seeds, inhibited *C. albicans* growth *in vitro* and reduced corneal fungal burden and inflammation in a mouse model of *C. albicans* keratitis (79). In corneal epithelial cell experiments, Nrf2

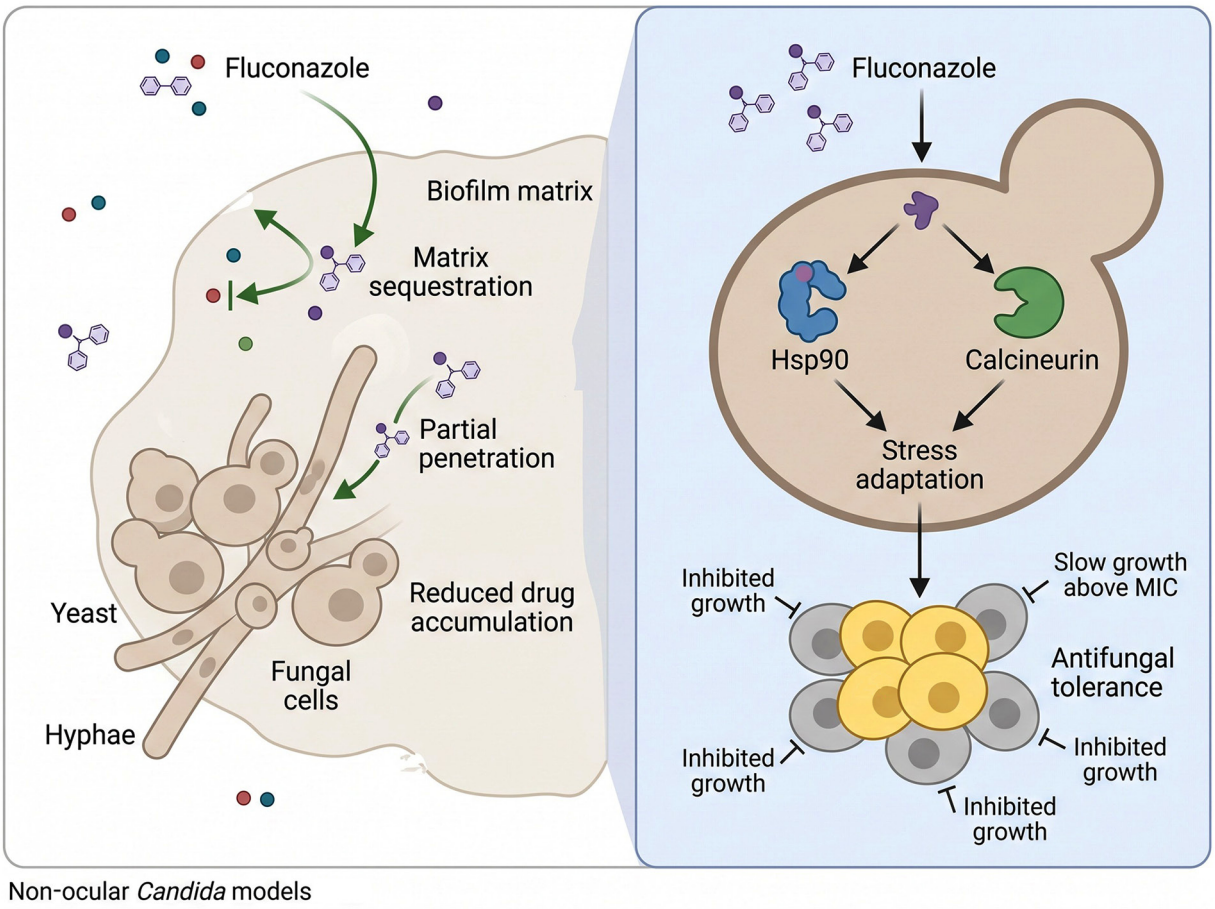


Figure 8. Biofilm protection and antifungal tolerance in non-ocular *Candida albicans* models. The biofilm matrix sequesters fluconazole without completely preventing penetration. Cellular stress adaptation involves Hsp90 and calcineurin. Yellow cells represent slow growth above the MIC, while gray cells show growth inhibition. These mechanisms do not establish the cause of treatment failure in fungal keratitis. Hsp90, heat shock protein 90; MIC, minimum inhibitory concentration.

or HO-1 inhibitors attenuated the anti-inflammatory effects of perillaldehyde and thymoquinone, supporting involvement of this pathway (36,79). Reduced inflammation *in vivo* can reflect both host modulation and a lower fungal burden. Isorhamnetin also suppressed inflammatory mediator expression in cells exposed to inactivated fungal hyphae, supporting an effect beyond fungal killing (114). However, changes in receptor expression or downstream phosphorylation alone do not establish direct inhibition of a specific molecular target. These findings support further investigation of natural compounds as adjunctive candidates, but do not establish clinical efficacy. Translation will require effective corneal exposure, acceptable ocular toxicity and reproducible activity across clinical isolates. Because the cited corneal studies of these compounds primarily examined *A. fumigatus* or *C. albicans*, and antifungal susceptibility and host responses vary among fungal species, activity against *Fusarium* and dematiaceous fungi needs separate evaluation. Comparisons with standard antifungal treatment should establish whether adding a candidate compound improves fungal clearance and corneal healing. Treatment timing also requires evaluation to avoid weakening protective antifungal immunity.

Prospects of immunomodulatory therapy. Host-directed immunomodulation aims to limit corneal injury while preserving antifungal defense. Dectin-1 illustrates the difficulty of separating these effects. In mice infected with an *A. fumigatus* corneal isolate, Dectin-1 deficiency reduced early corneal opacity but increased fungal survival (11). This increase in fungal survival was not observed with the laboratory strain Af293.1RFP. Laminarin reduced corneal inflammation in a separate murine *A. fumigatus* model, but dosing began before infection and fungal burden was not quantified (12). These findings do not establish a safe window for therapeutic Dectin-1 inhibition. Preserving neutrophil function is also important because NADPH oxidase-dependent killing contributes to the control of *Aspergillus* and *Fusarium* in experimental keratitis (14). Candidate interventions should therefore be assessed for both tissue protection and fungal clearance. Nrf2-directed interventions provide another experimental approach. Dimethyl itaconate treatment initiated after infection reduced corneal inflammation and fungal burden in mice with *A. fumigatus* keratitis (37). Pharmacological inhibition of Nrf2 or HO-1 weakened its anti-inflammatory effect in stimulated corneal epithelial cells (37). Dimethyl fumarate also reduced fungal burden and inflammatory injury in murine *A. fumigatus* keratitis (76). In corneal epithelial cells, Nrf2 silencing attenuated its suppression of pyroptosis-related proteins, including cleaved caspase-1 and cleaved gasdermin D (76). In cell-free *A. fumigatus* cultures, dimethyl itaconate

Table I. Factors affecting antifungal susceptibility and treatment response in fungal keratitis.

Fungal species	Antifungal agent or class	Mechanism or contributing factor	Evidence in keratitis	Clinical relevance and limitations
F. solani species complex	Azoles	CYP51 sequence variation and increased *CYP51A* expression following azole exposure	Mechanistic studies in non-ocular isolates and fungal cultures	Candidate contributors to low azole susceptibility. These findings do not establish acquired resistance during corneal infection
A. fumigatus	Itraconazole	Cyp51A G54E substitution associated with itraconazole resistance	A resistant isolate recovered from a patient with keratitis	The patient had no prior azole exposure. The isolate remained susceptible to voriconazole and posaconazole
C. albicans	Fluconazole and voriconazole	Target alterations encoded by *ERG11* variants	Functional testing of clinically derived variants in laboratory strains. Keratitis was not evaluated	Supports a molecular basis for reduced azole susceptibility. Its contribution to corneal treatment failure requires direct investigation
F. keratoplasticum	Azoles	Drug efflux supported by functional expression of ATP-binding cassette transporter 1	Transporter studies using a clinical isolate and a heterologous yeast host. Keratitis was not evaluated	Supports an efflux mechanism. Its contribution to drug response during corneal infection remains unverified
C. lusitaniae	Amphotericin B	Disruption of *ERG6* and altered ergosterol biosynthesis	Non-ocular laboratory and clinical-isolate studies	Supports sterol-dependent amphotericin B resistance. The findings do not establish an equivalent mechanism for natamycin in keratitis
Fusarium spp., *Cladosporium sphaerospermum* and *Acremonium implicatum*	Natamycin and amphotericin B	Reduced antifungal susceptibility during biofilm development	Biofilms formed by corneal isolates. A 14-isolate *Fusarium* series also included clinical outcomes	Drug effects varied by species. Associations with more complex treatment do not establish biofilms as an independent cause of clinical failure
C. albicans	Fluconazole	Sequestration by the extracellular biofilm matrix, with reduced intracellular drug accumulation	Non-ocular biofilm experiments	Provides a drug-specific explanation for biofilm protection. Direct confirmation in corneal infection is lacking in the cited study
A. fumigatus	Voriconazole and amphotericin B	Hypoxic microenvironments within mature biofilms reduce drug susceptibility	Non-ocular laboratory biofilm models	Supports a physiological contribution to reduced susceptibility. Its importance in corneal biofilms remains unverified
C. albicans	Amphotericin B	Survival of small biofilm subpopulations after high drug exposure, described as persistence	Conflicting findings from non-ocular biofilm studies	Improved drug exposure reduced apparent survivors in a later study. Persister-mediated treatment failure has not been established in keratitis
C. albicans	Fluconazole	Slow growth of a subpopulation above the MIC. Temperature and chromosome copy number influence this phenotype	Non-ocular laboratory and clinical-isolate studies	Distinct from resistance measured by MIC. Tolerance cannot be classified solely by reversibility, and its predictive value in keratitis remains uncertain
C. albicans	Fluconazole	Hsp90-dependent biofilm drug responses and calcineurin-associated stress survival	Hsp90 studied in non-ocular biofilms. Calcineurin evaluated in mouse keratitis	Calcineurin inhibition improved fluconazole treatment response in mice. These results do not directly establish Hsp90-mediated treatment failure in keratitis

Table I. Continued.

Fungal species	Antifungal agent or class	Mechanism or contributing factor	Evidence in keratitis	Clinical relevance and limitations
C. albicans	Echinocandins	Increased chitin synthesis compensates for inhibition of cell wall glucan synthesis	Non-ocular laboratory experiments	Supports an adaptive cell wall response. Its contribution to treatment failure in keratitis remains unverified
Not species-specific	Topical natamycin	Formulation-dependent corneal penetration	Permeation experiments using excised goat corneas	Nanoparticles increased permeation compared with an unformulated suspension. This addresses drug delivery rather than fungal resistance and does not establish improved clinical outcomes

CYP51, sterol 14α-demethylase; ERG, ergosterol biosynthesis gene; MIC, minimum inhibitory concentration; Hsp90, heat shock protein 90; *C. albicans*; *Candida albicans*; *A. fumigatus*; *Aspergillus fumigatus*.

at 2-20 mM inhibited growth during 48 h incubation at 37˚C in minimal medium containing 2% glycerol as the sole carbon source (37). Dimethyl fumarate also inhibited *A. fumigatus in vitro* in growth-inhibition assays used to determine the MIC and reduced biofilm formation and conidial adherence (76). Their effects on fungal burden therefore cannot be attributed solely to host immune modulation. Enhancing protective immune functions offers a complementary strategy. In murine *F. solani* keratitis, oral vitamin C increased neutrophil recruitment (91). Complementary cell experiments showed no increase in ROS production or NET formation (91). Combining vitamin C with amphotericin B reduced corneal fungal burden more than either treatment alone (91). These findings remain preclinical. Future studies should compare antifungal monotherapy with adjunctive treatment initiated at defined stages of infection. Early fungal control and later tissue recovery should be evaluated together, without assuming a clear separation between protective and damaging inflammation. Serial measurements of viable fungal burden should accompany assessments of epithelial healing and stromal scarring. Dose, treatment duration, pathogen identity and ocular drug exposure require evaluation to define a therapeutic window.

Anti-biofilm strategies and drug delivery systems. Biofilms formed by corneal isolates can show reduced antifungal susceptibility, providing a rationale for investigating biofilm-targeted adjuncts (55). In a non-ocular *C. albicans* biofilm model, adding DNase enhanced amphotericin B activity but did not improve fluconazole susceptibility (116). This drug-specific effect does not establish efficacy in corneal infection. A nanosystem combining lyticase with gallium degraded extracellular polysaccharides and showed activity against mature *C. albicans* biofilms *in vitro* (117). Therapeutic benefit was also reported in a mouse keratitis model, providing preclinical support for combining matrix disruption with antifungal activity (117). Drug delivery systems aim to improve antifungal exposure at the site of infection. Natamycin-loaded solid lipid nanoparticles provided sustained release and greater permeation across excised goat corneas than an unformulated natamycin suspension (118). A natamycin-loaded niosomal gel containing ketorolac and 4% sodium carboxymethyl cellulose reduced corneal infiltration, ciliary injection and hypopyon in rabbits with *Aspergillus* keratitis. Histological examination showed less inflammation and fewer fungal colonies than with the alternative gel formulation or mixed marketed eye drops (119). These outcomes do not demonstrate elimination of a mature corneal biofilm. Improved drug delivery also does not establish reversal of genetic resistance. Photodynamic activity depends on the photosensitizer and irradiation conditions. Rose bengal with green light inhibited the growth of *A. fumigatus*, *F. solani* and *C. albicans* keratitis isolates *in vitro* (120). Riboflavin with ultraviolet A irradiation did not produce notable inhibition under the tested conditions (120). This assay did not evaluate established corneal biofilms. A randomized trial involving 330 participants, including 301 with filamentous fungal keratitis, found no overall improvement in 6-month visual acuity with adjunctive rose bengal photodynamic therapy (121). At 12 months, no benefit was detected for visual acuity, scar size or the combined outcome of corneal perforation and therapeutic penetrating keratoplasty (122).

Table II. Evidence and translational status of selected therapeutic strategies for fungal keratitis.

Therapeutic strategy	Evidence source and pathogen	Main findings	Translational status and limitations
Topical natamycin 5%	Randomized clinical trial involving 323 patients with filamentous fungal keratitis	Improved visual outcomes and fewer perforations or therapeutic keratoplasties than topical voriconazole 1%. Much of the benefit was attributable to *Fusarium* infections	Established treatment supported by clinical evidence. The comparative benefit was not uniform across fungal groups
Natural flavonoids including isorhamnetin, kaempferol and rutin	Separate mouse models of *A. fumigatus* keratitis and complementary laboratory experiments	Reduced corneal fungal burden and inflammation. Reported changes in inflammatory signaling differed among compounds	Preclinical evidence. Changes in receptor expression or phosphorylation do not establish direct molecular targeting. Clinical efficacy remains unestablished
Dimethyl itaconate and dimethyl fumarate	Mouse models of *A. fumigatus* keratitis and corneal epithelial cell experiments	Reduced fungal burden and inflammatory injury, with evidence supporting Nrf2-associated anti-inflammatory effects. Both compounds also inhibited fungal growth *in vitro*	Preclinical evidence. Host modulation and direct antifungal activity require further separation. Effective ocular exposure and treatment timing remain unresolved
Vitamin C combined with amphotericin B	Mouse *F. solani* keratitis and complementary cell experiments	Vitamin C increased neutrophil recruitment. Combined treatment reduced corneal fungal burden more than either treatment alone	Preclinical evidence. The study included vitamin C supplementation before infection. Benefit from supplementation initiated after diagnosis requires evaluation
Fluconazole combined with cyclosporine A	*C. albicans* keratitis in immuno-suppressed mice	Combined topical treatment accelerated disease resolution compared with either drug alone. Genetic experiments also implicated fungal calcineurin in pathogenicity	Preclinical evidence. Fungal effects and effects on host immunity require separate evaluation before clinical translation
DNase combined with amphotericin B	Non-ocular *C. albicans* biofilms studied *in vitro*	DNase increased amphotericin B activity against biofilm cells. It did not improve fluconazole susceptibility	Early mechanistic evidence. The effect was drug dependent and was not evaluated in corneal infection
Gallium and lyticase integrated nanosystem	*C. albicans* biofilm experiments and a mouse keratitis model	Degradation of extracellular polysaccharides accompanied activity against mature biofilms. Therapeutic benefit was also reported in infected corneas	Preclinical evidence. Activity against mature biofilms *in vitro* does not establish their eradication within infected corneas
Natamycin-loaded niosomal gel containing ketorolac	Rabbit *Aspergillus* keratitis	The tested formulation improved corneal infiltration and histological findings compared with the comparator formulations	Preclinical evidence. The findings do not establish eradication of mature biofilms or reversal of genetic resistance
Adjunctive rose bengal photodynamic therapy	Randomized clinical trial involving 330 participants, including 301 with filamentous fungal keratitis, with follow-up to 12 months	No overall visual benefit at 6 or 12 months. At 12 months, scar size and perforation or therapeutic keratoplasty outcomes also showed no benefit	Clinical evaluation without demonstrated overall benefit for the tested protocol. Other photosensitizers and treatment schedules require separate assessment
Topical manogepix	Clinical keratitis isolates of *C. albicans*, *F. solani* and *Aspergillus flavus*, and rabbit *F. solani* keratitis	The Gwt1 inhibitor inhibited fungal growth *in vitro*. Topical treatment reduced corneal fungal counts and disease severity compared with untreated controls	Direct preclinical evidence in keratitis. Human ocular dosing, safety and comparative efficacy remain unestablished

Table II. Continued.

Therapeutic strategy	Evidence source and pathogen	Main findings	Translational status and limitations
Systemic fosmanogepix	Open-label phase 2 study in adults with invasive mold disease and registered phase 3 trials in systemic fungal infections	The prodrug of manogepix has undergone clinical evaluation through intravenous and oral administration. Phase 3 programs address invasive mold infections and candidemia or invasive candidiasis	Clinical development for systemic infections. These studies do not establish effective ocular dosing or clinical efficacy in fungal keratitis
Fosmanogepix combined with liposomal amphotericin B	Mouse models of invasive aspergillosis, mucormycosis and fusariosis	Combined treatment improved survival compared with either agent alone	Indirect preclinical evidence from non-ocular infections. Benefit in corneal infection requires direct evaluation

A. fumigatus, *Aspergillus fumigatus*; *C. albicans*, *Candida albicans*; *F. solani*; *Fusarium solani*; Nrf2, nuclear factor erythroid 2-related factor 2; Gwt1, glycosylphosphatidylinositol-anchored wall protein transfer 1.

Alternative photosensitizers and treatment protocols require separate evaluation. Future studies should distinguish prevention of biofilm formation from activity against established biofilms. Viable fungal counts and matrix measurements should accompany drug-release and corneal-exposure studies. Comparisons with standard antifungal treatment should assess microbiological clearance, epithelial healing, visual outcomes and toxicity across relevant fungal species. Reproducible manufacture, formulation stability and feasible dosing schedules are also needed for clinical translation.

Emerging antifungal agents and combination therapy. Fosmanogepix is an investigational prodrug of manogepix, which inhibits the fungal inositol acyltransferase glycosylphosphatidylinositol-anchored wall protein transfer 1 and disrupts glycosylphosphatidylinositol anchor biosynthesis (123,124). An open-label phase 2 study evaluated systemic fosmanogepix in adults with invasive mold disease (125). Phase 3 trials are evaluating its efficacy in invasive mold infections (NCT06925321) and candidemia or invasive candidiasis (NCT05421858). These trials address systemic infections and do not establish efficacy in fungal keratitis.

Direct evidence from corneal infection models is emerging. Manogepix inhibited clinical keratitis isolates of *C. albicans*, *F. solani* and *A. flavus in vitro* (124). In a rabbit model of *F. solani* keratitis, topical manogepix reduced corneal fungal counts and disease severity compared with untreated controls (124). Checkerboard testing identified synergy between manogepix and amphotericin B in one of three *C. albicans* isolates (124). Separately, fosmanogepix combined with liposomal amphotericin B improved survival compared with either drug alone in mouse models of invasive mold infection (126). These non-ocular findings provide further support for investigating combination therapy in corneal infection. A randomized clinical trial also evaluated a natamycin-based combination, and results demonstrated that adding topical voriconazole 1% to natamycin 5% did not significantly improve ulcer healing compared with natamycin plus placebo; of note, both groups also received oral ketoconazole (127). Table II summarizes selected therapeutic strategies, the sources of supporting evidence and their main translational limitations.

8. Conclusions and future perspectives

Fungal keratitis involves fungal invasion and host responses that can both contain infection and damage the cornea (11,14,15). The present review associates these processes to antifungal susceptibility and tissue drug exposure to frame the interpretation of treatment failure. Why some patients control infection while others develop destructive inflammation remains unresolved. In mouse *A. fumigatus* keratitis, the requirement for Dectin-1 in fungal clearance varied with the infecting strain (11). Comparisons across *Aspergillus*, *Fusarium*, *Candida* and dematiaceous fungi should clarify how pathogen variation and host factors shape disease outcomes.

The timing and extent of immune modulation require direct evaluation. Neutrophil oxidative killing supports fungal control in experimental keratitis (14). Protective and damaging responses should therefore not be assigned to strictly separate disease stages. Future studies should test adjunctive

treatment at defined times after infection and alongside effective antifungal therapy. Serial viable fungal counts should accompany measurements of epithelial healing, stromal injury and visual function. Reduced inflammation alone cannot establish therapeutic benefit when impaired fungal clearance remains possible (11).

Clinical treatment failure also requires precise interpretation. Acquired resistance, intrinsic low susceptibility and biofilm-associated tolerance should be distinguished from inadequate corneal drug exposure, as summarized in Table I. In clinical keratitis, the association between susceptibility and treatment outcome differed between natamycin and voriconazole (26). Larger infiltrates and deeper stromal involvement at presentation have also been associated with poor outcomes (32,33). Prospective studies should integrate susceptibility testing with pathogen identification, drug exposure and clinical assessment to determine when laboratory findings predict treatment response.

Therapeutic strategies differ in their readiness for clinical use (Table II). Topical natamycin is supported by randomized clinical evidence in filamentous fungal keratitis (28). Natural compounds, agents that modulate Nrf2 and biofilm-directed nanosystems have shown benefit in experimental keratitis (37,114,117). Topical manogepix has direct preclinical evidence in keratitis, whereas the cited clinical fosmanogepix study addressed invasive mold disease (124,125). The lack of overall benefit with adjunctive rose bengal photodynamic therapy reinforces the need for controlled clinical validation (121,122). Candidate adjuncts require evaluation of corneal exposure, local toxicity and benefit over antifungal monotherapy.

The narrative design of the present review and its reliance on experimental models limit clinical inference. Its focus on PubMed and English-language publications also creates a risk of selection bias. Mechanisms derived from non-ocular infections require validation in the cornea. Human corneal samples and prospective clinical data should be used to test associations between fungal burden, host responses and treatment outcomes. Biomarkers require validation before they can guide immune intervention and clinical translation will require evidence of improved infection control and preservation of corneal function.

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Authors' contributions

NW was responsible for writing the original draft, designing the methodology and investigation (literature searching and study selection). LL was responsible for reviewing and editing the manuscript, and for conceptualization, supervision, project administration and validation (checking the accuracy of the literature synthesis and its interpretation). All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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