A Review of Current and Novel Approaches for Treatment of Keratitis

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Keratitis is an ocular disease characterized by inflammation of the cornea. If not treated quickly and efficiently, keratitis can lead to ocular morbidity. It is categorized, based on causal agent, into non-infectious and infectious keratitis. Non-infectious keratitis is commonly due to ocular injury or prolonged use of contact lenses and can lead to opportunistic infection; these infectious agents consist of HSV-1, *Staphylococcus*, *Candida*, and *Acanthamoeba*, among others. Treatment of infectious/microbial keratitis differs depending on the pathogen and each patient requires unique care and treatment. Topical administration of drugs, via eyedrops, is the most common form of initial treatment for keratitis. However, physiological barriers within the eye lead to poor bioavailability of drug from eyedrops, and frequent administration is needed to overcome this challenge. Since conventional treatment is not ideal, it is important to develop novel drugs and delivery methods that are more appropriate for treating ocular conditions and protect the corneal epithelium. Novel pharmaceutical approaches, such as drug-loaded contact lenses, in situ gel formulations, and nanoparticle-carriers are some of the most promising novel drug delivery methods currently being investigated. They are being used to deliver common antimicrobial agents such as nucleosides, fluroquinolones, and steroids, but with greater ocular bioavailability. A novel non-pharmaceutical approach, corneal crosslinking, has also been developed to treat keratitis. This review will discuss these current treatment options and conventional challenges regarding keratitis, with an emphasis on keratitis due to microbial pathogens.

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1.0 Introduction

Keratitis is an ocular condition characterized by inflammation of the cornea. The cornea is the transparent covering of the eye that serves as a physical barrier and is responsible for refraction of light onto the retina. It is composed of five different layers listed in Figure 1 (Cleveland 2018), with the corneal epithelium as the outermost layer that interacts with the tear film to wash and protect the eye. The epithelium itself is composed of 5-7 layers of nonkeratinized cells and is about 50 μm in thickness (Voss, Nguyen et al. 2021). Infiltration of the corneal epithelium by inflammatory cells can lead to ocular morbidity, especially with deeper corneal infiltrates.

Inflammatory cell migration and proliferation can be in response to non-infectious factors, such as ocular trauma, improper contact lens use, or autoimmune diseases, as well as pathogens like various viruses, bacteria, fungi, or amoeba. These causes may be systemic or localized to the corneal epithelium; in the case of the former, systemic antigens and viruses can cause corneal damage by traveling through the trigeminal ganglia and ophthalmic nerves to the eye, facilitating corneal inflammation (Koganti, Yadavalli et al. 2021). Ocular trauma can cause disruption of the corneal epithelium and also compromise its protective function, facilitating local opportunistic infection. Local infection begins at the epithelium, recruiting leukocytes and macrophages; these inflammatory cells, in addition to the pathogen itself, release hydrolases and proteases that contribute to the condition (Singh, Gupta et al. 2021). Further progression can result in epithelial ulceration and stromal infiltration, damaging the structural integrity of the cornea.

Although keratitis can develop in any individual, it is predominantly found in contact lens wearers; prolonged/improper contact lens use is the primary risk factor for developing keratitis

and it is estimated that almost 17% of adults wear contact lenses daily in the United States (Scruggs, Quist et al. 2019). Furthermore, it is estimated that $175 million is spent annually on keratitis treatment, with nearly one million doctor's office or emergency room visits throughout the US (Collier, Gronostaj et al. 2014).

Typical symptoms of keratitis include eye pain/burning, redness, and blurred vision. In severe cases of keratitis, or if infection progresses without adequate treatment, partial or total vision loss can occur. Therefore, it is important to develop efficacious treatments that can prevent progression of the condition in an individual. Although the eye is accessible and immunologically isolated, various physiological barriers, including the corneal epithelium itself, pose a challenge for drug delivery. Conventional treatment involves the use of drug-loaded eyedrops administered multiple times throughout the day; however, as will be discussed in this review, topical treatment is not ideal. As a result, various novel drugs and delivery methods are currently being investigated, both in animal models as well as human patients; these novel approaches for treatment of keratitis will be reviewed.

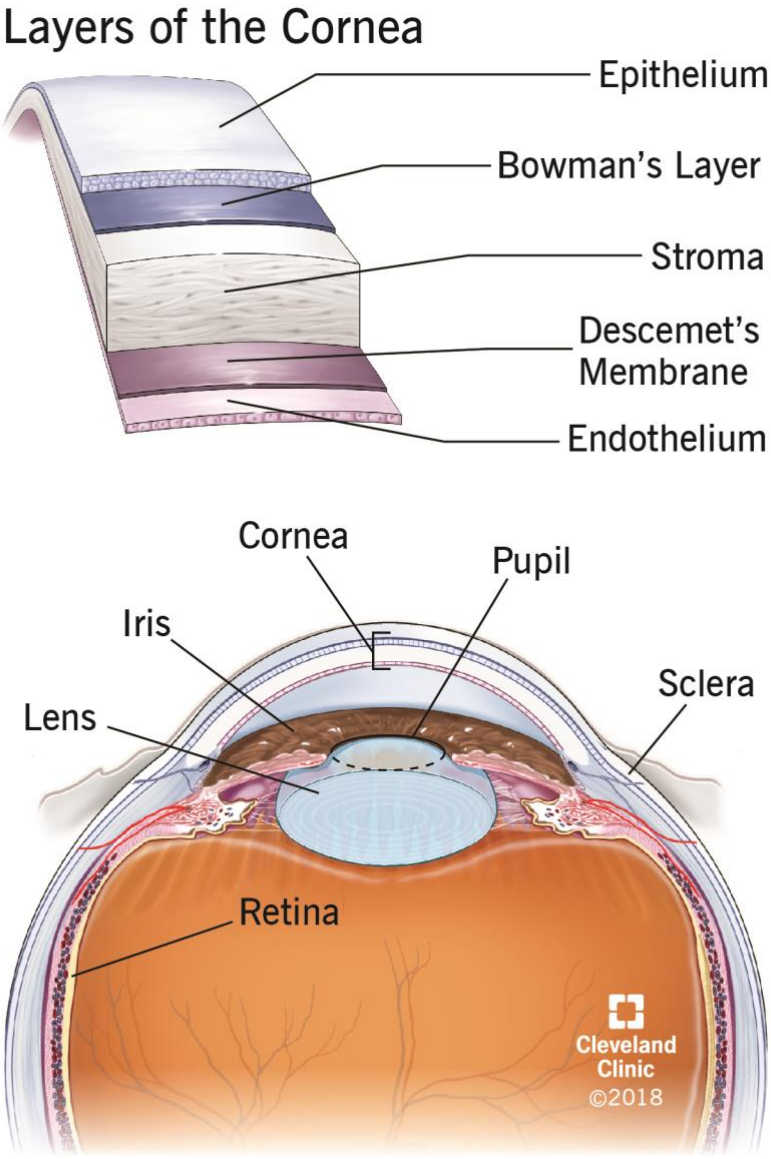

Figure 1. Anatomy of the eye and layers of the cornea. (Taken from Cleveland Clinic. https://my.clevelandclinic.org/health/treatments/17714-cornea-transplant)

2.0 Non-infectious Keratitis

Non-infectious keratitis has a variety of potential causes, separate from infection from a pathogen. A common cause is ocular injury, be it via fingernail scratching, contact lenses, or another foreign object. Various disorders can also cause a loss of corneal sensitivity, which leads to a decrease in protective function like blinking, ultimately leading to keratitis. Similarly, in cases of systemic conditions like rheumatoid arthritis (RA) that result in severe dry eye, a lack of tear film that washes the cornea can lead to keratitis (Itty, Pulido et al. 2008). Exposure to intense UV light can also cause damage to the cornea, a condition known as photokeratitis, which can be caused by reflection of sunlight without proper eye protection. These causes of non-infectious keratitis are important risk factors for infectious keratitis if microbes are able to infiltrate the corneal epithelium, either by direct entry via injury, prolonged exposure from contaminated contacts, or epithelial susceptibility from an autoimmune disease (Voss, Nguyen et al. 2021).

Current treatment options for non-infectious keratitis depend on the respective cause, but general treatment consists of anti-inflammatory treatments and lubrication (Voss, Nguyen et al. 2021). Topical corticosteroids, in combination with artificial tears, are usually sufficient to treat mild cases of non-infectious keratitis due to foreign injury/irritation. If an individual is unresponsive to this initial treatment, steroid-sparing agents like 2% cyclosporine A or 0.02% tacrolimus have be utilized to safely resolve symptoms (Shoughy and Tabbara 2020). For systemic causes like RA, immunosuppressants such as methotrexate are used (Singh, Gupta et al. 2021). In more severe cases, systemic suppression or amniotic membrane grafting may be necessary. For neurotrophic keratitis, a specific type of non-infectious keratitis caused by damage to trigeminal innervation of the cornea, Cenergermin was recently approved by the FDA as a treatment option

(Deeks and Lamb 2020). This drug is an ophthalmic solution containing 20 ug/mL of recombinant human nerve growth factor. It is the first approved drug for treatment for this condition. When administered topically to patients six times a day, for eight weeks, corneal healing was significantly improved; however, it did not show significant improvement in corneal sensitivity or visual acuity (Deeks and Lamb 2020). This, combined with frequent administration, are current challenges with Cenergermin, but is a possible alternative to surgical treatment options that involve corneal gluing using cyanoacrulate-based adhesive, corneal transplant, or keratoplasty (Koganti, Yadavalli et al. 2021).

With all causes of non-infectious keratitis, novel drug delivery methods are being used to provide sustained release of the therapeutic agent. These methods, such as use of nanocarriers or drug-eluting ocular inserts, are also being investigated to treat infectious keratitis. Since emphasis is being put on their application for the latter type of keratitis, they will be discussed later in the review.

3.0 Infectious Keratitis

Infectious, or microbial, keratitis is the predominant form of keratitis. In 2010, 76.5% of keratitis clinic visits in the US resulted in an antimicrobial prescription; it is estimated that 71,000 cases of severe infectious keratitis occur each year (Collier, Gronostaj et al. 2014). Two cross-sectional studies in 2014 estimated the prevalence of infectious keratitis in China to be, on average, 0.170%. This amount was further categorized by viral, bacterial, and fungal causes, with an average prevalence of 0.0875%, 0.0715%, and 0.011%, respectively (Koganti, Yadavalli et al. 2021). Quick and effective treatment of infectious keratitis is critical in stopping the progression of the infection. Many first-line treatments target inflammation, but do not necessarily take care of the infection; novel treatments and delivery methods are being researched to directly target the respective infection and reduce the burden of administration. However, many of these novel treatments are in early stages of research. As a result, clinical study is limited, and a majority of development is currently being performed in animal models.

The most common animal models used to study microbial keratitis are mice and rabbits. Advantages of using mouse models are cost, convenience, and experimental consistency. Mice can also be genetically modified to lack certain aspects of a necessary immune response, such as cytokines and toll-like receptors, which increases susceptibility to infection. However, the main disadvantage of using mice is eye size. Rabbit eyes are larger and more similar to humans, which facilitates ocular investigation. The typical rabbit model used is the New Zealand White rabbit (NZW). For both mice and rabbits, bacteria and virulence factors can be injected into the cornea to produce infection, or topical inoculation can be utilized following corneal wounding.(Marquart 2011) These different types of infection are used to study pathogen or host factors as it pertains to

keratitis; the former simulates a direct microbial infection, whereas the latter simulates non-infectious keratitis that evolves through opportunistic infection, a pathology commonly seen in human patients.

3.1 Viral Keratitis

Herpes simplex virus type 1 (HSV-1) is the dominant cause of viral keratitis worldwide. The virus infects roughly two-thirds of the global population, with about 1.5 million individuals developing herpes simplex keratitis (HSK) every year (Koganti, Yadavalli et al. 2021). HSK is also a persistent disease, with 40% of infected individuals experiencing more than one relapse during their lifetime (Koganti, Yadavalli et al. 2021). Therefore, it is important to develop effective treatment methods that not only control symptoms of the disease to prevent ocular morbidity but prevent relapse as well.

HSK treatment typically begins with standard medications, such as antiviral or glucocorticoid treatments. Trifluridine and acyclovir are the two most common antiviral studied in randomized controlled trials and prescribed for treatment of HSK, with the American Academy of Ophthalmology HSK guidelines and Herpetic Eye Disease Study recommending topical administration of these drugs as a first-line defense (Pandey, Choudhury et al. 2020). Trifluridine was the first FDA-approved drug for HSK treatment as a topical 1% ophthalmic solution that could be used every two hours (Koganti, Yadavalli et al. 2021). Trifluridine is a pyrimidine nucleoside that acts non-selectively to inhibit DNA synthesis both in infected and non-infected cells. As a result of this mechanism, however, prolonged use has been met with complications; ocular irritation, local toxicity, and inflammation have been observed after three weeks of daily use

(Pandey, Choudhury et al. 2020). Trifluridine has thus fallen out of favor as a preferred treatment when compared to other antiviral options, but still remains effective for short-term use.

Similar to trifluridine, acyclovir (ACV) is a purine nucleoside but with higher viral selectivity, leading to reduced prevalence of ocular side effects and toxicity (Pandey, Choudhury et al. 2020). ACV is well tolerated both orally and topically. Topical administration of acyclovir can be given up to five times per day and is formulated as a 3% ointment due to ACV's high lipophilicity. However, ACV ointment administration results in lower bioavailability of ACV due to rapid nasolacrimal clearance, as well as blurred vision and difficulty during administration, leading to poor patient compliance (Pandey, Choudhury et al. 2020). As a result, ACV is preferred to be administered orally three-to-five times a day as 400-800mg tablets, in order to treat an active infection. Long-term prophylactic ACV therapy has also been shown to be effective in clinical trials but comes with the risk of systemic toxicity and inducing drug resistance, thereby reducing therapeutic efficacy (Koganti, Yadavalli et al. 2021). Antiviral-resistant strains of HSV in immunocompromised individuals are a particularly significant challenge.

Other analogs of ACV, such as ganciclovir (GCV), cidofovir, and valacyclovir, have been studied as possible treatments for HSK. Topical GCV administration was studied on New Zealand White rabbits; a modified form of GCV, divaline-GCV (VVGCV), was found to have improved transcorneal permeability, and a 1% formulation of this GCV prodrug had greater efficacy when compared to 1% trifluridine (Majumdar, Nashed et al. 2005). Cidofovir has been found to be more effective than GCV when given intravenously for the first two weeks following infection, but must be administered intravenously or topically due to oral toxicity (Koganti, Yadavalli et al. 2021). Topical cidofovir administration was also found to be significantly more effective in treating HSK rabbit models than topical trifluridine and ACV (Romanowski, Bartels et al. 1999).

Glucocorticoid drops such as 0.1% prednisolone, 0.1% dexamethasone, and 0.5% loteprednol are often used in antiviral therapy to increase effectiveness of treatment (Guess, Stone et al. 2007). They have shown to be effective in reducing optical symptoms and inflammation that accompany HSK, but do not target the viral infection directly, so must be combined with antiviral agents discussed above (Koganti, Yadavalli et al. 2021). Furthermore, these treatments also come with some of the challenges as typical topical delivery, such as low bioavailability and blurred vision following administration. Because of these challenges, there is a need for novel drug delivery methods in treating HSK.

A recent ocular insert that delivers G2-C, a cysteine-modified G2 peptide that blocks HSV-1 entry into cells, has shown efficacy in treating HSK *in vivo*. This drug-loaded contact lens provides a more stable form of the G2 peptide for delivery and was capable of extended release (Jaishankar, Buhrman et al. 2016). Cationic peptides like G2 and G1 have repeatedly demonstrated efficacy in *in vitro, ex vivo,* and *in vivo* models (Pandey, Choudhury et al. 2020, Koganti, Yadavalli et al. 2021), but come with their own delivery challenges. Efficacy of these drugs are reduced if applied the day after infection, and the physiochemical properties of peptides make delivery difficult and time-consuming to formulate.

Apart from optical inserts, various novel drug delivery systems are being researched to improve delivery of standard treatments, specifically ACV and GCV. One promising approach is the delivery of in situ gel formulations. These formulations exist as solutions before administration and undergo gelation when in presence of ocular environmental conditions like temperature, pH, and ionic strength. In situ gel systems exhibit a significant improvement in corneal drug contact due to prolonged release; this extended rate of release can be approximately five times slower than conventional topical drug administration (Pandey, Choudhury et al. 2020). Extended release time

equates to greater local bioavailability, improving the therapeutic effect of the drug. This sustained release is also found in nanoparticle formulations that are incorporated in an in situ gel formation. A relevant clinical study performed by Sirion Therapeutics, Inc. resulted in FDA approval in 2009 of an in situ ophthalmic gel named Zirgan, which contains 0.15% GCV. In addition to the antiviral drug, carbomer 974P, water, sodium hydroxide, mannitol, and benzalkonium chloride are included in the formulation. This product is proven to significantly improve clinical effectiveness for patients with HSK with minimal side effects (Kaufman and Haw 2012).

Various lipid-based nanocarriers are also being investigated for delivery of standard HSK treatments. Liposomes have a greater ability to interact with the corneal epithelium, and when combined with their small size, show improved ocular drug penetration and bioavailability; this especially beneficial when delivering poorly permeable antiviral drugs like ACV and GCV. Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) are similar to liposomes, but are more stable, less toxic, and able to be manufactured at a lower cost. These novel delivery methods have been shown to provide sustained release of antivirals, with NLCs exhibiting quicker drug release and more extensive corneal permeation when compared to SLNs, but both provide greater drug uptake by corneal epithelia cells than conventional suspensions (Pandey, Choudhury et al. 2020).

Current investigation regarding alternatives to nucleoside analog therapy consist primarily of CRISPR/Cas9 technology applications. CRISPR/Cas9 has been used to successfully target herpes simplex viruses in multiple *in vitro* studies by manipulating viral miRNA, which can reduce the recurrence of keratitis post-infection (Koganti, Yadavalli et al. 2021). While this therapy is promising, there is a lack of *in vivo* study regarding safety and efficacy.

3.2 Bacterial Keratitis

Bacterial keratitis is most commonly caused by *Staphylococcus aureus, Streptococcus pneumoniae,* and *Pseudomonas aeruginosa*. The primary risk factor for this type of infectious keratitis is prolonged use of contact lens; the risk of bacterial keratitis increases 9-15 fold if an individual sleeps with contacts overnight (Al-Mujaini, Al-Kharusi et al. 2009). Extended contact lens use, especially with those meant to be replaced daily, induces hypoxia, thereby increasing corneal temperature and decreasing tear flow over the corneal surface. The tear film serves a protective function for the cornea, so impaired tear production prevents the elimination of infectious microbes that come into contact with the corneal surface.

Conventional therapy for bacterial keratitis consists of hourly administration of broad spectrum antibiotics (fluoroquinolones). These topical antibiotics are used as an initial treatment while culture results are being analyzed. Some FDA-approved quinolone antibiotics for treatment of BK are ciprofloxacin 0.3%, ofloxacin 0.3%, and levofloxacin 1.5% (Egrilmez and Yildirim-Theveny 2020). After culture reports are available and response to initial treatment is evaluated, targeted treatment can be administered to the specific strain of bacteria present. For instance, moxifloxacin and gatifloxacin show higher potency against Gram-positive bacteria as opposed to Gram-negative organisms; if gram-negative organisms are found, ciprofloxacin may prove more effective (Lee, Somerville et al. 2021). In the case of fluoroquinolone-resistance bacteria, like MRSA, where an individual does not respond to initial antibiotic treatment, more potent antibiotics like vancomycin are effective in topical concentrations of 10-50 mg/mL (Egrilmez and Yildirim-Theveny 2020). If targeted treatment is successfully given, clinical trials have shown that monotherapy with fluoroquinolones can be as effective as combination therapy with fortified antibiotic eye drops but with less toxicity (Al-Mujaini, Al-Kharusi et al. 2009, Wong, Gangwani

et al. 2012). Although antibiotic eyedrops are a common first-line treatment, frequent administration is inconvenient and tedious for the patient, causing an overall reduction in compliance. As a result, novel drug delivery methods are needed to increase compliance and therefore efficacy of treatment.

Many of the novel drug delivery methods being studied to treat viral keratitis are also being studied for bacterial keratitis. Contact lenses have been developed to release antimicrobial drugs and drug-containing liposomes onto the cornea, such as ciprofloxacin, with demonstrated efficacy in *ex vivo* rabbit models (Hui, Willcox et al. 2014) A moxifloxacin (MF) and dexamethasone (DM)-eluting contact lens is another one of these non-standard treatment methods currently being investigated (Gade, Nirmal et al. 2020). In this study, a polymeric contact lens using chitosan, glycerol, and PEG was developed along with MF and DM. Drug-loaded contact lenses were tested with a combination of drugs as well as individually, and all three lenses were compared to treatment with individual drug solutions. The combined drug-loaded contact lens achieved the required therapeutic concentration of MF and showed significantly greater corneal drug distribution when compared to the individual drug solutions given topically to rabbits and humans. Furthermore, the contact lens showed mucoadhesion as well as both *in vitro* and *in vivo* antimicrobial activity (Gade, Nirmal et al. 2020). Moxifloxacin-loaded nanoparticles have also shown increased corneal penetration compared to solution, and in situ gel formulations can be combined with these nanoparticles for improved therapeutic effect (Lee, Somerville et al. 2021).

Apart from lenses offering sustained release of drugs, antimicrobial compounds have been incorporated into the lens itself; AGMNA, a metal organic framework featuring silver (a natural antimicrobial agent), has been developed both for inclusion into the contact lens structure and as a lens disinfecting agent, with high effectiveness and minimal toxicity (Rossos, Banti et al. 2020).

Furthermore, a combined *in vitro* and *in vivo* study showed that microemulsions demonstrate improvement in delivering antibiotic drugs to the epithelium in rabbit keratitis (Lee, Somerville et al. 2021). Combining conventional fluoroquinolone therapy with microemulsion delivery systems may improve the efficacy and tolerability of these drugs. Various novel antimicrobial peptides have been studied that are effective against *S. aureus*, which may prove beneficial in treating cases of BK caused by antibiotic-drug resistant bacteria. Some of these peptides include LyeTxI-b, D-Arg_4-Leu_{10}-Teixobactin, and RP442/3/4, which work by altering cellular interactions with the bacterial membrane. However, these peptides come with concerns over toxicity and stability, and thus need to be investigated further (Lee, Somerville et al. 2021).

Cysteine protease inhibitors have been implied as a potential therapeutic agent for treating BK; upon infection, bacterial pathogens secrete cysteine proteases which degrade proteins in the corneal environment. Surfactant protein D (SP-D), a removal agent typically present in the tear film, aggregates bacteria and facilitates pathogenic clearance. SP-D knockout mice were genetically modified to have increased susceptibility to bacterial keratitis, partly due to increased vulnerability to pathogenic secretion of these proteases. Treating SP-D KO mice with a cysteine protease inhibitor recovered the antimicrobial effects of (SP-D) (Zhang, Abdel-Razek et al. 2015). Further investigation is needed to assess whether these inhibitors can be effective therapeutic agents.

3.3 Fungal Keratitis

Fungal keratitis (FK) is primarily caused by filamentous (*Fusarium*, *Aspergillus*) and yeast-like (*Candida*) fungi. The major risk factor for developing fungal keratitis is ocular trauma

with vegetative matter, typically within rural farming areas of developing countries. In developed countries like the US, contact use is the primary risk factor (Ng, Fraunfelder et al. 2013).

Treatment of fungal keratitis involves oral/topical antifungal medications specific to the type of fungi responsible for the condition. Natamycin is the preferred first-line treatment for filamentous fungi and was the first approved antifungal agent for FK. It is administered hourly as 5% eyedrops; however, natamycin has poor penetration into the cornea, so is typically combined with 1% topical voriconazole if no significant therapeutic benefit is observed. Voriconazole features a wider therapeutic window along with broad antifungal activity, but has been reported to have minimal effect when given alone, an effect that may not be significantly different than placebo (Singh, Gupta et al. 2021). Clinical study has shown a similar therapeutic benefit to natamycin when the two drugs are given together; since natamycin is commercially available, it is usually preferred as initial treatment (Ansari, Miller et al. 2013). However, because natamycin has poor penetration and is ineffective against *Candida* fungi, it is limited to superficial FK.

In the case of *Candida* infection, Amphotericin B can be administered topically (0.15%) or intracamerally with significant benefit. Although, it is less effective against *Fusarium* species, has similar penetration to natamycin, and can be toxic if administered intravenously in high doses (Ansari, Miller et al. 2013). Fluconazole is another option for elimination of *Candida* in cases where greater intraocular penetration is required. It can be given topically, orally, or intravenously with minimal side effects. Topical fluconazole has been shown to effectively treat FK in individuals who do not respond to Amphotericin B and can be given as an adjunct to natamycin or Amphotericin B, depending on the species of fungi (Ng, Fraunfelder et al. 2013). This broadens antifungal effects and improves the chance of a successful treatment. Cyclosporine has also been implicated as a possible management option for FK; it was shown to have antifungal and anti-

immune properties in patients with FK undergoing corneal transplant, but sample size was limited (Ansari, Miller et al. 2013).

3.4 Amoebic Keratitis

Amoebic keratitis (AK) is a more uncommon type of infectious keratitis caused primarily by the genus of amoeba known as *Acanthamoeba*. Given this distinct causal pathogen, the primary risk factor is exposure to soil or contaminated water while wearing contact lenses; this risk can be compounded by ocular damage. The incidence of AK in the United States is estimated to be two new annual cases per one-million contact lens wearers, although a recent investigation in Iowa reported a tripling of AK cases during 2010-2017 (Scruggs, Brittni et al. 2019). This study was performed in Iowa but raises the possibility of similar surges in AK cases in other states.

The clinical pathology of AK is similar to that of other forms of keratitis, including intense pain, ocular oedema, epithelial defect, and eventual loss of vision (Lorenzo-Morales, Khan et al. 2015). Ring infiltrates are commonly seen in amoebic keratitis, which can progress to create a disease state indistinguishable from bacterial and fungal keratitis (Singh, Gupta et al. 2021). Because AK can present itself similarly to more common types of keratitis, misdiagnosis is a main issue. The other significant issue with treatment is the pathogen itself; *Acanthamoeba* has two forms, a metabolically-active trophozoite and a dormant, resistive cyst stage (Lorenzo-Morales, Khan et al. 2015). While the former is much more effectively handled with treatment, the latter is more resistant; successful treatment is determined by elimination of both forms of *Acanthamoeba.*

The first line treatment for AK are biguanides such as 0.02% polyhexamethylene biguanide (PHMB) and 0.02% chlorhexidine. They have proven to be effective for treating the amoebic

infection and are active against both trophozoites and cysts when combined (Lorenzo-Morales, Khan et al. 2015). These drugs are administered topically every hour for the first few days post-diagnosis, and then tapered off over the course of several weeks depending on clinical response. Although these drugs are effective, PHMB in particular can be damaging to corneal cells, and epithelia toxicity can develop with frequent/extended use of the drug. The increased inflammation and side effects that come with this condition may require the use of topical steroids, but there is evidence that steroid use in the presence of *Acanthamoeba* may actually increase protozoal abundance (Lorenzo-Morales, Khan et al. 2015). If biguanides are not initially successful, possibly due to resistant *Acanthamoeba,* PHMB and chlorhexidine may be combined with antimicrobial agents like pentamidine or levofloxacin to speed up treatment and reduce the risk of developmental toxicity (Singh, Gupta et al. 2021). If all of these pharmaceutical treatments do not work, corneal transplant is usually performed to prevent progression of the condition. Because of these challenges, novel treatments for AK are necessary, but literature on this topic is minimal.

Phosphocholines have been shown to have antineoplastic activity *in vitro* and *in vivo.* A particular drug of this class, miltefosine, has been shown to be effective against *Acanthamoeba.* Miltefosine was combined with polyhexamethylene biguanide for enhanced treatment of AK in rats, healing approximately 86% of infected eyes (Polat, Walochnik et al. 2014). Recently, a "pharmaceutical phylogeny" started for *Acanthamoeba* to identify new therapeutic sites that can be targeted by novel or conventional drugs in order to improve efficacy of treatment (Lorenzo-Morales, Khan et al. 2015). Although the current number of novel drugs and delivery methods for treating AK are few, it is possible that efficacy of current treatment options like biguanides may be enhanced in the future through previously-mentioned delivery methods being studied for other, more common types of infectious keratitis.

3.5 Non-Pharmaceutical Treatment

Despite the development of many promising delivery methods for the treatment of infectious keratitis, expense, delivery complications, and lack of established safety/efficacy data limit clinical use. A novel, non-pharmaceutical treatment method under clinical investigation for infectious keratitis is corneal crosslinking (CXL). CXL has been utilized, albeit in small numbers, to treat infectious keratitis when patient response to pharmaceutical drugs is nominal (Lee, Somerville et al. 2021). CXL takes advantage of free radical generation from the combination of UV light and riboflavin to damage microbial DNA, and produces corneal resistance to proteolytic enzymes generated by pathogenic microbes; this technique has demonstrated effectiveness both *in vitro* and *in vivo* in treatment of bacterial keratitis, but can be combined with antibiotic therapy to further improve therapeutic effect (Egrilmez and Yildirim-Theveny 2020). In cases of fungal keratitis, CXL has been shown to have significant therapeutic benefit, healing corneal epithelium and improving visual acuity within two weeks post-treatment (Ng, Fraunfelder et al. 2013). CXL has also significantly improved AK within 24 hours in four individuals, further resolving all symptoms/epithelial defects within three months; no *Acanthamoeba* was detected in corneal tissue following this period of time. Concern has arisen over exposing the cornea to UV light, which can possibly result in corneal melt, a severe symptom of infectious keratitis itself. To negate this, a topical CXL approach has been developed that utilizes sodium hydroxymethylglycinate as the cross-linking agent instead of UVA/riboflavin *in vitro*, but further clinical investigation is required if this is to become a viable alternative (Lee, Somerville et al. 2021).

4.0 Conclusion

Despite recent advancements in the field, treatment of keratitis remains a difficult task. Corticosteroid eye drops are the typical first-line treatment for most cases of keratitis, including non-infectious keratitis, where cyclosporine and tacrolimus can be used if the patient is unresponsive to treatment. Cenergermin, a topical solution consisting of recombinant human nerve growth factor, was recently approved by the FDA. Although these topical treatments are welcome alternatives to surgical intervention, the frequent administration and low bioavailability necessitates novel delivery methods.

Topical trifluridine and acyclovir are common first-line treatments for viral keratitis caused by HSV-1 infection, as well as other analogs of ACV. Glucocorticoid drops are used in combination with these therapeutic agents to increase effectiveness of treatment. Ocular inserts consisting of antimicrobial drugs and peptides have been developed, both for treatment of viral and bacterial keratitis. Moxifloxacin is one of the fluoroquinolones used to treat bacterial keratitis and has been included as part of a nanoparticle carrier to increase corneal penetration. In situ gel formulations are being developed to prolong the release of drugs onto the cornea, as well as the use of lipid carriers. Topical antifungal agents are used in treatment of fungal keratitis, including Natamycin, Voriconazole, and Amphotericin B, depending on the type of fungi. Cyclosporine is currently being investigated as a novel treatment method for this specific kind of keratitis. Lastly, biguanides are conventionally used to treat *Acanthamoeba* keratitis; phosphocholines such as miltefosine are being investigated as novel treatment options. For most types of keratitis, corneal crosslinking has been used successfully, but total case numbers are low. Overall, novel treatments for ocular conditions, especially keratitis, remain an important topic in the field of drug delivery.

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