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An exploration of the ocular mysteries linking nanoparticles to the patho-therapeutic effects against keratitis

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Abstract

Microbial keratitis, a sight-threatening corneal infection, remains a significant global health concern. Conventional therapies using antimicrobial agents often suffers from limitations such as poor drug penetration, side effects, and occurrence of drug resistance, with poor prognosis. Novel treatment techniques, with their unique properties and targeted delivery capabilities, offers a promising solution to overcome these challenges. This review delves into timely update of the state-of-the-art advance therapeutics for keratitis treatment. The diverse microbial origins of keratitis, including viral, bacterial, and fungal infections, exploring their complex pathogenic mechanisms, followed by the drug resistance mechanisms in keratitis pathogens are reviewed briefly. Importantly, the emerging therapeutic techniques for keratitis treatment including piezodynamic therapy, photothermal therapy, photodynamic therapy, nanoenzyme therapy, and metal ion therapy are summarized in this review showcasing their potential to overcome the limitations of traditional treatments. The challenges and future directions for advance therapies and nanotechnology-based approaches are discussed, focusing on safety, targeting strategies, drug resistance, and combination therapies. This review aims to inspire researchers to revolutionize and accelerate the development of functional materials using different therapies for keratitis treatment.

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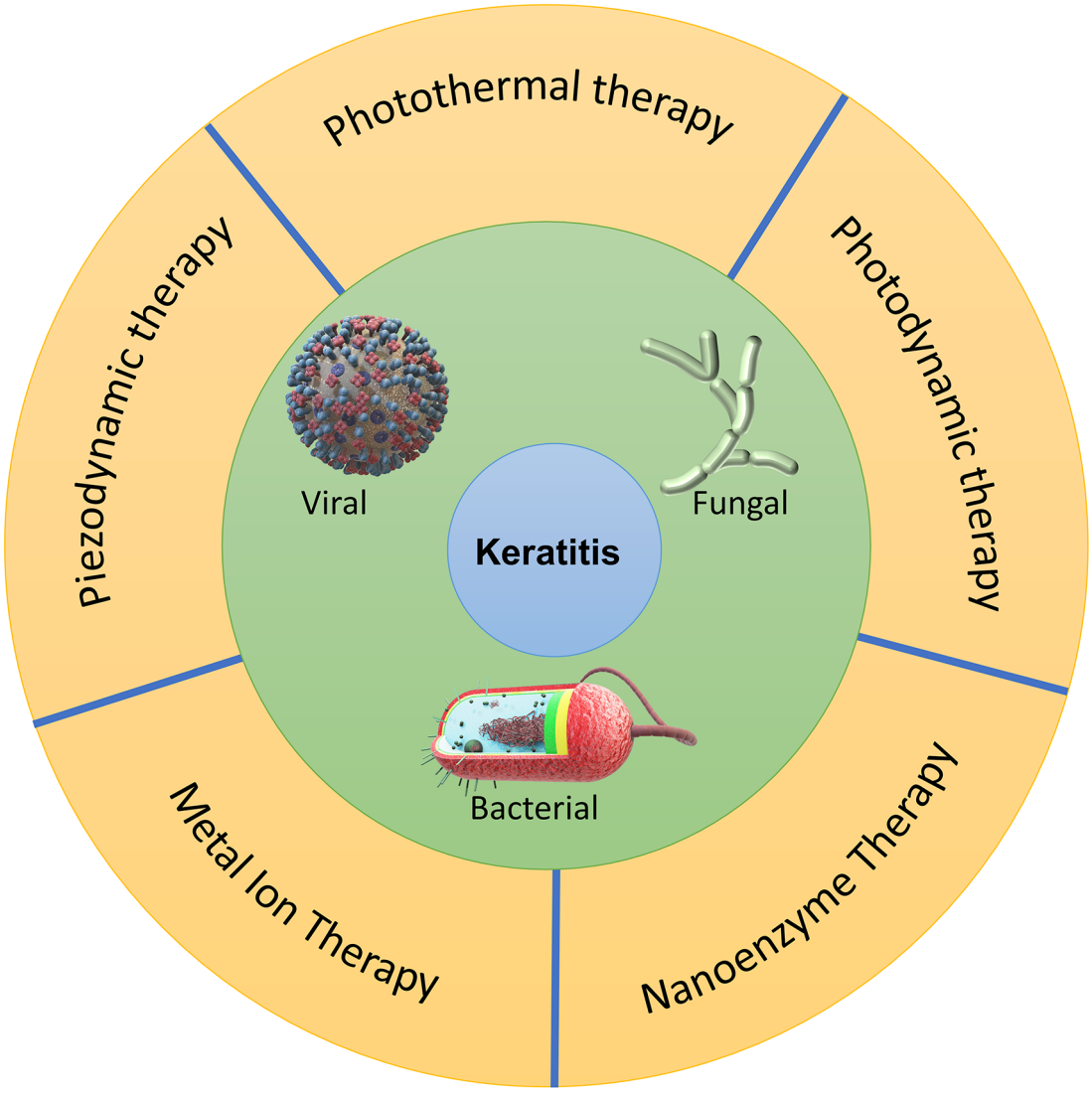
Graphical abstract



Keywords Keratitis, Drug delivery, Microbial infection, Advance therapies, Drug resistance

Introduction

Eye is a remarkable organ with a complicated structure and highly specialized tissues that work together to enable vision. Its diameter is roughly 2.5 cm and its volume is approximately 6.5 mL [1]. Functionally, the eye has two main segments, the rear segment, which comprises the vitreous humor, retina, choroid, and sclera, and the prior segment known as anterior part, consists of the conjunctiva, cornea, iris, aqueous humor, ciliary body, lens, and lacrimal system [2]. Among these, cornea plays a vital role in vision; it is a translucent and avascular tissue that acts as the primary obstacle and contributes to nearly two-third of the refractive strength of eye [3]. Cornea is comprised of three layers: the outer epithelial layer, which provides protection; the stromal layer, that accounts for 90% of the cornea's depth and contains collagen and other structural components; and the innermost endothelial layer, which maintains the hydration and transparency of cornea [4]. Corneal disorders can be inherited or acquired through various factors such as trauma, infections, or surgery [5]. These conditions often result in loss of transparency and visual impairment. Under normal conditions, the cornea initiates an intricate wound-healing response to maintain its structure and transparency [6]. However, in pathological situations, excessive healing can result in issues like corneal

scarring, vision loss, and fibrosis [7, 8]. This excessive healing is driven by an imbalance in the production of growth factors, cytokines, and other molecules, which leads to disruptions in the corneal structure [9].

Infectious keratitis is the leading cause of non-trachomatous corneal opacification and ranks as the fifth most common cause of blindness globally, accounting for 3.5% of all blindness cases (36 million people) up to 2015. The prevalence of corneal blindness resulting from infectious keratitis has declined from approximately 1.6 million cases in 1990 to 1.3 million cases in 2015. Similarly, the number of individuals with vision impairment due to this condition decreased from 3.3 million to 2.9 million during the same period, though these figures are likely underreported [10–12]. Keratitis is a corneal infection triggered by bacteria, viruses, fungi, or amoebae, that can significantly impair vision if left untreated [13]. The incidence of microbial keratitis varies significantly across regions. In developed countries, the rates reported include 27.6 per 100,000 person-years in the United States (1999), 40.3 per 100,000 in England (2006), and 6.6 per 100,000 in Australia (2015). However, in developing nations in Asia, infectious keratitis poses a substantial public health challenge. Limited access to healthcare, poor health metrics, and a higher proportion of agricultural workers contribute to significantly higher incidences, such as 113 per 100,000 in Madurai, Tamil Nadu, India; 339 per 100,000 in Bhutan; 710 per 100,000 in Myanmar; and 799 per 100,000 in Nepal [14–17]. Although the natural defense mechanisms of eye usually protect it from infection, factors like contact lens wear, corneal injuries, and certain ocular diseases can compromise these defenses, making the cornea more susceptible to infection [18]. Keratitis causes redness, pain, blurred vision, and light sensitivity [19]. Timely treatment is crucial, as delays can result in ulcers, corneal scarring, and perforation that may result in permanent vision loss [20]. The first degradation caused by a corneal infection is ulcer of cornea. Following this, polymorphonuclear and lymphomononuclear cells infiltrate the stroma [21]. This infiltration results in stromal necrosis and the destruction of Bowman's layer that can lead to blockage of arteries that feed the cornea. Descemetocele, or concentrated corneal thinning, can occur when these necroses intensify and make the Descemet layer visible [22]. In order to stop the corneal layers from necrotizing, the immune response of body simultaneously causes apparent corneal vascularization and epithelial regeneration. Based on bacterial pathogenesis and the compromised immune response during that period, the pathogen may potentially colonize the entire cornea. The resultant corneal epithelial defects facilitate the ingress of microorganisms into the deeper ocular structures. Progressive infections not only occlude the pupil and iris but also compromise the delicate conjunctival tissue, leading to the formation of a pseudocornea [23]. Contingent upon the severity of the condition, corneal perforations and opacification may result in visual impairment or complete loss of vision. Since opacification and corneal perforations are typically irreversible, to repair corneal function, corneal transplantation is required [24]. The successful treatment of keratitis is seriously threatened by the rise of drug-resistant microbiological strains, especially *Staphylococcus aureus* and *Pseudomonas aeruginosa* [25]. These resistant strains, driven by factors like genetic mutations and overuse of antibiotics, complicate treatment strategies. Virulence factors produced by these pathogens, such as toxins, proteases, and adhesins, contribute to the severity of corneal infections [26]. The choice of appropriate antibiotic for keratitis is crucial, but challenges arise due to the lack of well-defined clinical breakpoints and the complex interplay between antibiotic susceptibility, corneal penetration, and bacterial factors. Therefore, a comprehensive understanding of both microbial resistance mechanisms and virulence factors is essential for developing effective treatment strategies and preventing the spread of drug-resistant infections [16, 27]. Keratitis must be treated in order to avoid complications including visual, ulcers, corneal scarring, and even irreversible vision loss if left untreated. Whether the origin of the infection is bacterial, fungal, viral, or non-infectious, immediate diagnosis can effectively treat it. Early action lowers the chance of long-term eye injury, encourages healing, and reduces inflammation [13, 28].

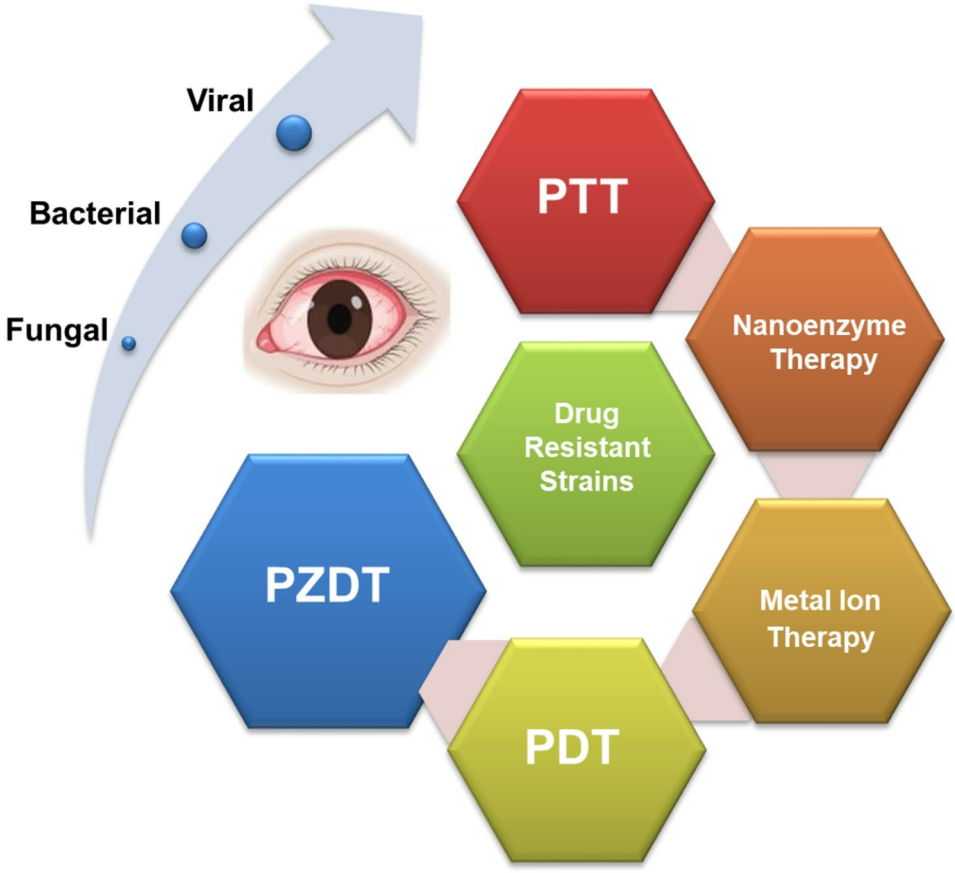
Common treatments for keratitis include topical eye drops, but these have limitations, particularly in terms of ocular bioavailability [29]. Due to rapid clearance by the tear film and lacrimal drainage, only a small fraction of the medication remains on the corneal surface, often requiring frequent applications that can be inconvenient for patients and may lead to side effects [30, 31]. Current development in the clinical strategies aims to prevail these challenges. For therapeutic modalities, they have many advantages, such as enhanced ocular bioavailability, targeted drug delivery, improved drug stability, and reduced side effects [32]. Novel approaches to treating keratitis have been developed as a result of recent developments in conventional therapies, a serious eye infection that can cause blindness [33]. These strategies focus on overcoming the limitations of traditional antifungal and antibacterial treatments, such as drug resistance and limited penetration into infected tissues [22, 34]. One promising approach involves the use of therapeutics to supply antimicrobial agents directly to the area of infection [35]. For instance, researchers have developed nanomedicines that can encapsulate antifungal drugs and release them slowly over time, increasing their efficacy and minimizing side effects [36]. Additionally,

nanomedicines can be functionalized with targeting molecules to specifically bind to infected cells, enhancing drug delivery and minimizing damage to healthy tissues [37]. Moreover, some nanomedicines can produce reactive oxygen species (ROS), that can destroy fungi and bacteria directly [38]. Another innovative strategy utilizes functionalized materials to disrupt the structure of bacterial and fungal biofilms, which are complex communities of microorganisms that are resistant to conventional treatments [39]. By targeting the extracellular matrix of biofilms, these materials can weaken their structure and increase their susceptibility to antimicrobial agents [40]. By overcoming the limitations of conventional treatments, emerging therapeutic modalities hold the potential to revolutionize the management of keratitis and other ocular diseases [41].

Many nanomedicine-based therapeutic paradigms have been developed like piezodynamic therapy (PZDT), photothermal therapy (PTT), photodynamic therapy (PDT), nanoenzyme therapy, and metal ion therapy [42]. Nanomedicines combined with these therapeutic modalities, enhance their antibacterial and antifungal effects [43, 44]. For example, gold nanoparticles (Au-NPs) can be used to transform light energy into heat, killing bacteria and fungi through thermal ablation [45]. Additionally, photosensitizers can be incorporated into NPs to generate ROS upon exposure to light, further damaging microorganisms [46]. PZDT is a non-invasive treatment utilizing high-frequency acoustic waves, and often shows promise in managing corneal inflammation by stimulating cellular repair, boosting collagen production, and modulating the immune response [47]. While traditional treatments have limitations, innovative approaches like multifunctional hydrogel patches incorporating NPs offer a promising solution. These patches combine self-healing, tissue adhesion, and antibacterial properties, mimicking the structure of cornea and function. PTT and PDT are developing as promising non-invasive treatments for keratitis. These therapies involve the use of photosensitizing agents that, upon activation by light, produce ROS, which can destroy bacteria and aid in tissue healing [48, 49]. While PDT is efficient in preventing a wide range of microorganisms, PTT offers the advantage of rapid heat generation, which can directly inactivate pathogens. Combining these two therapies can provide a synergistic approach, enhancing their efficacy and reducing the risk of bacterial resistance [50–52].

Innovations in therapies using nanotechnology to deliver drugs or treat diseases at the molecular level have shown promise for improving the treatment of corneal disorders [53]. NPs and other nanostructured materials have been explored to enhance drug penetration, reduce side effects, and improve patient compliance [54]. Nanozyme-based treatments, particularly nanozymes delivered *via* microneedle patches, show promise in combating keratitis [55]. These nanozymes possess multiple enzyme-like activities, effectively targeting pathogens, reducing oxidative stress, and promoting tissue repair [56]. This approach offers advantages over traditional treatments, including improved drug delivery and reduced risk of microbial resistance [57]. Metal ion therapy, particularly utilizing metal-based NPs, has emerged as a promising approach for treating keratitis. These NPs, often composed of metals like silver, copper, or zinc, possess potent antimicrobial properties [58]. They work through various mechanisms, including ROS generation, disruption of bacterial cell membranes, and interference with essential metabolic processes [13]. One of the key advantages of metal ion therapy is its broad-spectrum antimicrobial activity, enabling it to target a wide range of pathogens, including bacteria, fungi, and viruses [59]. Additionally, metal-based NPs can be designed to have sustained release properties, ensuring prolonged therapeutic effects and reducing the frequency of administration and these NPs can be functionalized with targeting ligands to specifically deliver the antimicrobial agents to the infected corneal tissue, minimizing systemic side effects [60, 61]. These strategies include the use of NPs to deliver antimicrobial agents directly to the infected site, the development of nanomedicine with targeted delivery capabilities, and their exploration with multifunctional properties, such as antimicrobial and anti-inflammatory effects [62, 63]. While significant progress has been made, further research is necessary to fully realize the potential of pre-existing therapies in the treatment of keratitis. Challenges such as toxicity, biocompatibility, and regulatory hurdles must be carefully addressed to ensure the safe and effective translation of these technologies into clinical practice.

In this review article, we will discuss all types of keratitis like bacterial, fungal and viral followed by their drug resistance mechanisms and comprehensively all the therapies like, PTT, PDT, nanoenzyme therapy, and metal ion therapy which perform a crucial role in keratitis treatment. The objective of this review is to explain various innovative drug delivery methods with the formulation of different active substances and describe some novel drug delivery methods that have an efficacy for a successful keratitis treatment. However, there are still challenges in the treatment of microbial keratitis owing to drug resistant pathogens and other intricacies. Unlocking the full potential of nanomedicine-based therapeutic strategies that can lead us to potential solutions and future research directions for treating keratitis (Scheme 1 and 2).



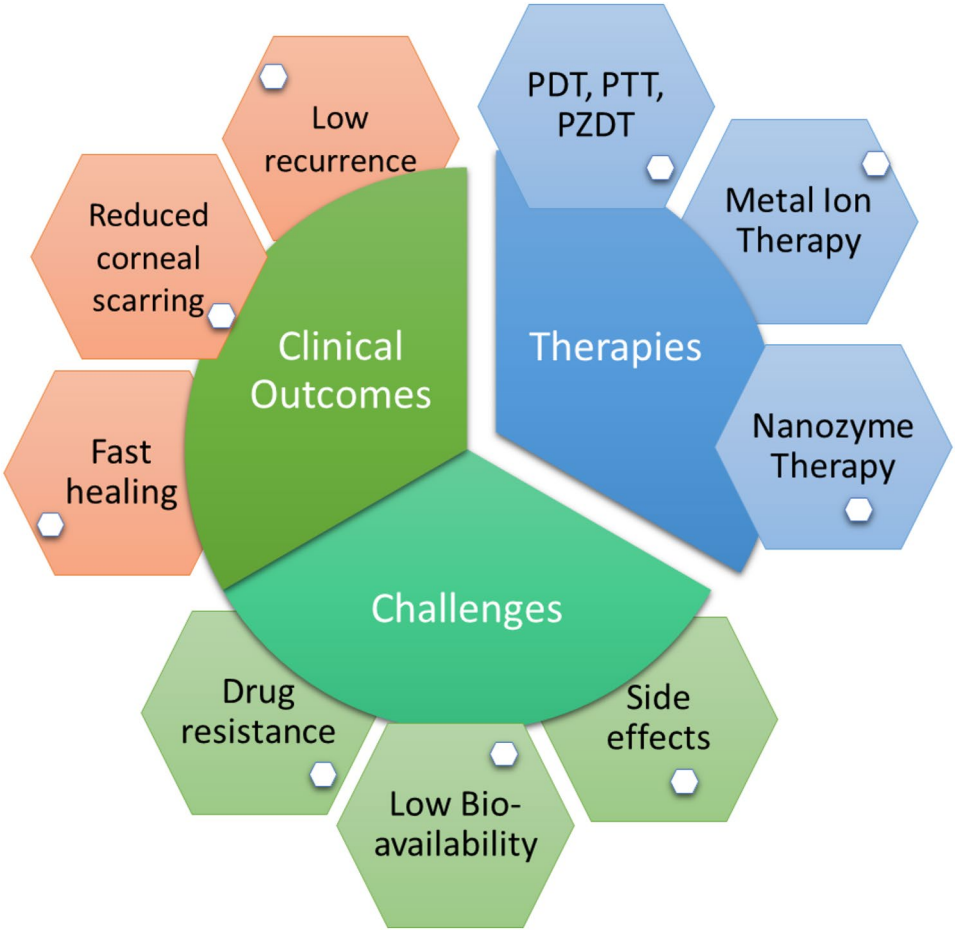
Scheme 1 Schematic representation of keratitis types, drug resistant strain, and featured therapies

Microbial origin of keratitis

The microbial origins of keratitis typically involve viruses, bacteria, fungi, or parasites that invade the corneal tissue, often due to trauma, contact lens wear, or underlying health conditions. Microbial keratitis affects 11/100,000 people in the United States, compared to 799/100,000 people in impoverished nations each year [64]. Corneal epithelial antibodies and mucins provide the key eye defense against keratitis caused by microbes. Therefore, conditions including physical or chemical assault, scarring, and contact lens wear that compromise the corneal epithelial barrier, dramatically increase the chance of developing microbial keratitis [65]. Some of the most common microbial origins for keratitis have been listed in this section.

Fungal keratitis

Fungal keratitis is a general corneal infection that affects around 1 million individuals annually, and results as 1–45% occurrences of infectious keratitis [66]. Typically, young healthy farmers or outdoor laborers are infected who have been injured by organic waste. Furthermore, previous ocular surface disorders, ocular surgery, using contact lenses and having a background of systemic or topical corticosteroid use issues are known to increase the chance of developing filamentous fungal keratitis [67]. The prevalence and epidemiological patterns of fungal keratitis are closely linked to geographical regions, showing significant variation worldwide, even within different areas of the same country and among distinct population groups. Tropical regions predominantly report *Aspergillus*, *Fusarium*, and *Curvularia* species, whereas yeasts are more commonly associated with temperate climates. The prevalence of *Candida* keratitis has been documented at 60.6% in London and 32.7% in Melbourne [68]. In Paraguay, *Acremonium* species are identified as the primary causative agents. *Aspergillus* species are the leading pathogens in northern and eastern India, whereas *Fusarium* species are more prevalent in the western and southern regions [69]. Notably frequent pathogenic fungi that cause keratitis are Fusarium and Aspergillus. Fungal keratitis morbidity was shown to be more in men than in women, with a ratio of 1.6:1. The incidence of fungal keratitis changes greatly across species and geographical regions. Fungal keratitis manifests clinically as stellate ulcers, conjunctival hyperemia, an overlying epithelial deficiency, and infiltration with irregular feathery margins [70]. Treating fungal keratitis is difficult since



Scheme 2 Infographic summarizing therapeutic challenges, solutions, and clinical outcomes for keratitis

delayed diagnosis, insufficient therapy, or carelessness can enable and accelerate fungal growth. Fungi gradually invade the flexible layer of cornea, eventually causing endophthalmitis and possibly eyeball enucleation [17, 71].

The initial therapy for fungal keratitis in medical facilities is natamycin 5% suspension, which is accessible on marketplaces [72]. As of right now, the Food and Drug Administration (FDA) has only given permission for this medication to treat fungal keratitis. Antifungal natamycin operate by binding to the ergosterol found on the fungal cell surface. This engagement is believed to alter membrane permeability, increase membrane porosity, and finally cause fungal cell death, possibly as a result of the loss of crucial intracellular components [73–75]. Extensive and continued studies assessing the effectiveness of specific azole antifungals against natamycin have produced assorted findings, most of which support superiority of natamycin. Topical ophthalmic solutions comprising 0.2% posaconazole, 0.1% voriconazole, 1% miconazole, 2% fluconazole, and 2% econazole have also been used to treat fungal keratitis [2]. While fluconazole is superior in treating *Candida*, voriconazole is effective against *Fusarium*, *Aspergillus*, and *Candida.* Echinocandins, which contains micafungin, caspofungin, and anidulafungin, are also a new class of antifungal medication compounds. Micafungin can also be given intravenously in doses of 100–150 mg each day for the therapy of fungal keratitis [76]. Metal-based anti-infection medications have garnered a lot of interest because of their high efficacy and multitarget antibacterial activity, that comprises preventing ROS production, protein dysfunction, and nutrition absorption [77]. The antifungal activity of representative silver against eye pathogenic filamentous fungus seemed encouraging, reducing gonorrhea infants and other ophthalmic infectious disorders. Despite most metals have a high antifungal impact, their toxicity must be considered and carefully chosen [78–80]. Gallium (Ga-III), which has almost similar ionic radius to iron, could operate as a "Trojan Horse" in the cell, mimicking iron but not effectively replacing it. The FDA has allowed injectable gallium nitrate $Ga(NO_3)_3$ for the treatment of cancer-related hypercalcemia with very minimal side effects when taking the recommended dosages [81,

82]. Ga-based prospective medications disrupted iron metabolism and showed broad-spectrum antibacterial activity, however there are little studies on their antifungal properties, not to be listed in animal models. Nevertheless, the usage of Ga^+ alone must additionally address the polysaccharide barriers found in fungal biofilms and cell walls, which reduce their antifungal effectiveness [83]. Thus, exopolysaccharide degradation and a Ga^+ co-delivery system show a great deal of promise for treating fungal keratitis with the right design. We then introduce a polydopamine-modified mesoporous silicon nanosystem (MLPGa) that is co-integrated with lyticase and Ga^+. This system can break down the exopolysaccharides found in cell walls and biofilms, and the liberated Ga^+ can then kill *Candida albicans* [84]. Additionally, the inherent Raman signals from the chelation action between the polydopamine (PDA) and Ga allow for real-time monitoring of the release behavior of Ga^+ both in-vitro and in-vivo. Metal-based anti-infection drugs have received widespread interest due to their high effectiveness and multitarget antibacterial activity, which includes protein malfunction, the formation of ROS, and interference with nutrient uptake. Representative silver demonstrated promising antifungal action against ocular pathogenic filamentous fungus, reducing gonorrhea infants and other ophthalmic infectious disorders. Although most metals have a high antifungal impact, their toxicity must be considered and carefully chosen [85].

Bacterial keratitis

Keratitis caused by bacteria is the most prevalent kind of microbial keratitis, making up 90% of all cases. It is estimated that 2.0 to 3.5 million cases of bacterial keratitis occur each year, although the actual incidence is believed to be much higher due to underreporting, particularly in developing countries [86]. Since 1995, the reported incidence of bacterial keratitis in developed countries has been significantly lower, ranging from 4.5 to 37.7 cases per 100,000 population per year in regions such as US, UK, Australia, and Taiwan [87]. Bacterial keratitis is a dangerous eye infection, which occurs when bacteria invade the cornea, often following corneal trauma or underlying eye conditions [88]. This infection can lead to rapid corneal ulceration, severe pain, and significant vision loss if left untreated. The infection triggers inflammation, characterized by high levels of ROS, which further damages the cornea [89]. To combat this, researchers have developed innovative nanotechnology-based drug delivery systems. These systems, such as polymer nanomicelles, can encapsulate antibiotics and other therapeutic agents, enhancing their delivery to the infected cornea and improving their efficacy [90]. By targeting the bacteria and reducing inflammation, these nanomicelles offer a promising approach to treating bacterial keratitis and preventing vision loss. The microbes that induce bacterial keratitis differ significantly dependent on the patient's location [91]. Nonetheless, *Staphylococci*, *Streptococci*, *Pseudomonas*, and *Serratia* species are the most commonly found bacteria in bacterial keratitis situations. Keratitis due to bacteria presents with numerous clinical signs. Bacterial keratitis frequently manifests as photophobia, ocular discharge, redness, discomfort, blurred vision, and heavy tears. There is strong and compelling evidence in the American academy of ophthalmology (AAO) 2018 study that fluoroquinolone treatment by itself is nearly as efficient as combination therapy with increased drops [92, 93].

Fluoroquinolone drugs, including ofloxacin 0.3%, levofloxacin 1.5%, and ciprofloxacin 0.3% have also been approved by the FDA for the treatment of bacterial keratitis [94]. Bacterial keratitis is now treated with fourth-generation fluoroquinolones such moxifloxacin and gatifloxacin due to the advancement of resistance to second-generation fluoroquinolones. While the FDA has yet to approve gatifloxacin or moxifloxacin for this evidence, they have been investigated for treatment of bacterial keratitis. *S. aureus* which is methicillin-resistant was found in 72.8% of the 621 isolates of *S. aureus*, while 33.6% of the 1,695 isolates showed doxifloxacin tolerance [95]. Comparable studies show that resistance to fourth-generation fluoroquinolones is growing. Nonetheless, in-vitro tests indicated that the newly marketed ocular fluoroquinolone besifloxacin 0.6% was more effective than earlier ophthalmic fluoroquinolones against *Staphylococcus* epidermidis and methicillin- and ciprofloxacin-resistant *S. aureus*. It was discovered that besifloxacin was superior to moxifloxacin and gatifloxacin in lowering keratitis due to *Pseudomonas aeruginosa* [96]. A mixture of polycurcumin (pCur) and carbon quantum dots (CQDs) made from curcumin and l-arginine hydrochloride (Arg) was developed [97]. The Arg-CQDs/pCur combination demonstrated encouraging anti-inflammatory, antibacterial, antioxidative, fibroblast migration stimulation, and corneal endothelial cell proliferation properties. A straightforward one-step mild pyrolysis was used to create Arg-CQDs from Arg, which were subsequently *in-situ* conjugated with pCur in an alkaline setting to create Arg-CQDs/pCur nanocomposites [98]. Additionally, it is crucial for cell division, immunological responses, and wound healing. A naturally occurring polyphenolic compound found in turmeric, curcumin, has been demonstrated to have anticancer, anti-inflammatory, and antioxidant properties. Moreover, combining curcumin with other antibacterial medications can enhance its antibacterial qualities since it possesses photocatalytic activity and can prevent bacteria from clumping together to create biofilms. Curcumin promotes re-epithelialization,

fibroblast migration, and TGF-β production, which helps in wound healing [99].

The increasing prevalence of bacterial keratitis is a serious eye infection caused by bacterial invasion of the cornea. While traditional treatments like fluoroquinolones are effective, emerging antibiotic resistance necessitates innovative approaches. Nanotechnology-based drug delivery systems, such as polymer nanomicelles, offer promising solutions by enhancing drug delivery to the infected cornea and improving therapeutic efficacy. Preclinical studies demonstrate the superior efficiency of Arg-CQDs/pCur nanomaterials in treating bacterial keratitis compared to conventional therapies, leading to significant improvements in corneal health and reduced inflammation. This research offers a potential breakthrough in the management of bacterial keratitis, providing a more effective and targeted treatment option for this challenging ocular infection.

Viral keratitis

Virus is a tiny infectious agent that can only reproduce within live cells of creatures. It is made up of genetic substances, either DNA or RNA, wrapped in a protein coat and occasionally an outer lipid envelope [100]. Viruses are incapable of carrying out metabolic operations on their own and must rely only on host cells for reproduction. Once inside the body, they connect to and infiltrate host cells, causing them to create more viruses and frequently damaging or killing the infected cells in the process [101]. HSV (herpes simplex virus) is the most prevalent reason of viral keratitis, which firstly attach to corneal cells and fuse with their membranes [79]. Viruses multiply inside cells by using the host's machinery. This replication and release of additional viruses frequently result in cellular lysis or elicit an inflammatory response because infected cells transmit danger signals [102].

Viral keratitis is a significant eye infection that can lead to severe vision impairment if left untreated [103]. While topical antiviral medications are the first-line treatment, their effectiveness is often limited by factors such as rapid tear turnover and poor drug infiltration into the cornea. Approximately 50% of adults in the United States are estimated to be seropositive for HSV, with rates as high as 90% in regions like Africa. On a global scale, 67% of individuals under 50 are exposed to HSV-1, while 11% are exposed to HSV-2 [103]. Keratitis resulting from HSV affects around 1.5 million persons worldwide and 500,000 in the United States of America. Although less frequent, CMV and varicella-zoster virus can both result in keratitis [104, 105]. According to estimates, the prevalence of ocular HSV infection is 150 cases per 10,000 people in wealthy nations and 5–20 cases per 10,000 people annually. HSV-1 is transmitted by an actively shedding and personal contact between a seronegative, seropositive individual via tissue transplants and bodily fluids [106]. Once the virus has attached itself to host surfaces, it moves to the trigeminal ganglia, a dormant location of infection. Although the precise origin of HSV-1 reactivation is unknown, it is generally accepted that a number of host stresses, like fatigue, immunosuppression, psychological stress and UV exposure cause HSV-1 to reactivate in the TG. Through the ophthalmic nerves and trigeminal ganglia, the reinstalled virus retrogradely makes its way to the eye, where chronic inflammation encourages corneal deterioration. HSV-1 can shed either asymptomatically or symptomatically [107–109]. Massive syncytial structures are created as a result of the controlled replication of virus and promotion of the recruitment of many cells during symptomatic shedding. Following replication, the viral genomes are put together to form virions. The virus is released into the surrounding tissue when the cell necrotizes toward the conclusion of the infectious cycle. Among the viruses that cause viral keratitis, only HSV-1-induced keratitis has been extensively researched (Fig. 1) [110]. HSV-1 proliferates in epithelial cells of cornea, generating massive syncytial frameworks. These cells finally burst necroptically and release HSV-1 virions into the adjacent tissues, resulting in extensive keratitis lesions in the stroma, epithelium, and endothelium of cornea. Interestingly, cornea epithelial cells exhibit unique toll-like receptor (TLR) expressional dynamics during HSV-1 infection. Active keratitis significantly overexpresses TLR-4, 7, 8, and 9. Knocking down TLR-2 has been shown to be an efficient method for preventing keratitis lesions in mouse models. In contrast, TLR-4 knock-out mice developed severe keratitis lesions more quickly. TLR-9 antagonists also have a significant function in avoiding illness aggravation in the course of the transmission of HSV-1 DNA. Although TLR-9 ligation with HSV-1 DNA triggers powerful immune responses for HSV-1 clearance, it also increases inflammatory mechanisms that cause corneal damage. As a result, blocking TLR-9, TLR-2 and activating TLR-4 using molecular mimics could be an innovative, effective technique for avoiding innate immune-mediated corneal injury. HSV keratitis can be intermittent and persistent, conversely to bacterial and fungal keratitis [111, 112]. Possible symptoms include light sensitivity, weeping, conjunctival injection, feeling like a foreign body, and blurred vision. Antiviral medications have been applied topically and collectively to treat HSV epithelial keratitis, depending on the severeness of the disease. Currently, topical medicines like as ganciclovir, trifluridine, and acyclovir are available. On the market, trifluridine is offered as an ocular solution, whereas ganciclovir is sold as gel [113]. To address these challenges, researchers have developed innovative drug delivery systems, including in-situ gelling systems, nanocarriers, prodrugs, and

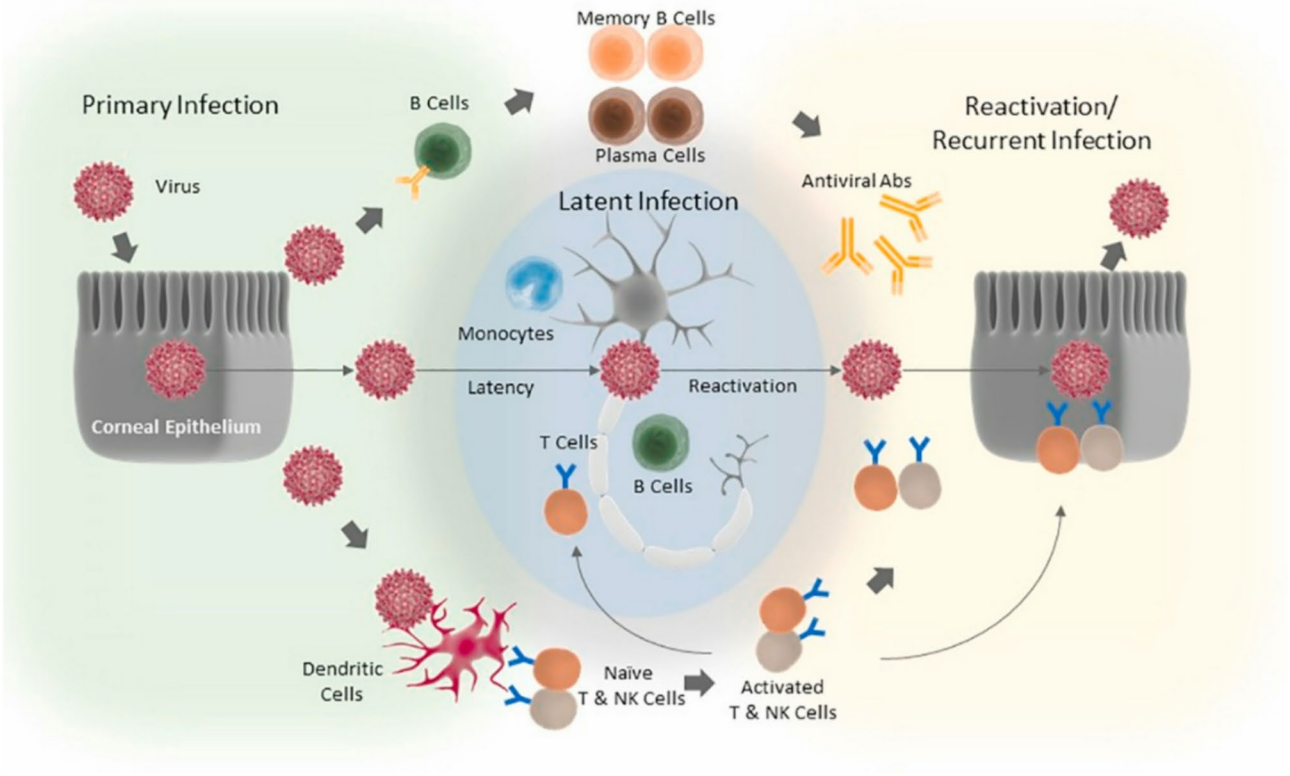


Fig. 1 The life cycle of HSV-1 and related immunological responses during ocular infection [110]. Reprinted with permission from ref. no. 119. Copyrights 2021, ELSVIER

peptide-based approaches. These systems offer the possibility to enhance drug delivery to the cornea, enhance drug retention time, and reduce systemic side effects. Nanotechnology offers innovative strategies to modulate immune responses in viral keratitis by targeting immune pathways at a molecular level. Nanocarriers, such as liposomes, NPs, and nanogels, can be engineered to deliver antiviral agents or immunomodulatory molecules directly to infected tissues, enhancing therapeutic efficacy while minimizing off-target effects. For instance, drug delivery systems like DECON (Drug Encapsulated CarbON) not only sustain antiviral drug release but also reduce viral load by trapping free viruses, indirectly attenuating inflammatory cascades. Moreover, emerging therapies involving RNA aptamers and CRISPR/Cas9 systems provide precision tools to disrupt viral genetic elements and modulate immune evasion mechanisms, such as viral microRNA activity, which are critical for persistent infection and reactivation [110]. These nanotechnology-driven approaches offer significant potential to reprogram immune responses, improve drug bioavailability, and reduce the risk of drug resistance in managing viral keratitis. Although encouraging, further clinical research is necessary to completely assess the prolong effects, safety, and effectiveness of these innovative strategies.

The complexities of viral keratitis, particularly that caused by HSV is a serious ailment of eye. Dendritic cells and other antigen-presenting cells in the corneal epithelium trigger the humoral and cellular immune pathways during the early infection. Activated naive T and NK cells seek for infected host cells to lyse. Similarly, B cells undergo activation and undergo differentiation into plasma cells and memory B cells. To fight infection, plasma B cells release antiviral antibodies. An immune system penetration in the corneal epithelium during repeated infection causes inflammation, and persistent corneal inflammation can trigger the development of keratitis. Memory B and T cells stay at the area of latency to monitor reactivation and trigger a quicker immune response once reactivation is infuriated. While topical antiviral medications are a mainstay treatment, their limitations, such as rapid tear turnover and poor corneal penetration, necessitate innovative approaches. Researchers are exploring various strategies, including in-situ gelling systems, nanocarriers, prodrugs, and peptide-based therapies, to enhance drug delivery and efficacy. These advancements aim to improve patient outcomes by optimizing drug concentration at the place of infection and minimizing systemic side effects. By addressing the challenges associated with traditional treatments, these

innovative approaches offer hope for more effective and targeted management of viral keratitis.

Drug resistant microbial strains

The reduced capacity of an antimicrobial agent to eradicate or stop the growth of a pathogen is known as antimicrobial resistance. Since many pathogenic organisms have become resistant to effective antimicrobial treatments, drug-resistant microbial strains have been acknowledged as a significant public health concern throughout the last 20 years [114, 115]. Numerous factors contribute to its development, such as commercial pressure-induced overuse and misuse of antibiotics in the agricultural industry, diagnostic uncertainty (e.g., bacterial versus viral infection) that leads to inappropriate antibiotic use, financial rewards for prescribing antibiotics, and public use of non-prescription antibiotics, particularly in nations with low and moderate incomes [116]. From a genetic perspective, horizontal gene transfer and genetic mutational resistance are the two main ways that produce antimicrobial drug resistance. Drug resistant microbial strains pose a significant threat to effective keratitis treatment. These strains, resistant to multiple antibiotics, complicate the management of corneal infections [117]. Common drug-resistant organisms in keratitis include *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Traditional antibiotics like fluoroquinolones and aminoglycosides may be ineffective against these strains [118]. As a result, clinicians often turn to alternative therapies such as topical colistin and imipenem, which have shown efficacy against drug-resistant pathogens [118]. However, these agents may have potential side effects and require careful monitoring. Several key virulence factors of *Staphylococcus aureus* contribute to the pathogenesis of keratitis. These comprise surface proteins including collagen-binding adhesion, fibronectin-binding protein, Staphopain A, and Eap, as well as toxins such α-hemolysin, β-toxin, γ-toxin, and Panton-Valentine leucocidin. These factors promote bacterial adhesion, invasion, and evasion of host immune responses, leading to increased inflammation and tissue damage [87]. Additionally, recent research has emphasized the role of *Staphylococcal enterotoxins* in exacerbating keratitis by inducing cytotoxicity and immune dysregulation. On the other hand, *Pseudomonas aeruginosa* has a number of virulence factors as well that include proteases like Protease IV, which degrades host proteins, and exotoxins such as ExoS and ExoU, which induce cytotoxicity and inflammation. Additionally, factors like PASP, elastases, and lipopolysaccharide play significant roles in promoting tissue damage, bacterial adhesion, and immune activation, ultimately leading to the development of severe corneal infections [87].

Drug resistance development is a complicated phenomenon driven by various mechanisms. One primary mechanism is the acquisition of genetic mutations that alter the target site of an antibiotic, reducing its effectiveness. Pathogens can also develop resistance through the overexpression of efflux pumps, which actively expel antibiotics from the cell. Additionally, the production of enzymes that inactivate antibiotics, such as β-lactamases, can contribute to drug resistance. These mechanisms, coupled with the excessive and misuse of antibiotics, have led to the rise of multidrug-resistant microbes, posing a significant challenge to public health [119]. Figure 2a represents the effect of different microbial virulence factors for keratitis. The choice of appropriate antibiotics for keratitis is crucial for optimal patient outcomes [118]. However, challenges arise in interpreting susceptibility test results, as clinical breakpoints for topical antimicrobials are often not well-defined. The effectiveness of a particular antibiotic can be influenced by factors such as corneal penetration, tear film clearance, and the specific bacterial strain involved (Fig. 2b) [87].

While laboratory-based MIC values provide valuable information, their direct correlation to clinical efficacy in the context of corneal infections may be limited. Therefore, it is essential to consider the specific characteristics of the infection, the clinical presentation of patients, and the local epidemiology of resistant organisms when selecting antimicrobial therapy [120]. There are some other factors that limit drug bioavailability in the eye, including anatomical barriers. Ocular barriers play a crucial role in protecting the eye from external threats, but they also present significant challenges for delivering therapeutic agents effectively. These barriers, including the tear film, corneal epithelium, conjunctiva, sclera, and blood-retinal barrier (BRB), limit the bioavailability of drugs and complicate their delivery to targeted tissues or cells. The most common among them is tear film, which serves as an initial protective layer, rapidly clears topically applied drugs due to its high turnover rate. Tear glands generate tears, which help to keep the eyes functioning properly. The tear film comprises of three layers: an exterior lipid layer released by a middle hydrophilic layer, meibomian glands, and an interior mucous layer [121, 122]. These tear factors negatively affect the absorption of ocular medications even if they significantly improve visual performance. Tears can significantly dilute topical medications, with a cul-de-sac containing approximately 30 µL and a solitary tear containing approximately 7 µL [123]. Tear proteins and mucins, which attach to drug molecules and lessen the concentration of drug in contact with the cornea, are another factor affecting bioavailability. The conjunctiva serves to preserve and lubricate the eye surface by generating mucus and antibacterial peptides. Because tight connections form on the outside

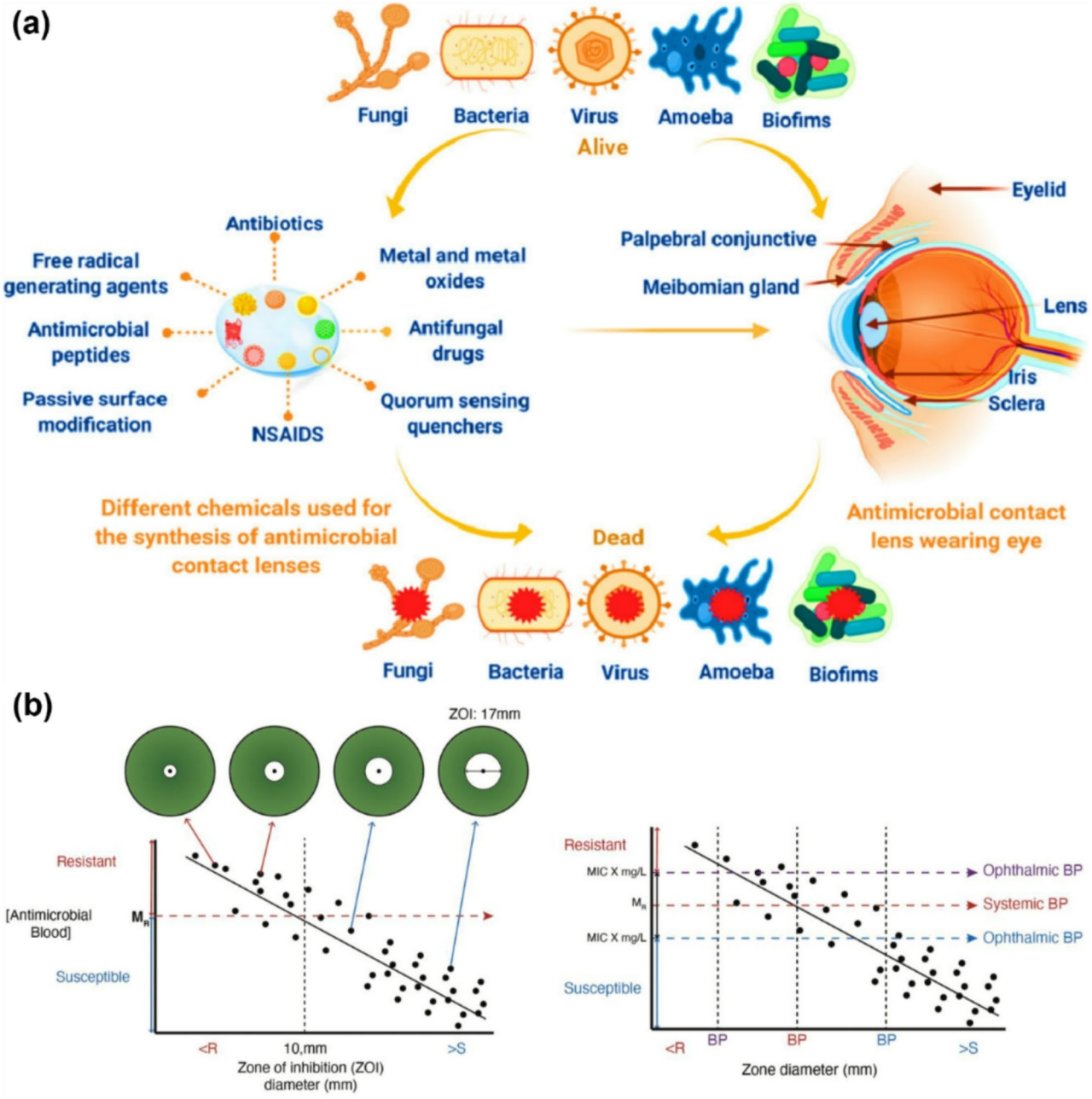


Fig. 2 (**a**) Surface Modifications to Active Agents Targeting Pathogens [118]. Reprinted with permission from ref. no. 132. Copyrights 2020, ELSVIER. (**b**) Pathogens causing the keratitis classified as susceptible or resistant [87]. Reprinted with permission from ref. no. 133. Copyrights 2022, ELSVIER

of its cells, the conjunctiva is highly vascularized and serves as a crucial barrier of defense on the surface of the eyes. Additionally, the corneal epithelium forms a tight diffusion barrier for most molecules The surface of cornea is mostly composed of endothelium, stroma, and epithelium [124]. The cornea enables around two-thirds of the optical capability in addition to protecting the eyes. Because of their high lipid content, endothelium and epithelial membranes operate as a physical barrier to the transfer of hydrophilic substances, making it impossible for drug molecules to move through them [125]. The blood-ocular barriers, such as BRB, further restrict drug movement, with the iris and ciliary body presenting additional obstacles through their specialized transport systems, including ATP-binding cassette transporters that actively remove unwanted compounds [126].

Furthermore, corneal permeation to hydrophilic medications is reduced because of the close connections among corneal epithelial cells, which restrict paracellular drug permeability. A highly watery extracellular

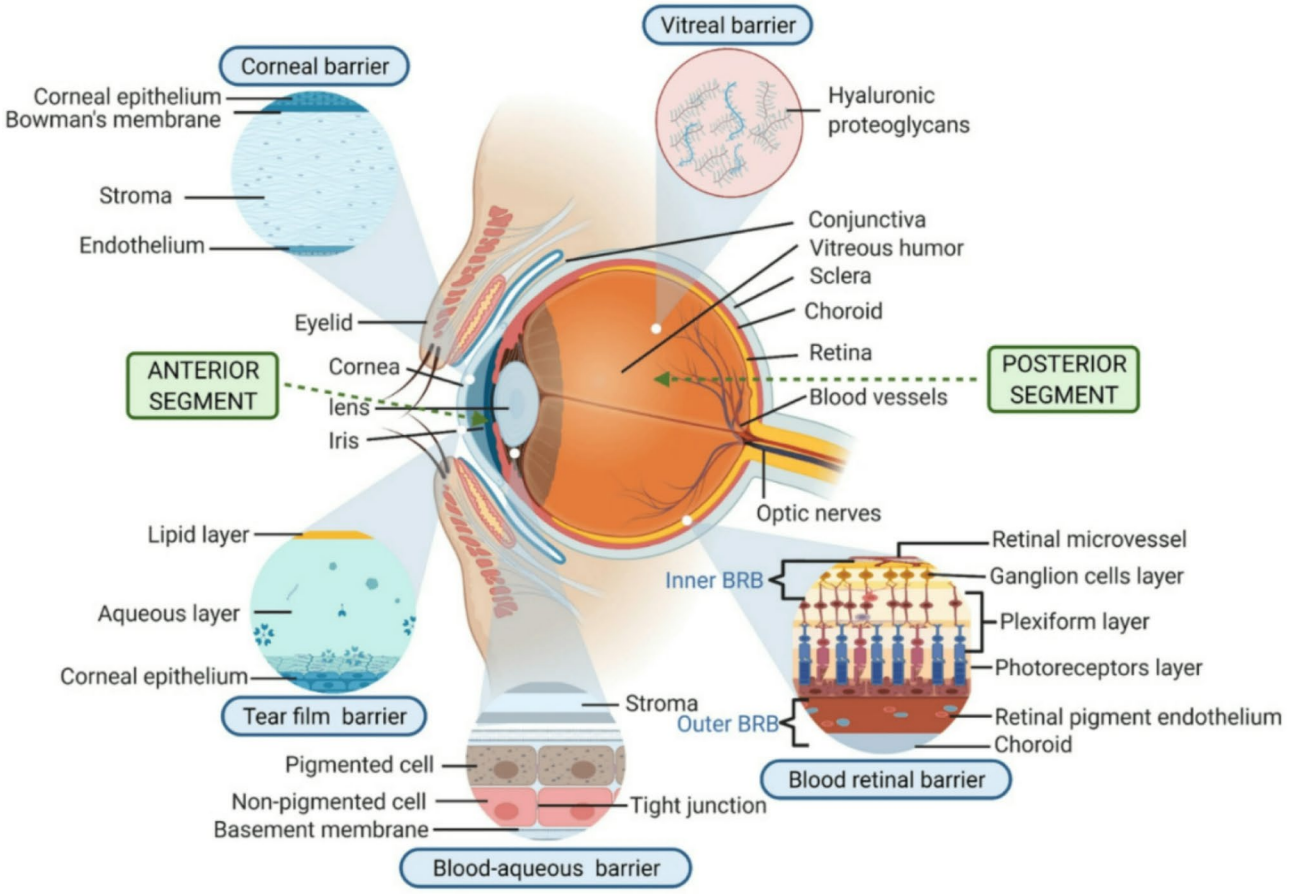


Fig. 3 Ocular medication delivery obstacles [130]. Reused under creative common attribution license

matrix with a collagen lamellar structure makes up the stroma of the cornea, making it more resistant to lipophilic medicines [127]. A single-cell membrane called the endothelium of cornea connects the aqueous humor. Lipophilic chemicals can pass through the corneal endothelium, which is primarily made up of phospholipids, whereas hydrophilic molecules cannot. To recap, for a medicine It needs to have the ability to pierce the cornea requires amphiphilic disposition, which is the presence of both hydrophobic and hydrophilic components within a single framework. This tight connection between the ciliary unpigmented epithelium of body, the junctions of the iris tissues, and the iris blood vessels is known as the blood aqueous barrier [128, 129]. Because of its anatomical placement, this barrier stops drugs from penetrating the anterior region of the eye. Drug delivery barriers in ocular route have been shown in Fig. 3 [130]. The corneal barriers, such as the tear film, corneal epithelium, conjunctiva, and blood aqueous barrier, may originate from the anterior part. The blood-retinal barriers, which include the vitreous barrier, retinal endothelium, ganglion cells, pigment cells, and retinal arteries, may be the cause of the posterior segment barrier. Overall, these obstacles decreased the availability of drugs in the posterior part ocular tissues.

The treatment of microbial keratitis often involves broad-spectrum antibiotics like fluoroquinolones. However, increasing antibiotic resistance is a growing concern. This is due to factors like overuse of antibiotics, biofilm formation by bacteria, and the presence of genetic elements that are mobile and capable of transmitting resistance genes. While antibiotic resistance in keratitis isolates is still relatively uncommon, there is a need for careful monitoring and prudent use of antibiotics to prevent further advancement of resistance. Combined treatment may be important in some cases, but it is important to select combinations that have synergistic or additive effects to maximize efficacy. Early analysis, prompt initiation of appropriate therapy, and close follow-up are crucial in managing keratitis caused by drug-resistant strains to prevent vision loss and other complications. Understanding the specific roles of various virulence factors in keratitis pathogenesis may help develop novel therapeutic strategies to combat this sight-threatening infection.

Advance therapeutic paradigms for keratitis

Conventional therapies like antibiotics for keratitis primarily involve topical medications, as antiviral drugs for fungal infections, for viral infections and antibiotics for bacterial infections [131]. These medications are often administered multiple times daily to maintain therapeutic levels in the eye. While effective in many cases, conventional therapies have limitations, including rapid tear turnover, poor drug penetration, and potential systemic side effects. Additionally, frequent dosing can be inconvenient for patients and may lead to non-compliance. A range of therapeutic modalities, including PZDT, PDT, PTT, nanoenzyme therapy, and metal ion therapy, offer promising avenues for the treatment of keratitis which are listed below.

PZDT for keratitis treatment

PZDT, a treatment that uses high-frequency acoustic waves, is gaining popularity because of its ability to treat corneal inflammation. This therapy may help reduce inflammation, speed up healing, and improve blood circulation in the corneal tissue [132, 133]. Shockwaves produced during therapy can stimulate cellular repair processes, boost collagen production, and encourage tissue regeneration, possibly aiding in the healing of corneal injury caused by keratitis [134, 135]. Furthermore, PZDT may modify the immune response, aiding in the management of inflammatory processes in the cornea. Current keratitis therapies mostly concentrate on tissue restoration and bacterial removal [136]. While antibiotic drops for eye are widely used, they have drawbacks such as low absorption and the ability to promote antibiotic-resistant bacterial strains. Using various techniques, including hydrogel, biomedical patches have recently been formed to integrate bioactive or antibacterial components for damaged corneas. microneedle, 3D printing, electrospinning, and microfluidics. Yet, these patches are usually monofunctional and lack enough mechanical strength and tissue attachment, among other shortcomings [137–139]. More significantly, the majority of studies do not incorporate the complex fiber structure of cornea and real components into their design, which may diminish the effectuality of treatment. Therefore, for the therapy of keratitis, a mutable patch with excellent tissue adhesion and mechanical strength to keep stability, efficient bacterial eradication, and corneal structure mimicking to encourage restoration is greatly desired [140, 141]. Dealing with these issues, Barium titanate ($BaTiO_3$, BTO), a traditional piezodynamic material, is widely employed in biosensors, piezocatalysis, and biomedical applications [142]. The catalytic efficacy of piezodynamic materials is reportedly enhanced by the generation of a Schottky barrier by metal and piezoelectric materials. Furthermore, ROS can be generated by piezocatalysis through the reaction of charged carriers with nearby H_2O and O_2 molecules (Table 1 and 2).

Kong et al. [143] imbued the fiber hydrogel with an antibacterial function, and produced BTO@Au NPs by coating BTO with Au-NPs. (Fig. 4a). BTO@Au NPs has a zeta potential of -52 mV and a hydrodynamic magnitude of 278 nm (polydispersity (PDI) = 0.22). It is evident from the TEM image in (Fig. 4b) that Au-NPs are dispersed haphazardly across the cubic surface of BTO. XRD verified the perovskite framework of the BTO and BTO@Au NPs, and due to the low Au concentrations, no discernible change occurred upon Au deposition. Using X-ray photoelectron spectroscopy (XPS), the valence states and surface compositions of BTO@Au were identified. (Fig. 4c). ROS production was mediated by piezocatalytic activity, primarily facilitated by BTO coated with Au-NPs. When subjected to ultrasound (US) stimulation, the piezoelectric properties of BTO generate localized electric charges due to mechanical deformation. These charges, in the presence of H_2O and oxygen O_2, undergo redox reactions to produce ROS, such as hydroxyl radicals (•OH) and singlet oxygen ($^1O_2$). The incorporation of Au-NPs on the BTO surface enhances this process by forming a Schottky barrier, which improves charge separation and transfer efficiency, thereby boosting the catalytic performance under US stimulation. This synergistic effect of BTO and Au-NPs significantly increases the production of ROS, which interact with surrounding molecules to exert antibacterial effects by damaging bacterial membranes and cellular structures. It is possible to attribute all of the peaks to Au, Ba, Ti, O, and C, correspondingly. Initially, the production of •OH using terephthalic acid (TA) was measured. When TA reacts with •OH, it can produce the fluorescent 2-hydroxyterephthalic acid (HTA), which is a probing molecule (Fig. 4d). HTA showed an emission peak at 426 nm in the proximity of BTO@Au NPs and US irradiation, and the peak fluorescence intensity progressively rose over time (Fig. 4e). A 10-minute US stimulation was 3.2 times more powerful than a 1-minute one, suggesting that •OH production is time-dependent. Additionally, under the same US irradiation environment, BTO@Au NPs demonstrated a greater •OH production capacity than BTO NPs (Fig. 4f). Additionally, since singlet oxygen sensor green can produce fluorescence when it reacts with $^1O_2$, it was utilized as a commercial ROS probe to measure the production of $^1O_2$. Likewise, when BTO@Au NPs and US irradiation were present, The fluorescence intensity of SOSG at 522 nm progressively declined over time, and BTO@Au NPs outperformed BTO NPs in terms of $^1O_2$ generation (Fig. 4g). According to these findings, Au NP decorating may improve sonosensitivity and •OH and $^1O_2$ production. Seven days following treatment, as seen in (Fig. 4h), BTO + the fiber gel + US and fiber gel + BTO@Au + US

Table 1 Common pathogens with diagnostic tests and clinical features [11]. Reused under creative common attribution license

Disease	Common pathogen	Symptoms	Transmission	Diagnostic tests	Treatment
Bacterial keratitis	*CoNS* *Staphylococcus aureus* *Streptococcus pneumoniae* *Pseudomonasaeruginosa*	Blurred vision, redness, photophobia.	Exposure to pathogens CL wear Ocular surface disease Ocular trauma Topical steroid use Previous microbial keratitis	**Gram staining Sensitivity**: 60-75% **Culture Sensitivity**: 38-66% **PCR Sensitivity**: 25-88%	Broad-spectrum topical antibiotics15 Monotherapy with fluoroquinolone OR Fortified antibiotics: Cephazolin 5% plus gentamicin 0.9% Consider adjuvant topical steroid at least 2–3 days of improvement when Organism has been identified and corneal infiltrate compromises the visual axis
Herpes simplex keratitis	Herpes simplex virus type 1	redness, discharge, watery eyes, irritations, itching, pain and photophobia	Direct contact with infected lesions or their secretions.	**PCR Sensitivity**: 70-100% **Specificity**: 67.9-98%	Australian HSK recommendations16 Occ ACV 3% five times daily for 1–2 weeks OR VLC 500 mg BD, 7 daysb VLC 500 mg once daily during topical steroid use PLUS Prednefrin Forte 4–6 times daily tapered over > 10 weeks
Fungal keratitis	*Fusarium spp*. and *Aspergillus spp* *Candida spp*	Redness, tearing, pain, sensitivity to light, discharge, decreased vision	Corneal injury Contact lens wear Ocular surface disease conditions: dry eye, blepharitis, bullous ke	**Gram and Giemsa Sensitivity**: 65-75% **10% KOH Sensitivity**: 61-99.23% **Specificity**: 91-97%. **Culture**: Blood and chocolate and Sabouraud dextrose agar **PCR Sensitivity**: 75-100% **Specificity**: 50-100% **IVCM** **Sensitivity**: 80-94% **Specificity**: 78-91.1%.	Topical natamycin 5% Topical voriconazole 1% Amphotericin B 0.15%

groups had noticeably fewer bacteria than the other groups. According to the results of the in-vitro experiments, the best synergistic effect for killing bacteria was that of BTO@Au and US irradiation exposure.

This study presents a novel multifunctional hydrogel patch designed to mimic the framework and function of the cornea. The patch incorporates several key features: self-healing and tissue adhesion properties for secure application, a nanofiber structure that promotes cell growth and mechanical strength, and embedded antibacterial nanoparticles for infection control. These combined properties make the patch a promising therapeutic option for keratitis, offering potential benefits in terms of wound recovery, tissue regeneration, and infection prevention. Although promising, the precise mechanisms and clinical efficacy of PZDT for keratitis are still being investigated. Additional study is important to determine its safety and efficiency for ocular applications. It may eventually function as a complimentary treatment to current therapies, providing a non-invasive approach to improve keratitis recovery.

PDT in keratitis

PDT uses certain wavelengths of light to stimulate the oxygen, generating ROS and photosensitizer (PS) that can cause apoptosis, pyroptosis, or oxidative damage to cellular constituents [144]. PDT has been thoroughly researched and developed as an anticancer treatment in recent years. In the future era of drug resistance, the potential of PDT as an alternate antibacterial method has also been highlighted [145]. High quantum yields of ROS and selectivity for microbial cells over host mammalian cells are expected from PS, a class of non-toxic light-activatable dyes. Toluidine blue, methylene blue, and indocyanine green have all obtained clinical approval, with being reported as an adjuvant to antimicrobial periodontal therapy [146]. In addition, riboflavin (vitamin B2), chlorin-e6 (Ce6), curcumin, and other compounds are widely explored in the anti-cancer and antibacterial properties. For illumination, visible red light is commonly used because most PS absorb light with a wavelength of 630 nm or greater [147]. Because PDT does not penetrate other parts of the body, its use may be restricted in those areas. As a result of their ability to penetrate tissue, near-infrared and visible light are frequently used as light sources. The high light transmission capacity of eye makes it possible to effectively overcome this shortcoming when PDT is applied externally to the corneal surface or inside the eyeball [148–150].

Table 2 Summary of therapeutic modalities with their advantages, and limitations

Materials	Therapy	Advantages	Limitations	References
Graphene oxide Au-AgNS-DTTC $AuAgCu_2O$ NS	PTT	Accurate heat distribution to specific regions. Very little tissue injury. Efficient elimination of germs.	Restricted durability and piercing depth of nanoparticles	[169]
ZIF-8-PAA MB@ AgNPs@ Van-PEG (ZPMAVP) MMP-S NPs	PDT	efficient in combating germs that are resistant Minimal toxicity Encourages the healing of tissue	Concerns about photosensitiser neurotoxicity and oxygen-dependent reactions	[153]
$BaTiO_3$	PZDT	non-intrusive methodology lowers inflammation and encourages tissue repair.	Insufficient clinical research and additional optimization are required.	[187]
MLPGa, Ga $(NO_3)_3$ MSN@PDA	Metal Ion Therapy	anti-inflammatory and antimicrobial qualities Wide-ranging action	Possible hazard at elevated levels	[184]
GDY MnOx/GDY	Nano-enzyme Therapy	The ability of ROS to scavenge impact on tissue repair and inflammation reduction	Concerns about biocompatibility and durability in biological contexts	[180]

Chen et al. [151]. developed a metal organic framework for TB targeted delivery that is pH-responsive and contains (ZIF-8-PAA) zeolitic imidazolate framework-8-polyacrylic acid. ZIF-8-PAA-MB@AgNPs@Van-PEG (ZPMAVP), a composite nanomaterial double-modified with Ag-NPs and vancomycin/NH_2-polyethylene glycol (Van/NH_2-PEG), showed a definite advantage over PDT/Ag-NPs over any single technique. ZPMAVP's high pH responsiveness to bacterial diseases allows it to kill germs in 5 min using PDT. Conversely, ZPMAVP is able to release Ag-NPs and exhibit bactericidal properties without ongoing laser exposure. The biocompatibility and antibacterial activity of composite material were shown in both in-vitro retinal pigment epithelial cellular biocompatibility studies and in-vivo animal endophthalmitis models. Additionally, toluidine blue-O demonstrated antibacterial action in models of rabbit bacterial keratitis. MDR P. aeruginosa keratitis in mice in-vivo and mycobacterium fortuitum have also been reported to respond to MB- and Ce6-mediated PDT. The eradication of biofilms was found to be successful with Ce6-mediated PDT. NPs like titanium dioxide (TiO_2) have photocatalytic and antimicrobial properties, indicating tremendous potential in the biomedical field. PDT has also been shown to have amoebicidal effects. In-vitro, the TiO_2/UV-A combination effectively inhibited cysts and *Acanthamoeba sp*. trophozoites. Recently, Walvekar et al. [152]. developed MMP-S NPs, MMP-sensitive supramolecular NPs to improve photodynamic antibacterial efficacy against biofilm-associated bacterial keratitis. MMP-S NPs were produced using host-guest self-assembly of MMP-9-sensitive peptides ending in adamantane (Ad) (Ad-MMP-S PEPs) and Ce6 coupled β-cyclodextrin (β-CD) prodrug (β-CD-Ce6). Adhesion to healthy corneal cells and the normal ocular surface was inhibited by the negatively charged surface. Higher MMP-9 levels in the keratitis microenvironment were seen when MMP-S NPs penetrated infected lesions exposed cationic peptides, increasing NP interaction with gram negative bacterium *P. aeruginosa*. The photodynamic antibacterial action was significantly enhanced as the NPs pierced and bound to bacteria while also accumulated in biofilms. PDT uses have grown quickly in the antibacterial sector. Since the corneal surface is continuously exposed to the outside environment, it receives a large amount of oxygen and emits a large amount of light. Such traits facilitate the development of ROS [153–155].

Han et al. [153] generated MMP-S NPs that are produced through host-guest interactions between coupled β-cyclodextrin (β-CD) chlorin e6 (Ce6) MMP-9-sensitive peptides (YGRKKKRRQRRR-GPLGVRG-EEEEEE) and prodrugs (β-CD-Ce6) concluded with adamantane. Ad-MMP-S PEPs served as the hydrophilic shell, with Ce6 in β-CD-Ce6 serving as the hydrophobic center (Fig. 5a). MMP-S NPs with an EEEEEE peptide shell had negatively charged surfaces that hindered binding to regular ocular surfaces or healthy corneal cells, hence increasing tear retention time. After reaching the ocular infection areas, the protective EEEEEE layer of MMP-S NPs was disrupted by breaking apart GPLGVRG peptides by overexpression of MMP-9 in biofilms. (Fig. 5b). To investigate the penetration behavior of MMP-S NPs, which are capable of surface charge reversal in infected biofilm settings, were used to cure biofilms. As shown in (Fig. 5c), In biofilms treated with MMP-S NPs, a significant red fluorescence from Ce6 was seen, demonstrating the efficient penetration and accumulation of NPs. In contrast, biofilms treated with MMP-IS NPs only showed mild red fluorescence. (Fig. 5c). ROS production involved the activation of MMP-S NPs by 660 nm light irradiation. Upon cleavage of the GPLGVRG peptide shell by MMP-9, the cationic peptides were exposed, enhancing the penetration and retention of the NPs within the biofilms. This increased accumulation facilitates higher ROS

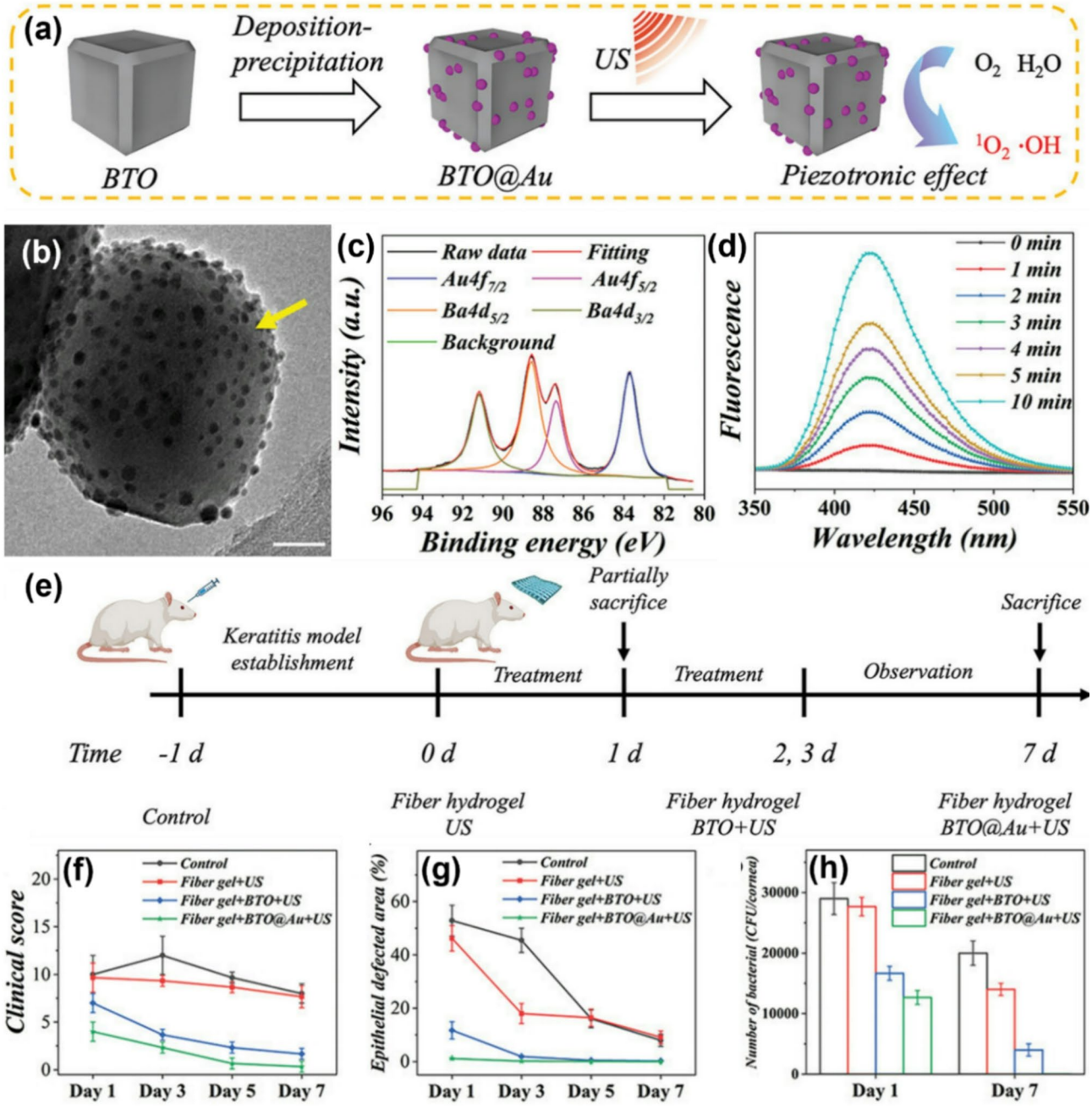


Fig. 4 Synthesis, characterization, and therapeutic efficacy of BTO@Au nanoparticles for the treatment of keratitis. (**a**) Schematic diagram BTO@Au and ROS. (**b**) TEM image of BTO@Au nanoparticles. (**c**) Au (4f) and Ba (4d) XPS spectra. (**d**) •OH. BTO@Au-loaded fiber hydrogel patch's fluorescence spectrum (**e**) Rat model of keratitis and treatment protocol development. (**f**) Clinical scores following various treatments. (**g**) Fluorescein staining quantitative analysis of the epithelial defect region. (**h**) Bacterial number histogram for days 1 and 7 [143]. Reprinted with permission from ref. no. 158. Copyrights 2023, WILEY

generation under light irradiation due to the photosensitizing properties of Ce6 in the NPs. The ROS production was amplified in MMP-S NPs compared to MMP-IS NPs, which lack this surface charge reversal and biofilm-specific responsiveness, leading to enhanced antibacterial efficiency in *P. aeruginosa* biofilms. β-CD-Ce6 was synthesized through a multi-step process involving the activation of β-CD with OTs, followed by therapy with triethylenetetramine to obtain β-CD-NH_2. Subsequent amidation with lipophilic Ce6 yielded β-CD-Ce6, with an average of one Ce6 molecule grafted per β-CD molecule, as confirmed by NMR (Fig. 5d-e). MMP-9-cleavable (MMP-S) and non-cleavable (MMP-IS) peptides with Ad termination were made to order and confirmed by mass

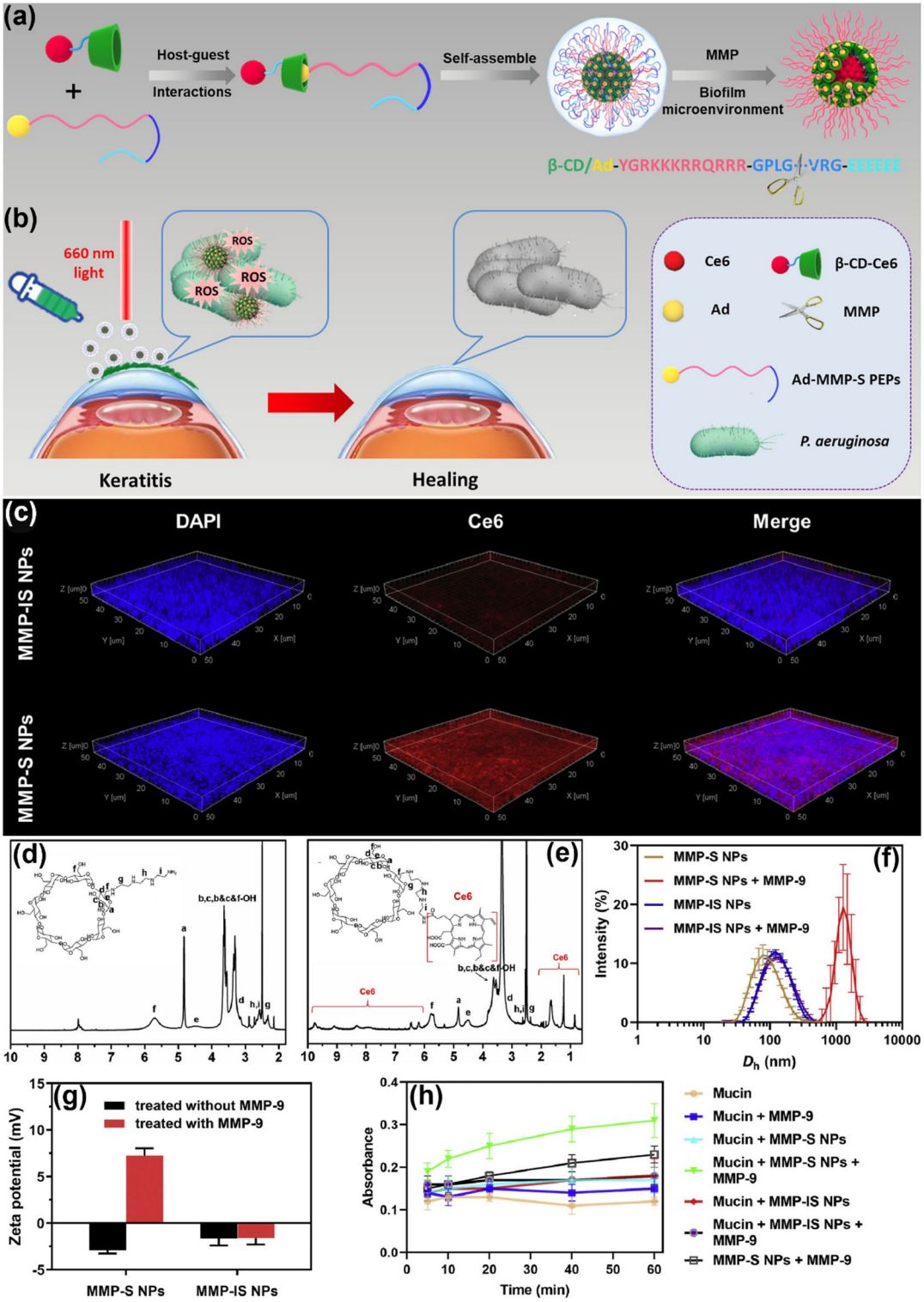


Fig. 5 Synthesis and characterization of matrix metalloproteinase-sensitive nanoparticles (MMP-S NPs) for targeted drug delivery to bacterial biofilms in the cornea. (**a**) Schematic representation of MMP-S nanoparticle synthesis (**b**) Design of MMP-activated improved antimicrobial PDT using MMP-S NPs to eradicate cornea biofilms. (**c**) Nanoparticle penetration into P. aeruginosa biofilms medicated with MMP-IS and MMP-S NPs for four hours at a Ce6 concentration of 30 μM. 1 H NMR spectra of β-CD-NH_2(**d**)and β-CD-Ce6 (**e, f**) Measurements of the hydrodynamic diameter and (**g**) Zeta potential of MMP-S and MMP-IS NPs with and without MMP-9 treatment. (**h**) Mucoadhesion studies employing turbidimetric readings [153]. Reprinted with permission from ref. no. 172. Copyrights 2020, ELSVIER

spectrometry. Supramolecular NPs, using the significant host-guest connection among β-CD and Ad, MMP-S NPs and MMP-IS NPs were created by self-assembly of β-CD-Ce6 with either MMP-9-sensitive or -insensitive peptides. Given the overexpression of MMP-9 in biofilm-associated bacterial keratitis, the MMP-9 sensitivity of MMP-S NPs was examined. DLS analysis revealed a significant size increase of MMP-S NPs (from 100 nm to above 1800 nm) upon cultivation with MMP-9 at pH 5.0, mimicking the acidic microenvironment for biofilms. In contrast, MMP-IS NPs remained relatively unchanged (Fig. 5f). TEM imaging confirmed this size increase and morphological transformation of MMP-S NPs. Additionally, following MMP-9 treatment, the zeta potential of MMP-S NPs changed from − 2.5 mV to + 7.3 mV, signaling that the cationic peptide was exposed. (Fig. 5g). These findings imply that MMP-S NPs experience size changes and surface charge reversals in the biofilm environment, which promotes penetration and retention. Because the EEEEEE peptide inhibits contact with mucin, turbidimetric studies showed that MMP-S NPs have superior antimucoadhesive characteristics. (Fig. 5h). A slight increase in absorbance was observed for MMP-9-treated MMP-S NPs due to the size-induced changes.

This is a novel approach to combat *P. aeruginosa* biofilms using supramolecular MMP-S NPs. These NPs are designed to be stable in healthy tissues but undergo significant size and charge changes upon exposure to MMP-9, which is overexpressed in infected tissues. This enables targeted delivery and enhanced penetration into biofilms. Once within the biofilm, the NPs release a photosensitizer, Ce6, which generates highly ROS upon light irradiation, leading to bacterial cell death and reduced inflammation. The study demonstrates the potential of MMP-S NPs as a potentially effective treatment approach for infections linked to biofilms, particularly bacterial keratitis.

PTT in keratitis

PTT is a type of therapy which utilizes photothermal transduction agents (PTAs) thermal conversion action. By converting the energy from an external light source into heat, target cells (such bacteria, cancer cells, and other pathogens) can be killed and the ambient temperature raised [156, 157]. PTT is currently gaining popularity as a powerful method for combating bacteria-induced illnesses, thanks to its wide-ranging bactericidal effectiveness and robust prevention of biofilm development *via* physical heat. Photothermal preservation procedure is somewhat quick, typically taking just a few minutes. NIR light, which has a wavelength range of 700–1100 nm, is thought to be the ideal wavelength for therapeutic PTT because of its exceptional strength to enter tissues with little impact on healthy human tissue [158–160]. The bactericidal effect primarily implicates the production of oxidative stress. Radiated PTAs generate hyperthermia, which causes protein denaturation, nucleic acid degradation, and cell membrane disintegration [161]. Typical PTAs comprise noble metallic nanoparticles, particularly Au-NPs, Nanoconjugates based on carbon (such as graphene and graphene oxide), organic compounds (diketopyrrolopyrrole, DPP, and ICG, for example), as well as polymeric NPs, among others. The combination of several types of PTAs can improve the photothermal effects and antibacterial activity [162]. Noble metallic NPs have been testified to be effective in treating eye contagions, and the use of other promising PTAs in this sector is still being researched and developed. The remarkable localized surface plasmon resonance properties of noble metallic nanocomposites, such as those of silver, gold, ruthenium, and palladium, are the main cause of their high photothermal conversion efficiency [163–165]. The delocalized conduction electrons in metal nanostructures begin to oscillate coherently with respect to the framework of positive nuclei at the same frequency as the incoming light when they are exposed to electromagnetic radiation with the right wavelength. Au-NPs are quite good metal-based PTAs for a number of applications, especially antibacterial ones [166]. The Changes with pH-sensitive zwitterion with mixed charges can provide Au-NPs with bacterial targeting characteristics, potentially reducing harm to healthy tissues. Although Ag-NPs have lower photothermal efficiency than Au-based NPs, their remarkable intrinsic bactericidal capabilities have led to the development of a combination of chemo-photothermal antibacterial methods [167, 168].

It has been demonstrated that gold-silver nanoshells (AuAgNS-DTTC) that are conjugated enhance injury healing. (Fig. 6a). Surprisingly, in order to enhance the healing process of cutaneous chronic injury and non-recoverable keratitis with multi drug resistant (MDR) bacterial infection, using a hollow AuAg core and a Cu_2O shell. Qiao et al. [169] demonstrated a synthetic (photothermal, released Ag^+, ROS production, and improved healing by released Cu^{2+}) cupriferous hollow nanoshell ($AuAgCu_2O$ NS). (Fig. 6b). Photoactivation of $AuAgCu_2O$ NS under NIR laser irradiation induced the generation of ROS. The unique composition and structure of the NSs enhance their ability to interact with bacterial cells, facilitated by their high electrical intensity and localized heating effect. Upon laser irradiation, the NSs generate ROS, as confirmed by the strong fluorescence signal detected using DCFH-DA. These ROS disrupt bacterial cell membranes, damage intracellular components like DNA and proteins, and impair biofilm formation, leading to bacterial death. Along with its exceptional antibacterial activity in-vitro, $AuAgCu_2O$ NS has demonstrated strong anti-infective and healing efficiency in-vivo against keratitis

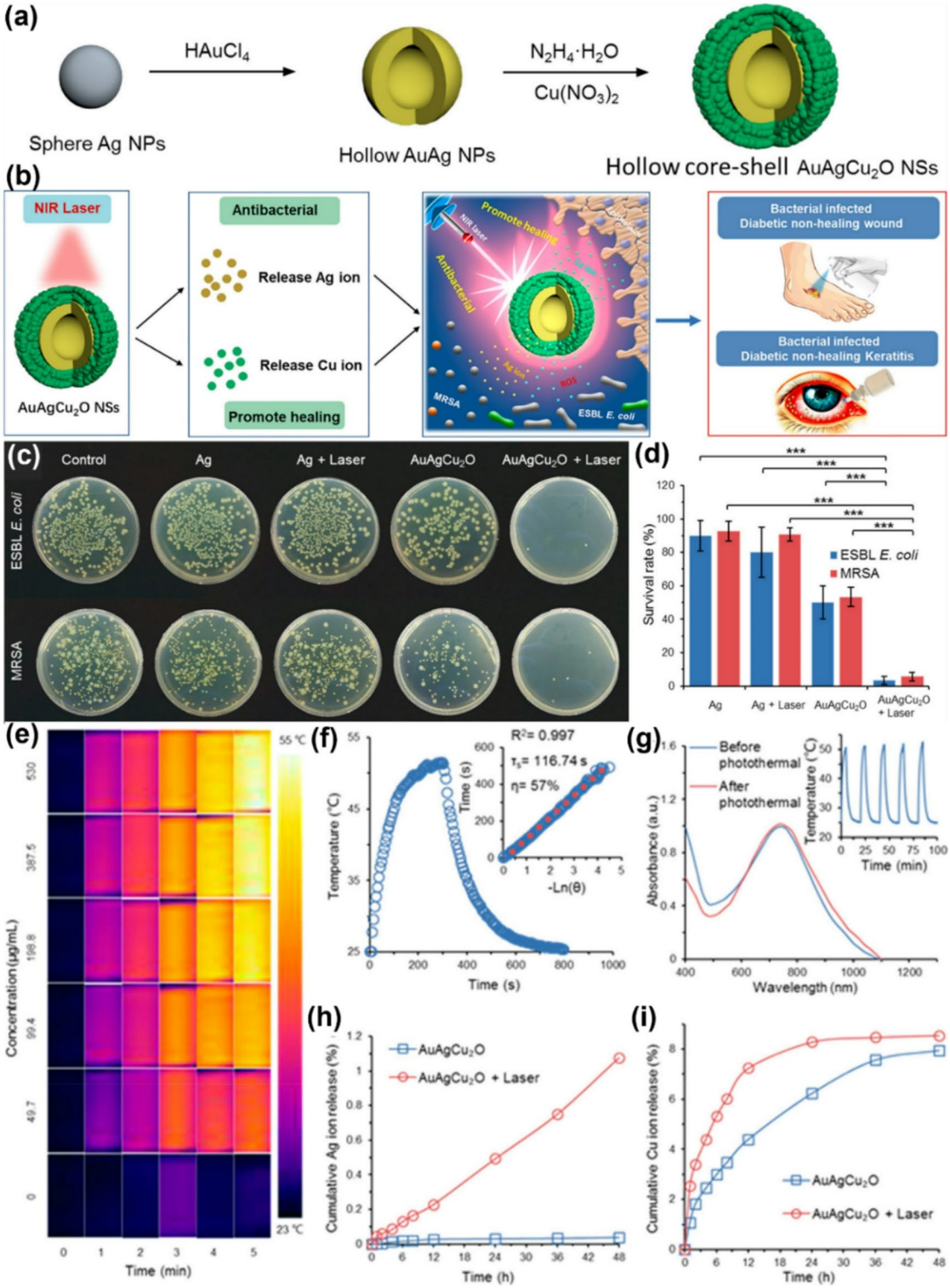


Fig. 6 Synthesis, characterization, and antimicrobial activity of $AuAgCu_2O$ nanoshells. (**a**) Graphical representation of the $AuAgCu_2O$ NSs synthetic mechanism. (**b**) Cupriferous hollow nanoshell with a composite structure ($AuAgCu_2O$ NS) (**c**) Images of the microorganism colony-forming units following a 24-hour standard incubation period with 100 mL of planktonic growth solution (1:106 dilution) from only 26.4 μg/mL groups of the OD600 assay. (**d**) Histogram demonstrating the quantitative rate of bacterial survival (**e**) Thermal imaging parade of various $AuAgCu_2O$ NS concentrations over a 5-minute irradiation period. (**f**) Water-floating $AuAgCu_2O$ NSs' photothermal evolution profile (25 °C, 397.5 μg/mL) (**g**) Comparison of $AuAgCu_2O$ NSs UV-vis-NIR absorption spectra before and after NIR irradiation (**h-i**) Ag and Cu^+ cumulative release quantities from $AuAgCu_2O$ NS hydrogel solution, either with or without NIR laser exposure [169]. Reused under creative common attribution license

infected with MRSA, which has the consequence of non-healing corneal wounds. Two MDR bacterial strains, *S. aureus* (MRSA) methicillin-resistant and extended-spectrum β-lactamase-positive *E. coli* (ESBL *E. coli*), which belong to gram-positive and gram-negative bacteria, respectively, were assessed against $AuAgCu_2O$ NSs with and without laser. The bactericidal qualities of traditional Ag-NPs were evaluated using them as a standard. Assays for optical density and turbidity at 600 nm (OD600). For ESBL *E. coli*, Ag-NPs had 70.02 and 99.45% inhibition at 35 μg/mL; for MRSA, Ag-NPs + laser had 63.84 and 99.54% inhibition at 35 μg/mL effectively suppressed the reproduction of both strains when $AuAgCu_2O$ NSs were used with or without a laser. Ag-NPs, which show the antibacterial activity both with and without the laser, was, however, weak against both strains ESBL *E. coli* was inhibited by Ag-NPs at 24.16 and 26.05% at 35 μg/mL; Ag-NPs + laser, 22.21. Under laser irradiation, $AuAgCu_2O$ NSs efficiently eradicated both organisms at less than 26.4 μg/mL, which is the lowest inhibitory concentration (MIC) of $AuAgCu_2O$ NSs PTT for MRSA and ESBL *E. coli*. Additional research was done on the colony-forming unit (CFU) test and survival rate analysis. (Fig. 6c, d**).** For all bactericidal groups, the $AuAgCu_2O$ NS-based PTT's MIC of 26.4 μg/mL was employed. There was no evidence of a synergistic effect between normal NIR laser irradiation and Ag-NPs, since live microbes were nearly same between the Ag-NPs with and without laser groups. By drastically reducing the number of colonies, $AuAgCu_2O$ NSs treatment, on the other hand, showed antibacterial action. Therefore, the complex structure of the empty AuAg core, which shows greater Ag ion release in comparison to regular Ag-NPs in suspension, is responsible for the behaviors mentioned above. More significantly, $AuAgCu_2O$ NSs could kill all MDR bacteria by mediating synergistic antibacterial effects through PTT and Ag^+ release with the aid of laser irradiation. At the same concentration (26.4 μg/mL), the bactericidal activity of clinical antibiotics was compared between $AuAgCu_2O$ NS lasers and penicillin versus MRSA and ESBL *E. coli*. Both MDR bacterial strains were more strongly inhibited by $AuAgCu_2O$ NS laser irradiation than by penicillin. Next, the photothermal capability of $AuAgCu_2O$ NSs was properly investigated by maximizing the laser power density, NS concentration, and temperature rise. (Fig. 6e). The calculations demonstrate that by controlling the laser power density and concentration of NSs, an exceptional photothermal efficiency of $AuAgCu_2O$ NSs could be achieved and modified, the temperature of $AuAgCu_2O$ NSs in under five minutes (Fig. 6f) increased to 51.2 °C. $AuAgCu_2O$ NSs had an efficiency of 57% photothermal conversion. (Figs. 6g). Laser irradiation after five cycles at 2.55 W/cm^2, the $AuAgCu_2O$ NSs demonstrated exceptional photostability, with virtually no decrease in absorbance and consistent photothermal conversion efficiency. Driven by the remarkable NIR photothermal transformation effect and the tiny size of Cu_2O protrusions of $AuAgCu_2O$ NSs, the hyperthermia and ion release (Ag and Cu ions) may be achieved after laser irradiation. The released Ag and copper Cu ions from the $AuAgCu_2O$ NSs solution were analyzed with and without NIR laser irradiation throughout a predefined time range. When compared to $AuAgCu_2O$ NSs without laser irradiation, the presence of the laser led to a noticeably greater amount of Ag and Cu ion release in the $AuAgCu_2O$ NS solution. (Fig. 6h, i). This suggests that Cu_2O protrusions' minuscule size and the photothermal action may help $AuAgCu_2O$ NSs release Cu and Ag ions.

This study introduces a novel dual-purpose $AuAgCu_2O$ nanogel that offers a multi-pronged approach to combat MDR-infected chronic injuries and nonhealing keratitis. The uniform-sized NPs of nanogel release Ag and Cu ions, and produce heating effect under NIR laser irradiation, synergistically targeting and eliminating bacteria. The gel support enables precise and targeted therapy within the lesion, promoting wound healing through copper ion-mediated tissue regeneration. Notably, the nanogel demonstrates exceptional biocompatibility, making it a promising candidate for clinical translation. This innovative nanoplatform could revolutionize the treatment of chronic infections, offering a powerful tool to combat drug-resistant bacteria and accelerate wound healing. PTT is a promising alternative for bacterial eradication because of its great efficacy and noninvasive nature. Significant ablation necessitates hyperthermia, although non-localized high temperatures typically cause significant harm to adjacent healthy tissues. Over and above 70% of the corneal matrix comprises of collagen, which is also an essential part of other ocular frameworks. Thermal scission and dehydration in PTT can extremely degrade ultra-structures of collagen, resulting in recurrent corneal opacity. It might be feasible to create customized mild-temperature PTT nano-platforms and create synergistic antibacterial methods such as photodynamic-photothermal therapy and chemo-photothermal therapy.

Nanoenzyme therapy

A family of nanomaterials known as nanozymes has proven to be an excellent alternative to natural enzymes due to its enzyme-like properties, high stability, low cost, and ease of storage. It has also been applied to biosensing, diagnostics, disease treatment, and other fields [164]. A number of keratitis nanozyme treatments have recently been created, and they have shown impressive results in reducing corneal inflammation and preventing infection [170, 171]. However, despite significant progress in these treatments, the efficiency of these treatments in treating

infectious keratitis is mainly limited by the diverse therapeutic requirements of disease and the effectiveness of the nanozymes involved [172]. Interestingly, multienzyme-like nanozymes that have many enzyme-like activities seem to be promising options for the treatment of infectious keratitis [173]. However, there are currently few studies on antibacterial and anti-inflammatory combination keratitis treatments based on multienzyme-like nanozymes. Enhancing delivery efficiency is a significant problem in the therapy of ocular illnesses, in addition to multienzyme-like nanozymes [174]. The microneedle approach is painless, bloodless, effective having patient-friendly features and transdermal drug release effectiveness enable ocular drugs to cross the ocular barrier, enhancing the effectiveness of treatment [175]. Studies on nanozyme ocular microneedle are still rare, nevertheless. Manganese oxide (MnO) nanoclusters, decorated on graphdiyne nanosheets, were developed as multienzyme-like nanozymes. These nanozymes were then incorporated into polymethyl methacrylate-based ocular microneedle patches and hyaluronic acid. The resulting microneedle patches were made to cure keratitis brought on by fungus or bacteria [176, 177]. In-vitro, ex-vivo, and in-vivo studies demonstrated that the microneedles effectively penetrated the corneal epithelium, targeted pathogens, and activated the nanozymes within the biofilm microenvironment. The activated nanozymes exhibited oxidase, peroxidase, superoxide dismutase, and catalase-like activities, leading to the destruction of pathogens, reduction of oxidative stress, and promotion of tissue repair. Compared to commercial ophthalmic voriconazole, the microneedle patches showed superior therapeutic efficacy without inducing microbial resistance or cytotoxicity [178, 179].

Liu et al. [180]. created graphdiyne (GDY) powders using a Glaser-Hay coupling process and the hexaethynylbenzene (HEB) monomer. Thin-layered GDY nanosheets were produced by ultrasonically exfoliating the powders. Manganese chloride, sodium hydroxide, and GDY nanosheets were hydrothermally produced as MnOx/GDY using a 1:1:5 ratio in ethylene glycol solution for three hours at 160 °C. The MGMN was produced by vacuum casting MnOx/GDY, HA, and PMMA onto polydimethylsiloxane (PDMS) MN molds. The molds were then separated at 5000 rpm for 30 min and cured for 24 h at 25 °C (Fig. 7a). The oxidase (OXD) like activity of MnOx/GDY was assessed using the TMB method (Fig. 7b). The nanozyme efficiently catalyzed the conversion of oxygen to superoxide radicals, oxidizing TMB, resulting in a characteristic absorption peak. The OXD-like activity was significantly influenced by oxygen concentration, pH, and enzyme and substrate concentrations. ROS production by MnOx/GDY nanosheets involved their activation by the biofilm and inflammatory microenvironments at the site of corneal infection. Under optimal conditions, MnOx/GDY exhibited high catalytic activity catalysing the generation of ROS during the antimicrobial process. This is evidenced by the significant ROS fluorescence staining observed in treated bacterial and fungal groups. The adherence of MnOx/GDY nanosheets to microbial surfaces facilitated their enzymatic activation, inducing localized ROS production without causing substantial physical damage to microbial membranes or cytoplasm. These ROS play a key role in eradicating pathogens by disrupting essential cellular processes while maintaining minimal cytotoxic effects. An in-vitro investigation was used to assess MnOx/GDY's antibacterial efficacy against MRSA and MDR *Candida albicans* (*C. albicans*). The suppression growth curves revealed that increasing concentrations of MnOx/GDY (0–25 μg mL^{-1}) led to enhanced suppressive effects on both fungi and bacteria (Fig. 7c-d). According to live/dead staining, a 20 μg mL^{-1} concentration of MnOx/GDY resulted in nearly complete eradication of both bacteria and fungi, as indicated by strong red fluorescence. At this concentration, only 10% of bacteria and 14% of fungi survived in the MnOx/GDY group (Fig. 7e). To evaluate the decrease in biofilm biomass, MRSA and *Candida albicans* biofilms were stained with crystal violet. MnOx/GDY treatment reduced bacterial and fungal biofilms by up to 80% and 67%, respectively (Fig. 7f). As shown in (Fig. 7g) green fluorescence decreased and red fluorescence increased with increasing MnOx/GDY concentration. At 20 μg mL^{-1}, strong red fluorescence was seen, indicating significant damage to both fungal and bacterial biofilms. At this concentration, only 16% of bacterial and 6% of fungal biofilms survived. The MnOx/GDY nanozyme catalyzed the formation of superoxide radicals, which were detected using electron spin resonance spectroscopy. This confirmed the OXD-like activity of the nanozyme (Fig. 7h). The electrochemical analysis revealed that MnOx/GDY exhibited the highest catalytic activity and oxygen activation efficiency compared to other nanomaterials (Fig. 7i). Additionally, MnOx/GDY demonstrated superior electronic conductivity and carrier transfer efficiency (Fig. 7j).

Nanozymes, particularly multi-enzyme-like nanozymes, offer a promising approach due to their diverse catalytic activities and ability to target and eliminate pathogens, reduce inflammation, and promote tissue repair. The integration of nanozymes into microneedle patches provides a convenient and effective delivery system, enhancing drug penetration and therapeutic efficacy. The development of MnOx/GDY-based microneedle patches represent a significant development in the field of keratitis treatment, providing a potential solution to combat both bacterial and fungal infections. To properly assess the safety and effectiveness, more

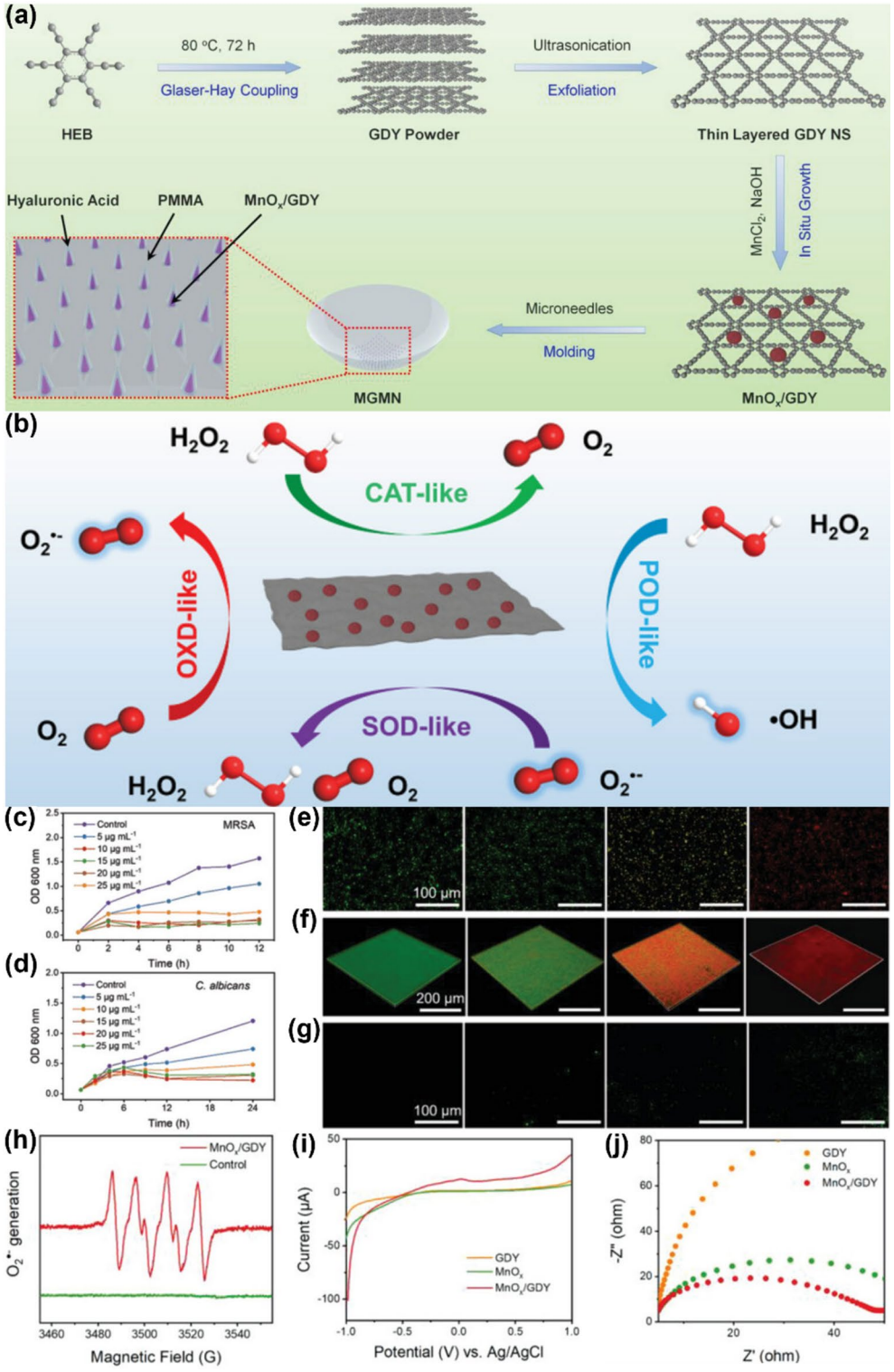


Fig. 7 Synthesis and characterization of a multifunctional nanomaterial (MnOx/GDY) for the treatment of bacterial and fungal infections. (**a**) Schematic illustration of MGMN fabrication process. (**b**) Schematic illustration of MnOx/GDY multienzyme-like activity. (**c**) Microbial growth inhibition curve for MRSA. (**d**) Microbial growth inhibition curve for *C. albicans*. (**e**) Live/dead cell staining images. (**f**) Live/dead cell staining images of biofilms. (**g**) Intracellular ROS fluorescence images. (**h**) O_2•- generation by MnOx/GDY. (**i**) Polarization curves of GDY, MnOx, and MnOx/GDY. (**j**) EIS spectra of GDY, MnOx, and MnOx/GDY.[198] Reproduced with permission from ref. no. 198. Copyrights 2023, WILEY

investigation and clinical trials are required of these innovative therapies and to explore their potential for personalized medicine.

Metal Ion therapy

Metal-based anti-infective drugs have gained a lot of attention with the quick development of nanomedicine because of their potent and multi-targeted antibacterial properties, which include metabolic disruption, the generation of ROS, and genetic material damage [181]. Since ancient times, metals have been employed as antibacterial agents; nevertheless, their exact mechanisms of action have been unknown for the majority of history. According to recent research, oxidative stress, protein malfunction, or membrane damage are the three main ways that certain metals harm microbial cells [83, 182]. Certain metals are essential to the biochemistry of life in all species because they perform cellular tasks that organic molecules cannot. Certain metal ions are essential for the structure of DNA and cell membranes; it is estimated that almost half of all proteins are reliant on metal atoms for both their structural integrity and their involvement in essential biological functions like catalysis and electron transfer. However, when these necessary metals are present in excess, they are fatal to all cells. Additionally, some non-essential metals, such Te, Ag, and Hg, are exceedingly toxic to the majority of bacteria and exhibit microbicidal action at very low doses [183].

MLPGa-based antifungal technique was created to break down the exopolysaccharide in the biofilm matrix and cell walls. The intrinsic catalytic activity of the Ga^{+} integrated within the polydopamine-modified mesoporous silicon nanosystem induced the ROS production. Upon degradation of exopolysaccharides in fungal cell walls and biofilms by lyticase, the MLPGa system adhered to fungal surfaces, facilitating Ga^{+} release. These ions disrupt iron metabolism by mimicking iron, interfering with iron-dependent enzymatic processes critical for antioxidative defense, such as superoxide dismutase (SOD3). This disruption triggers oxidative stress, amplifying ROS production within the fungal cells. The cascade of oxidative stress is further supported by upregulated antioxidant-related genes (e.g., CAT1, TTR1) as a compensatory response to increased ROS levels. This ROS generation, combined with metabolic interference and cell wall degradation, contributes to the potent antifungal activity of MLPGa. The generation of intrinsic ROS and metabolic disruption by liberated Ga^{+}, eliminates both mature biofilms and planktonic *C. albicans* (Fig. 8a). He et al. [184]. investigated the antifungal effectiveness of co-incubating planktonic *C. albicans* with varying concentrations of MLPGa (0–50 μg mL^{-1}) for a full day. CFU test on sabouraud dextrose agar (SDA) plates and corresponding survival rate analysis were used to confirm and compare the antifungal property of various groups (MSN@PDA, control, free ly, free $Ga(NO_3)_3$, MLPGa) with a concentration of 40 μg mL^{-1} (Fig. 8b). Although MLPGa effectively eliminated fungi and prevented the development of CFUs, just around 10% of the fungus survived in the free $Ga(NO_3)_3$ group, compared to over 80% in the MSD@PDA, control, and freely handled groups. Additionally, live/dead double staining fluorescent images demonstrated the most potent antifungal effect of MLPGa in these groups. SEM and TEM techniques were then employed to further examine *C. albicans* morphological alterations following MLPGa treatment and other methods. (Fig. 8c) demonstrates that the fungus in the MSN@PDA and control groups had an oval-rounded form, thick and homogeneous cytoplasm, connective and an intact and cell wall. But after being medicated with free lyticase, their cell wall changed to thin and some of their cytoplasm was irregular. Although the cell wall structure of free $Ga(NO_3)_3$ groups was largely intact, a similar condition of inhomogeneous cytoplasm was noted, indicating potential internal interference of metal ions. MLPGa NPs combine the effects of Ga^{+} and enzymes to speed up fungal death by complying to the fungal surface, breaking down the structure of cell wall, and disrupting the internal cytoplasm to cause leakage. SEM pictures revealed that MLPGa caused similar surface adherence and structural damage to fungus. Planktonic fungus and mature biofilm had lower total carbohydrate contents after being used with free lyticase than in the control groups, and the content decreased even more after being treated with MLPGa. β-1,3-glucan, a major exopolysaccharide, was used for further studies. After being treated with free lyticase or MLPGa, the β-1,3-glucan-specific dye aniline blue with green fluorescence was nearly invisible for planktonic fungus in (Fig. 8d), but it was evident in the control, MSN@PDA, and free $Ga(NO_3)_3$ groups. The fluorescence of aniline blue dye was likewise significantly reduced in mature biofilms treated with lyticase or MLPGa. Furthermore, lyticase treatment reduced β-1,3-glucan contents in both mature *C. albicans* and planktonic biofilms compared to the MSN@PDA groups or control. MLPGa-treated fungus and mature biofilms had the lowest β-1,3-glucan concentrations compared to other therapies. These findings support MLPGa's capacity to degrade exopolysaccharides found in cell walls or biofilms, including β-1,3-glucan. The MLPGa has outstanding antifungal efficacy in both mature biofilms and planktonic fungi following polysaccharide degradation *via* metabolic interference and intrinsic cascade ROS generation. Additionally, in a mouse model of fungal keratitis, the MLPGa-based antifungal approach shows excellent benefits in biological safety and has a reasonable therapeutic efficacy; no overtly harmful side effects were noted. The dynamic release behavior of the Ga^{+} may

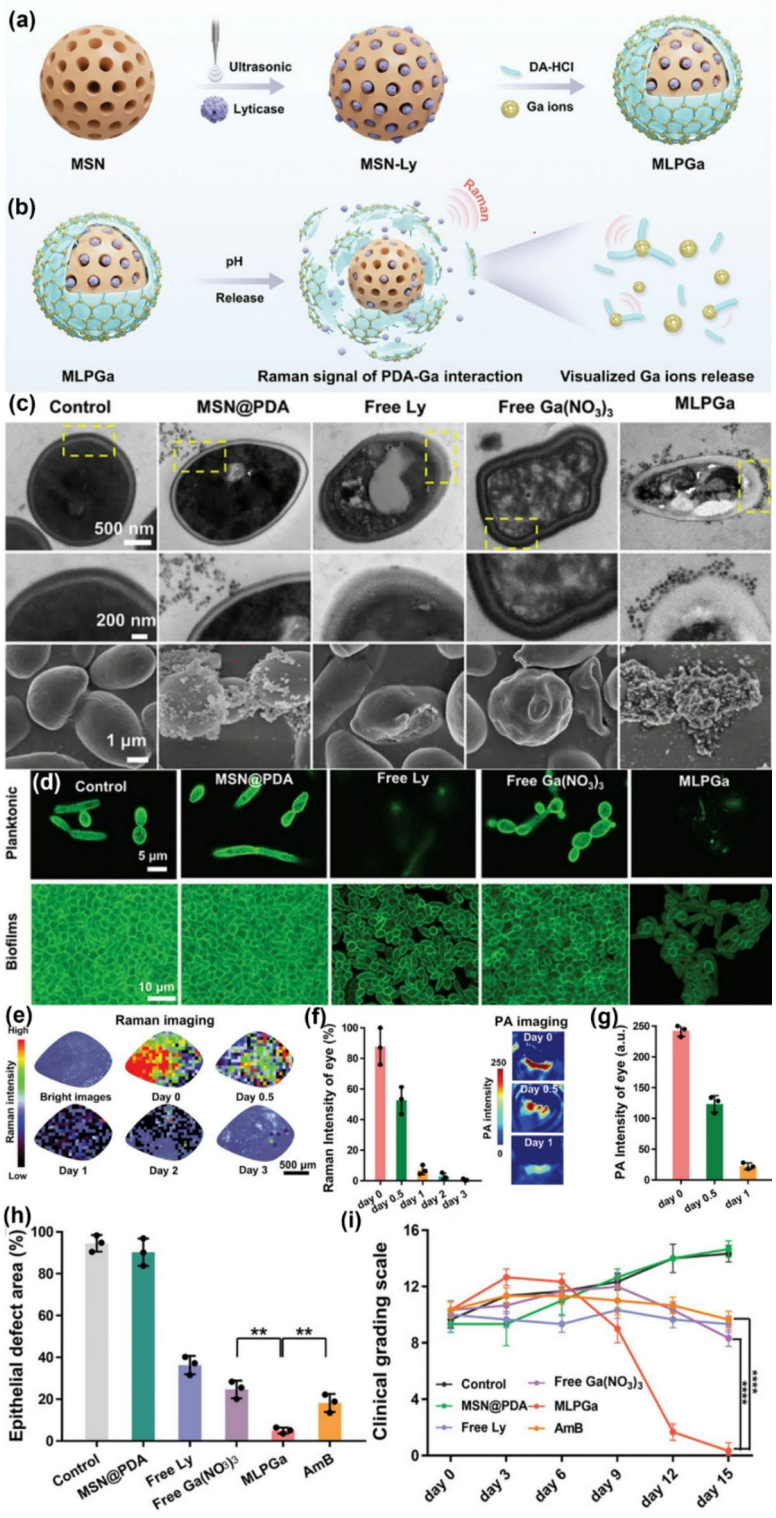


Fig. 8 Therapeutic efficacy of a metal-organic framework (MOF)-based drug delivery system (MLPGa) for fungal keratitis treatment. (**a**) The microstructure and design procedure of MLPGa. (**b**) Raman imaging is used to track the release of $Ga^{+}$and lyticase. (**c**) SEM and TEM pictures of *C. albicans* after co-incubation with different doses. (**d**) Confocal pictures of β-1,3-glucan stained with aniline blue (green fluorescence). (**e**) Raman mapping images in the fungal-infected eye at 1387 cm^{-1} and the quantitative analysis that goes along with them. (**f**) Quantitative intensity analysis and photoacoustic imaging. (**g**) Fluorescein staining quantitative analysis for the region of epithelial defects (**h-i**) Clinical grading scale for various treatments [184]. Reproduced with permission from ref. no. 203. Copyrights 2022, WILEY

be precisely shown by comparing the Raman signals created *via* the PDA layer in MLPGa and Ga^+ with the Ga concentration as determined by ICP-MS methods. Following dropwise pouring of the MLPGa solution (Day 0), the visible Raman signals were seen inside the diseased eyes. These signals weakened after two days of treatment and almost vanished for three days, implying that the Ga^+ were released, though a large amount of Ga was calculated by the ICP-MS (Fig. 8e). Additionally, the NIR-optical properties of PDA in MLPGa produced a photoacoustic signal that suggested the possibility of a gradual breakdown and emission of Ga^+ from MLPGa (Fig. 8f-g). Thus, we hypothesize that MLPGA might remain in the infected eyes for at least a day and continually release Ga ions for long-term therapy based on the outcomes of detailed ICP-MS and Raman/PA pictures. The cornea infection progressed with time, with hyperemia, edema, and cornea opacification with a specific amount of exudation and neovascularization observed after 15 days of no therapy or MSN@PDA treatment. Conversely, the cornea's state was marginally improved, and some of the edema and opacity gradually dissipated with lyticase or $Ga(NO_3)_3$ therapy alone. Cornea turned translucent, inflammation was successfully controlled, and nearly no new blood vessels developed following MLPGa therapy, indicating that the therapeutic efficacy could be further enhanced. On 15th day, the cornea in the groups medicated with MLPGa was almost entirely healed and effective than in the groups medicated with AmB. This was demonstrated by the smaller area of the epithelial defect (average: ≈3.62% versus ≈18.2%) and the lower clinical grading scale (average: ≈0.3 versus ≈9.7) (Fig. 8h-i), MLPGa-based antifungal strategy with very little toxicity showed impressive therapeutic success against fungal keratitis. Additionally, as a long-acting therapeutic approach, MLPGa-based nanodrops can be used to lower treatment pressure and dosage frequency. These findings could lead to a more effective way to destroying fungi and treating fungal keratitis in clinical settings.

There is an increasing prevalence of keratitis, particularly among young, healthy individuals, and lack of therapeutic modalities like limitations of current treatments like natamycin and azoles. A promising novel approach involves a lyticase and Ga^+ integrated nanosystem (MLPGa). This innovative system targets fungal cell walls and biofilms, degrading exopolysaccharides and subsequently releasing Ga^+ to disrupt fungal metabolism and induce cell death. Preclinical studies demonstrate superior efficacy of MLPGa in treating fungal keratitis compared to conventional therapies, leading to significant improvements in corneal health and reduced inflammation. This research offers a potential breakthrough in the therapy of fungal keratitis, providing a more effective and targeted treatment option for this challenging ocular infection.

Artificial intelligence and keratitis

Infectious keratitis is a serious eye condition that can lead to blindness if not diagnosed and treated promptly. Early detection is crucial to prevent progression, but the diagnosis and identification of the underlying cause of the infection are often challenging. Advances in artificial intelligence (AI), particularly in deep learning, offer promising solutions to address these challenges [185]. AI algorithms can analyze clinical images, such as slit-lamp images and confocal microscopy data, to accurately identify and classify corneal abnormalities, including keratitis. These systems not only help in the early detection of the disease but also assist in determining the most appropriate treatment strategies [30]. A deep learning system developed for automated classification of keratitis and corneal abnormalities showed high performance in distinguishing between infected and healthy corneal tissue. By leveraging extensive datasets of slit-lamp and smartphone images, the system outperformed cornea specialists in some instances [186]. Moreover, AI systems trained on confocal microscopy images have proven effective in detecting *acanthamoeba* keratitis with high accuracy. By employing convolutional neural networks, AI can efficiently classify images as either positive for *acanthamoeba* keratitis or nonspecific, aiding in faster and more accurate diagnoses [30]. To optimize these systems, various image processing techniques, including morphological operations and histogram equalization, were applied. Additionally, advanced algorithms like support vector machines (SVM) have been integrated to improve classification accuracy and sensitivity, particularly for grading the severity of corneal ulcers [187]. These AI-driven systems can also reduce computational costs by using techniques such as Global Average Pooling 2D, which enhances training efficiency while maintaining output quality [30]. Beyond diagnostic applications, AI is beginning to play a role in the development of novel therapies for infectious keratitis. AI algorithms can assist in designing targeted treatments, such as nanoparticle-based therapies, by simulating how nanoparticles interact with biological tissues. AI-driven nanoparticle design is emerging as a key technology that could accelerate the development of effective nanotherapies, offering a personalized approach to treating keratitis. As AI continues to evolve, its integration with nanoparticle design holds significant potential to revolutionize treatment strategies, ensuring faster, more precise, and effective interventions for patients with infectious keratitis. This synergy of AI and nanotechnology promises to greatly enhance the clinical outcomes of patients and could lead to more widespread adoption of nanotherapies in the future.

Conclusion and future perspective

Keratitis continues to be a significant global health challenge, necessitating the development of innovative and more effective therapeutic strategies. While traditional treatments have notable limitations, advance therapies offer a transformative potential for improving drug delivery and therapeutic outcomes. By utilizing these strategies, researchers can achieve enhanced targeted drug delivery, increased bioavailability, and reduced systemic toxicity, which is crucial for addressing the complexities of microbial keratitis. Key therapeutic modalities, including PZDT, PDT, and PTT, and metal ion therapy show great promise in combating keratitis, but their safety and efficacy must be optimized. Nanotechnology plays a pivotal role in refining these therapies by enabling precise drug release, minimizing adverse effects, and improving treatment precision. Moreover, recent advances such as nanoenzyme therapy offer new insights into the real-time monitoring of infection progression and treatment responses at the cellular level, further enhancing the accuracy of therapeutic interventions. To drive these innovations forward, research should focus on developing nanocarriers with controlled degradation rates, biocompatible materials, and mechanisms for targeted delivery to the cornea. Theranostic approaches, combining diagnostics with therapeutics, hold substantial promise for optimizing keratitis treatment. Addressing the growing challenge of drug resistance is equally critical, and nanocarriers can be engineered to deliver multiple drugs or drug combinations to bypass resistance mechanisms. Ongoing research into these strategies, alongside the integration of combination therapies, will be crucial to overcoming existing treatment barriers. Ultimately, advancements in the emerging therapies will not only improve clinical outcomes but also significantly reduce the burden of keratitis, enhancing the quality of life for affected patients worldwide. While innovative therapies like PZDT, PDT, and PTT offer promising avenues for keratitis treatment, ensuring their safety and efficacy is paramount. Nanotechnology, with its ability to engineer materials at the nanoscale, provides a powerful platform to address these challenges.

The development of advanced drug delivery systems with precisely controlled degradation rates can ensure timely drug release while minimizing tissue retention, utilizing natural and biodegradable materials to reduce toxicity and enhance patient safety. Modifying the surfaces of drug carriers with biocompatible polymers or ligands can further improve cellular uptake and reduce immune responses, enhancing the overall efficiency of the treatment. Integrating theranostic approach with controlled drug release mechanisms helps to optimize of precise drug exposure and therapeutic efficacy. Additionally, designing carriers with specific ligands to target receptors on BRB can improve drug transport. The use of biodegradable carriers that passively penetrate the BRB due to their small size offers another promising strategy. Combining these advanced delivery systems with other therapies, such as gene or stem cell therapy, can further enhance penetration and efficacy. To optimize delivery efficiency, it is crucial to investigate the mechanisms through which drug carriers are taken up by corneal cells. Detailed studies of intracellular trafficking pathways can ensure the effective delivery of therapeutic agents to their target sites, while strategies to promote endosomal escape can facilitate drug release directly into the cytoplasm. Another promising direction is designing carriers that release their medication payload in response to specific environmental triggers. For instance, delivery systems that respond to the acidic pH of infected tissues or elevated temperatures associated with inflammation could provide targeted drug release, offering significant advantages for treating conditions such as keratitis. Incorporating MDR inhibitors into drug carriers can overcome resistance mechanisms, while combining advanced delivery systems with conventional therapies or novel treatments can enhance therapeutic efficacy and prevent drug resistance. Exploring the potential of probiotics and prebiotics to modulate the ocular microbiome offers an additional avenue to improve the effectiveness of these therapies, further advancing treatment outcomes.

To address the regulatory challenges and biocompatibility testing for keratitis treatments, it is essential to outline a clear roadmap for clinical trials. The first step involves ensuring that the proposed treatment meets all regulatory requirements, such as adherence to good manufacturing practices and submission of preclinical data that demonstrates efficacy and safety. In vitro and in vivo biocompatibility studies should focus on assessing potential cytotoxicity of treatment, ocular irritation, and long-term safety, with particular attention to the potential for any adverse immune responses. Following successful preclinical evaluations, Phase I clinical trials should begin with small-scale studies to assess safety and dosage, particularly focusing on ocular toxicity and adverse events. Phase II trials can then move to larger populations to evaluate efficacy, optimal dosing treatments, and techniques to reduce infection-related inflammation without causing harm to the corneal tissue. Phase III trials would then expand to multicentre studies, comparing the novel treatment with current gold-standard therapies to assess its superiority or non-inferiority. Throughout these stages, regulatory agencies such as the FDA or EMA would require detailed reports on all clinical outcomes, as well as any potential long-term effects of the treatment. This structured approach to clinical trials, coupled with robust biocompatibility testing, will provide

the necessary evidence to support the clinical translation of new therapies for keratitis.

Author contributions
Siying Qu and Shuihua Zheng wrote the main manuscript text and prepared figures. All authors reviewed the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics and consent to participate
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Achouri D, Alhanout K, Piccerelle P, Andrieu V. Recent advances in Ocular Drug Delivery. Drug Dev Ind Pharm. 2013;39(11):1599–617. https://doi.org/10.3109/03639045.2012.736515.
2. Hashem SM, Gad MK, Anwar HM, Saleh NM, Shamma RN, Elsherif NI. Itraconazole-loaded ufasomes: evaluation, characterization, and anti-fungal activity against Candida Albicans. Pharmaceutics. 2023;15(1):26. https://doi.org/10.3390/pharmaceutics15010026.
3. Nguyen T, Ong J, Masalkhi M, Waisberg E, Zaman N, Sarker P, Aman S, Lin H, Luo M, Ambrosio R, Machado AP, Ting DSJ, Mehta JS, Tavakkoli A, Lee AG. Artificial Intelligence in corneal diseases: a narrative review. Contact Lens Anterior Eye. 2024;47(6):102284. https://doi.org/10.1016/j.clae.2024.102284.
4. Böhnke M, Masters BR. Confocal Microscopy of the Cornea. Prog Retin Eye Res. 1999;18(5):553–628. https://doi.org/10.1016/S1350-9462(98)00028-7.
5. Sood S, Tiwari A, Sangwan J, Vohra M, Sinha NR, Tripathi R, Sangwan VS, Mohan RR. Role of epigenetics in corneal health and disease. Prog Retin Eye Res. 2025;104:101318. https://doi.org/10.1016/j.preteyeres.2024.101318.
6. Jester JV, Moller-Pedersen T, Huang J, Sax CM, Kays WT, Cavangh HD, Petroll WM, Piatigorsky J. The Cellular basis of corneal transparency: evidence for 'Corneal crystallins. ' J Cell Sci. 1999;112(5):613–22. https://doi.org/10.1242/jcs.112.5.613.
7. Boroumand S, Rahmani M, Sigaroodi F, Ganjoury C, Parandakh A, Bonakdar A, Khani M-M, Soleimani M. The Landscape of clinical trials in corneal regeneration: a systematic review of tissue Engineering approaches in corneal disease. J Biomed Mater Res B Appl Biomater. 2024;112(8):e35449. https://doi.org/10.1002/jbm.b.35449.
8. Xia Y, Chen K, Yang Q, Chen Z, Jin L, Zhang L, Yu X, Wang L, Xie C, Zhao Y, Shen Y, Tong J. Methylation in Cornea and corneal diseases: a systematic review. Cell Death Discov. 2024;10(1):1–15. https://doi.org/10.1038/s41420-024-01935-2.
9. Kinoshita S, Adachi W, Sotozono C, Nishida K, Yokoi N, Quantock AJ, Okubo K. Characteristics of the human ocular surface epithelium. Prog Retin Eye Res. 2001;20(5):639–73. https://doi.org/10.1016/S1350-9462(01)00007-6.
10. Ung L, Bispo PJM, Shanbhag SS, Gilmore MS, Chodosh J. The Persistent Dilemma of Microbial Keratitis: global burden, diagnosis, and Antimicrobial Resistance. Surv Ophthalmol. 2019;64(3):255–71. https://doi.org/10.1016/j.survophthal.2018.12.003.
11. Cabrera-Aguas M, Khoo P, Watson SL. Infectious keratitis: a review. Clin Exp Ophthalmol. 2022;50(5):543–62. https://doi.org/10.1111/ceo.14113.
12. Flaxman SR, Bourne RRA, Resnikoff S, Ackland P, Braithwaite T, Cicinelli MV, Das A, Jonas JB, Keeffe J, Kempen JH, Leasher J, Limburg H, Naidoo K, Pesudovs K, Silvester A, Stevens GA, Tahhan N, Wong TY, Taylor HR, Bourne R, Ackland P, Arditi A, Barkana Y, Bozkurt B, Braithwaite T, Bron A, Budenz D, Cai F, Casson R, Chakravarthy U, Choi J, Cicinelli MV, Congdon N, Dana R, Dandona R, Dandona L, Das A, Dekaris I, Monte MD, Deva J, Dreer L, Ellwein L, Frazier M, Frick K, Friedman D, Furtado J, Gao H, Gazzard G, George R, Gichuhi S, Gonzalez V, Hammond B, Hartnett ME, He M, Hejtmancik J, Hirai F, Huang J, Ingram A, Javitt J, Jonas J, Joslin C, Keeffe J, Kempen J, Khairallah M, Khanna R, Kim J, Lambrou G, Lansingh VC, Lanzetta P, Leasher J, Lim J, Limburg H, Mansouri K, Mathew A, Morse A, Munoz B, Musch D, Naidoo K, Nangia V, Palaiou M, Parodi MB, Pena FY, Pesudovs K, Peto T, Quigley H, Raju M, Ramulu P, Rankin Z, Resnikoff S, Reza D, Robin A, Rossetti L, Saaddine J, Sandar M, Serle J, Shen T, Shetty R, Sieving P, Silva JC, Silvester A, Sitorus RS, Stambolian D, Stevens G, Taylor H, Tejedor J, Tielsch J, Tsilimbaris M, Meurs J. Global Causes of Blindness and Distance Vision Impairment 1990–2020: a systematic review and Meta-analysis. Lancet Glob Health. 2017;5(12):e1221–34. https://doi.org/10.1016/S2214-109X(17)30393-5. VarmaR.Virgili, G.; Wang, Y. X.; Wang, N.-L.; West, S.; Wiedemann, P.; Wong, T.; Wormald, R.; Zheng, Y.
13. Zhou L, Deng Y, Ren Y, Poon HL, Chu WY, Wang H, Chan YK. Antibiotics-free nanomaterials against bacterial keratitis: eliminating infections with reactive oxygen species (ROS). Chem Eng J. 2024;482:148978. https://doi.org/10.1016/j.cej.2024.148978.
14. Ung L, Acharya NR, Agarwal T, Alfonso EC, Bagga B, Bispo PJ, Burton MJ, Dart JK, Doan T, Fleiszig SM, Garg P, Gilmore MS, Gritz DC, Hazlett LD, Iovieno A, Jhanji V, Kempen JH, Lee CS, Lietman TM, Margolis TP, McLeod SD, Mehta JS, Miller D, Pearlman E, Prajna L, Prajna NV, Seitzman GD, Shanbhag SS, Sharma N, Sharma S, Srinivasan M, Stapleton F, Tan DT, Tandon R, Taylor HR, Tu EY, Tuli SS, Vajpayee RB, Van Gelder RN, Watson SL, Zegans ME, Chodosh J. Infectious corneal ulceration: a proposal for neglected Tropical Disease Status. Bull World Health Organ. 2019;97(12):854–6. https://doi.org/10.2471/BLT.19.232660.
15. Ting DSJ, Ho CS, Cairns J, Elsahn A, Al-Aqaba M, Boswell T, Said DG, Dua HS. 12-Year analysis of incidence, microbiological profiles and in Vitro Antimicrobial susceptibility of infectious keratitis: the Nottingham Infectious Keratitis Study. Br J Ophthalmol. 2021;105(3):328–33. https://doi.org/10.1136/bjophthalmol-2020-316128.
16. Stapleton F. The epidemiology of infectious keratitis. Ocul Surf. 2023;28:351–63. https://doi.org/10.1016/j.jtos.2021.08.007.
17. Sharma N, Bagga B, Singhal D, Nagpal R, Kate A, Saluja G, Maharana PK. Fungal keratitis: a review of clinical presentations, treatment strategies and outcomes. Ocul Surf. 2022;24:22–30. https://doi.org/10.1016/j.jtos.2021.12.001.
18. Arnalich-Montiel F, Corneal Endothelium. Applied anatomy. In: Alió JL, del Alió JL, Arnalich-Montiel F, editors. Corneal regeneration: therapy and surgery. Springer International Publishing: Cham,; 2019. pp. 419–24. https://doi.org/10.1007/978-3-030-01304-2_27.
19. Yagci A. Update on Peripheral Ulcerative Keratitis. Clin Ophthalmol. 2012;6(null):747–54. https://doi.org/10.2147/OPTH.S24947.
20. Niu P, Wu Y, Zeng F, Zhang S, Liu S, Gao H. Development of Nanodrug-Based Eye drops with good Penetration properties and ROS responsiveness for controllable release to treat fungal keratitis. NPG Asia Mater. 2023;15(1):1–15. https://doi.org/10.1038/s41427-023-00478-9.
21. Anwar A, Khan NA, Alharbi AM, Alhazmi A, Siddiqui R. Applications of photodynamic therapy in Keratitis. Int Ophthalmol. 2024;44(1):1–10. https://doi.org/10.1007/s10792-024-03062-4.
22. Wang R, Dong Y, Zhang J, Hao L, Zhou L, Sun L, Song Y, Jiang R, Qi X. A multifunctional nanoplatform with dual-targeted antibacterial and cascaded Immunomodulatory Strategy for the treatment of bacterial keratitis. Chem Eng J. 2024;498:155323. https://doi.org/10.1016/j.cej.2024.155323.
23. Xiang J, Zou R, Wang P, Wang X, He X, Liu F, Xu C, Wu A. Nitroreductase-responsive nanoparticles for *in situ* fluorescence imaging and synergistic

antibacterial therapy of bacterial keratitis. Biomaterials. 2024;308:122565. https://doi.org/10.1016/j.biomaterials.2024.122565.
24. Wilson SE, Chaurasia SS, Medeiros FW. Apoptosis in the initiation, modulation and termination of the corneal wound Healing Response. Exp Eye Res. 2007;85(3):305–11. https://doi.org/10.1016/j.exer.2007.06.009.
25. Chaurasia SS, Kaur H, de Medeiros FW, Smith SD, Wilson SE. Dynamics of the expression of Intermediate Filaments Vimentin and Desmin during Myofibroblast differentiation after corneal Injury. Exp Eye Res. 2009;89(2):133–9. https://doi.org/10.1016/j.exer.2009.02.022.
26. Austin A, Lietman T, Rose-Nussbaumer J. Update on the management of infectious keratitis. Ophthalmology. 2017;124(11):1678–89. https://doi.org/10.1016/j.ophtha.2017.05.012.
27. Shah S, Wozniak RAF. Staphylococcus Aureus and Pseudomonas Aeruginosa Infectious Keratitis: Key Bacterial Mechanisms That Mediate Pathogenesis and Emerging Therapeutics. Front. Cell. Infect. Microbiol. 2023:13. https://doi.org/10.3389/fcimb.2023.1250257
28. Sushma MV, Sankaranarayanan SA, Bantal V, Pemmaraju DB, Rengan AK. Ethosomal nanoformulations for Combinational Photothermal Therapy of Fungal Keratitis. Adv Ther. 2023;6(5):2200331. https://doi.org/10.1002/adtp.202200331.
29. Huang X, Li L, Chen Z, Yu H, You X, Kong N, Tao W, Zhou X, Huang J. Nanomedicine for the detection and treatment of ocular bacterial infections. Adv Mater. 2023;35(46):2302431. https://doi.org/10.1002/adma.202302431.
30. Shareef O, Soleimani M, Tu E, Jacobs DS, Ciolino JB, Rahdar A, Cheraqpour K, Ashraf M, Habib NB, Greenfield J, Yousefi S, Djalilian AR, Saeed H. N. A novel Artificial Intelligence Model for diagnosing *Acanthamoeba* Keratitis through Confocal Microscopy. Ocul Surf. 2024;34:159–64. https://doi.org/10.1016/j.jtos.2024.07.010.
31. Ye Y, He J, Wang H, Li W, Wang Q, Luo C, Tang X, Chen X, Jin X, Yao K, Zhou M. Cell Wall Destruction and Internal Cascade Synergistic Antifungal Strategy for Fungal Keratitis. ACS Nano. 2022;16(11):18729–45. https://doi.org/10.1021/acsnano.2c07444.
32. Lv Z, Li S, Zeng G, Yao K, Han H. Recent progress of Nanomedicine in managing Dry Eye Disease. Adv Ophthalmol Pract Res. 2024;4(1):23–31. https://doi.org/10.1016/j.aopr.2024.01.008.
33. Wu Y, Li X, Fu X, Huang X, Zhang S, Zhao N, Ma X, Saiding Q, Yang M, Tao W, Zhou X, Huang J. Innovative nanotechnology in Drug Delivery systems for Advanced treatment of posterior segment ocular diseases. Adv Sci. 2024;11(32):2403399. https://doi.org/10.1002/advs.202403399.
34. Liu X, Zhang Y, Peng F, Li C, Wang Q, Wang Z, Hu L, Peng X, Zhao G, Lin J. Macrophage membrane-coated nanoparticles for the delivery of natamycin exhibit increased antifungal and anti-inflammatory activities in fungal keratitis. ACS Appl Mater Interfaces. 2024;16(44):59777–88. https://doi.org/10.1021/acsami.4c11366.
35. Guo YX, He YX. Nanoparticle-based drug Delivery systems: an updated strategy for treating fungal keratitis. Colloid Interface Sci Commun. 2024;61:100794. https://doi.org/10.1016/j.colcom.2024.100794.
36. Meng T, Zheng J, Shin CS, Gao N, Bande D, Sudarjat H, Chow W, Halquist MS, Yu F-S, Acharya G, Xu Q. Combination Nanomedicine Strategy for preventing high-risk corneal transplantation rejection. ACS Nano. 2024;18(31):20679–93. https://doi.org/10.1021/acsnano.4c06595.
37. Fu X, Tian X, Lin J, Wang Q, Gu L, Wang Z, Chi M, Yu B, Feng Z, Liu W, Zhang L, Li C, Zhao G. Zeolitic Imidazolate Framework-8 offers an anti-inflammatory and antifungal method in the treatment of aspergillus fungal keratitis in Vitro and in vivo. Int J Nanomed. 2024;19:11163–79. https://doi.org/10.2147/IJN.S480800.
38. Li P, Kang J, Wu X, Wang D, Li Y, Liang N, Du R, Wang M, Dong A, Li Y. Mild phototherapy strategies for efficient treatment of bacterial keratitis while avoiding Side effects. Nano Today. 2024;57:102336. https://doi.org/10.1016/j.nantod.2024.102336.
39. Sathe P, Kailasam V, Nagarjuna V, Sharma H, Velpandian T, Garg P, Nirmal J. Nanomicelles empower Natamycin in treating fungal keratitis: an in Vitro, Ex vivo and in vivo study. Int J Pharm. 2024;656:124118. https://doi.org/10.1016/j.ijpharm.2024.124118.
40. Peng Y, Pang S, Zeng Y, Wei J, Lu J, Ruan Y, Hong X, He X, Chu X, Guo Y, Guo H, Qian S, Jiang Z, Jiang Z, Wang B. Antibiotic-free ocular sterilization while suppressing Immune response to protect corneal transparency in infectious keratitis treatment. J Controlled Release. 2024;374:563–76. https://doi.org/10.1016/j.jconrel.2024.08.038.
41. Cui W, Chen S, Hu T, Zhou T, Qiu C, Jiang L, Cheng X, Ji J, Yao K, Han H. Nanoceria-mediated cyclosporin a delivery for Dry Eye Disease Management through Modulating Immune–Epithelial Crosstalk. ACS Nano. 2024;18(17):11084–102. https://doi.org/10.1021/acsnano.3c11514.
42. Wang Z, Yu F, Hu F. Functional Chitosan and its derivative-related drug Delivery systems for Nano-Therapy: recent advances. Pharmaceutics. 2024;16(3):337. https://doi.org/10.3390/pharmaceutics16030337.
43. Krawczyk A, Stadler SM, Strzalka-Mrozik B. Nanomedicines for Dry Eye Syndrome: targeting oxidative stress with modern nanomaterial strategies. Molecules. 2024;29(16):3732. https://doi.org/10.3390/molecules29163732.
44. Yu C, He J, Tan M, Hou Y, Zeng H, Liu C, Meng H, Su Y, Qiao L, Lookman T, Bai Y. Selective enhancement of Photo-Piezocatalytic performance in BaTiO3 Via Heterovalent Ion Doping. Adv Funct Mater. 2022;32(52):2209365. https://doi.org/10.1002/adfm.202209365.
45. Nguyen DD, Lue SJ, Lai J-Y. Tailoring Therapeutic properties of Silver nanoparticles for effective bacterial keratitis treatment. Colloids Surf B Biointerfaces. 2021;205:111856. https://doi.org/10.1016/j.colsurfb.2021.111856.
46. Hamblin MR, Abrahamse H. Oxygen-independent antimicrobial photoinactivation: type III photochemical mechanism? Antibiotics. 2020;9(2):53. https://doi.org/10.3390/antibiotics9020053.
47. Shi S, Hu L, Hu D, Ou X, Huang Y. Emerging Nanotherapeutic approaches for Diabetic Wound Healing. Int J Nanomed 2024.
48. Tsui K-H, Li C-J. Mitoquinone Shifts Energy Metabolism to Reduce ROS-Induced oxeiptosis in female granulosa cells and mouse oocytes. Aging. 2023;15(1):246–60. https://doi.org/10.18632/aging.204475.
49. Zhang Y, Wang Q, Zhao X, Ma Y, Zhang H, Pan G. Molecularly imprinted nanomaterials with Stimuli responsiveness for applications in Biomedicine. Molecules. 2023;28(3):918. https://doi.org/10.3390/molecules28030918.
50. Zhang J, Zhuang Y, Sheng R, Tomás H, Rodrigues J, Yuan G, Wang X, Lin K. Smart stimuli-responsive strategies for Titanium Implant functionalization in bone regeneration and therapeutics. Mater Horiz. 2024;11(1):12–36. https://doi.org/10.1039/D3MH01260C.
51. Li D, Liu M, Li W, Fu Q, Wang L, Lai E, Zhao W, Zhang K. Synthesis of prussian blue nanoparticles and their Antibacterial, Antiinflammation and Antitumor Applications. Pharmaceuticals. 2022;15(7):769. https://doi.org/10.3390/ph15070769.
52. Shen S, Qiu J, Huo D, Xia Y. Nanomaterial-enabled Photothermal Heating and its use for Cancer Therapy via Localized Hyperthermia. Small. 2024;20(7):2305426. https://doi.org/10.1002/smll.202305426.
53. Dehghani B. Prevalence and nature of bacterial keratitis in Australia: A Systematic Review and Meta-Analysis.
54. Yan E, Kwek G, S Qing N, Lingesh S, Xing B. Antimicrobial photodynamic therapy for the remote eradication of Bacteria. ChemPlusChem. 2023;88(3):e202300009. https://doi.org/10.1002/cplu.202300009.
55. Liu X, Huang K, Zhang F, Huang G, Wang L, Wu G, Ren H, Yang G, Lin Z. Multifunctional Nano-in-Micro Delivery systems for targeted therapy in Fundus Neovascularization diseases. J Nanobiotechnol. 2024;22(1):354. https://doi.org/10.1186/s12951-024-02614-1.
56. He X, Qian Y, Wu C, Feng J, Sun X, Zheng Q, Li X, Shen J. Entropy-mediated high-entropy MXenes Nanotherapeutics: NIR-II-Enhanced intrinsic oxidase mimic activity to Combat Methicillin-Resistant Staphylococcus Aureus infection. Adv Mater. 2023;35(26):2211432. https://doi.org/10.1002/adma.202211432.
57. Zhang R, Yan X, Fan K. Nanozymes inspired by natural enzymes. Acc Mater Res. 2021;2(7):534–47. https://doi.org/10.1021/accountsmr.1c00074.
58. Zhou J, Zhang L, Wei Y, Wu Q, Mao K, Wang X, Cai J, Li X, Jiang Y. Photothermal Iron-based Riboflavin Microneedles for the Treatment of Bacterial Keratitis via Ion Therapy and Immunomodulation. Adv Healthc Mater. 2024;13(26):2304448. https://doi.org/10.1002/adhm.202304448.
59. Zhou W, Jiang X, Zhen X. Development of Organic Photosensitizers for Antimicrobial Photodynamic Therapy. Biomater Sci. 2023;11(15):5108–28. https://doi.org/10.1039/D3BM00730H.
60. Yao J, Shang X, Wei J, Guo L, Sheng F, Shao N, Chen L, Zhang Z, Zhou Y, Chen W. Light-activated silver Ion Release from Ag2Te nanoparticles for Treating Drug-resistant bacterial keratitis. ACS Appl Nano Mater. 2024;7(23):27325–35. https://doi.org/10.1021/acsanm.4c05174.
61. Ginn C, Burton MJ, Mohamed-Ahmed AHA. Anti-Infective Drug Nanosystems. In *Fundamentals of Pharmaceutical Nanoscience*; Uchegbu, I. F., Schätzlein, A. G., Lalatsa, A., Lopez, D. R. S., Eds.; Springer Nature Switzerland: Cham, 2024;451–484. https://doi.org/10.1007/978-3-031-59478-6_17
62. Gu L, Lin J, Wang Q, Meng F, Niu G, Lin H, Chi M, Feng Z, Zheng H, Li D, Zhao G, Li C. Mesoporous Zinc Oxide-Based Drug Delivery System offers an antifungal and immunoregulatory strategy for treating Keratitis. J Controlled Release. 2024;368:483–97. https://doi.org/10.1016/j.jconrel.2024.03.006.

63. Jiang X, Jin Y, Zeng Y, Shi P, Li W. Self-implantable core–Shell Microneedle Patch for Long-Acting treatment of Keratitis via programmed drug release. Small. 2024;20(29):2310461. https://doi.org/10.1002/smll.202310461.
64. Koh Y-Y, Sun C-C, Hsiao C-H. Epidemiology and the Estimated Burden of Microbial Keratitis on the Health Care System in Taiwan: a 14-Year Population-based study. Am J Ophthalmol. 2020;220:152–9. https://doi.org/10.1016/j.ajo.2020.07.026.
65. Hoffman JJ, Yadav R, Sanyam SD, Chaudhary P, Roshan A, Singh SK, Arunga S, Hu VH, Macleod D, Leck A, Burton MJ. Microbial Keratitis in Nepal: Predicting the Microbial Aetiology from Clinical features. J Fungi. 2022;8(2):201. https://doi.org/10.3390/jof8020201.
66. Kaur S, Sohnen P, Swamynathan S, Du Y, Espana EM, Swamynathan SK. Molecular nature of ocular surface barrier function, diseases that affect it, and its relevance for Ocular Drug Delivery. Ocul Surf. 2023;30:3–13. https://doi.org/10.1016/j.jtos.2023.08.001.
67. Aiello F, Gallo Afflitto G, Ceccarelli F, Turco MV, Han Y, Amescua G, Dart JK, Nucci C. Perspectives on the Incidence of *Acanthamoeba* Keratitis. Ophthalmology. 2024. https://doi.org/10.1016/j.ophtha.2024.08.003
68. Niu L, Liu X, Ma Z, Yin Y, Sun L, Yang L, Zheng Y. Fungal keratitis: Pathogenesis, diagnosis and Prevention. Microb Pathog. 2020;138:103802. https://doi.org/10.1016/j.micpath.2019.103802.
69. Prajna VN, Prajna L, Muthiah S. Fungal keratitis: the Aravind experience. Indian J Ophthalmol. 2017;65(10):912. https://doi.org/10.4103/ijo.IJO_821_17.
70. Stapleton F. Contact Lens-related corneal infection in Australia. Clin Exp Optom. 2020;103(4):408–17. https://doi.org/10.1111/cxo.13082.
71. Ghenciu LA, Faur AC, Bolintineanu SL, Salavat MC, Maghiari AL. Recent advances in diagnosis and treatment approaches in fungal keratitis: a narrative review. Microorganisms. 2024;12(1):161. https://doi.org/10.3390/microorganisms12010161.
72. Yang L, Li W, Zhong J, Liu X. Inhibitory effects and Mode of Antifungal Action of Isobavachalcone on *Candida Albicans* Growth and Virulence factors. Biomed Pharmacother. 2024;179:117352. https://doi.org/10.1016/j.biopha.2024.117352.
73. Szaliński M, Zgryźniak A, Rubisz I, Gajdzis M, Kaczmarek R, Przeździecka-Dołyk J. Fusarium Keratitis—Review of current treatment possibilities. J Clin Med. 2021;10(23):5468. https://doi.org/10.3390/jcm10235468.
74. Raj N, Vanathi M, Ahmed NH, Gupta N, Lomi N, Tandon R. Recent perspectives in the management of fungal keratitis. J Fungi. 2021;7(11):907. https://doi.org/10.3390/jof7110907.
75. Suriyaamporn P, Opanasopit P, Rangsimawong W, Ngawhirunpat T. Optimal design of Novel microemulsions-based two-layered dissolving microneedles for delivering fluconazole in treatment of Fungal Eye infection. Pharmaceutics. 2022;14(3):472. https://doi.org/10.3390/pharmaceutics14030472.
76. Soleimani M, Izadi A, Khodavaisy S, Santos CO. Tehupeiory-Kooreman, M. C.; Ghazvini, R. D.; Hashemi, S. J.; Mousavi, S. A. A.; Aala, F.; Abdorahimi, M.; Aminizadeh, M.; Abedinifar, Z.; Mahmoudi, S.; Mohamadi, A.; Rezaie, S.; Verweij, P. E. Fungal Keratitis in Iran: Risk Factors, Clinical Features, and Mycological Profile. Front. Cell. Infect. Microbiol. 2023:13. https://doi.org/10.3389/fcimb.2023.1094182
77. Lee Y, Robbins N, Cowen LE. Molecular mechanisms governing Antifungal Drug Resistance. Npj Antimicrob Resist. 2023;1(1):1–9. https://doi.org/10.1038/s44259-023-00007-2.
78. Arastehfar A, Gabaldón T, Garcia-Rubio R, Jenks JD, Hoenigl M, Salzer HJF, Ilkit M, Lass-Flörl C, Perlin DS. Drug-resistant Fungi: an emerging challenge threatening our limited antifungal armamentarium. Antibiotics. 2020;9(12):877. https://doi.org/10.3390/antibiotics9120877.
79. McCormick I, James C, Welton NJ, Mayaud P, Turner KME, Gottlieb SL, Foster A, Looker KJ. Incidence of herpes Simplex Virus Keratitis and other Ocular Disease: global review and estimates. Ophthalmic Epidemiol. 2022;29(4):353–62. https://doi.org/10.1080/09286586.2021.1962919.
80. Ling JYM, Yeung SN, Chan CC, Trinh T, Antaki F, Harissi-Dagher M, Sivachandran N, Fava M, Légaré M-È, Iovieno A. Trends and Clinical outcomes of Fungal Keratitis in Canada: a 20-Year retrospective Multicentre Study. Am J Ophthalmol. 2024;265:147–55. https://doi.org/10.1016/j.ajo.2024.04.009.
81. *Gallium disrupts bacterial iron metabolism and has therapeutic effects in mice and humans with lung infections| Science Translational Medicine*. https://www.science.org/doi/full/10.1126/scitranslmed.aat7520 (accessed 2024-12-17).
82. Harrison JJ, Ceri H, Turner RJ. Multimetal Resistance and Tolerance in Microbial Biofilms. Nat Rev Microbiol. 2007;5(12):928–38. https://doi.org/10.1038/nrmicro1774.
83. Lemire JA, Harrison JJ, Turner RJ. Antimicrobial activity of metals: mechanisms, molecular targets and applications. Nat Rev Microbiol. 2013;11(6):371–84. https://doi.org/10.1038/nrmicro3028.
84. Xu Y, Gao C, Li X, He Y, Zhou L, Pang G, Sun S. In Vitro Antifungal activity of silver nanoparticles against ocular pathogenic filamentous Fungi. J Ocul Pharmacol Ther. 2013;29(2):270–4. https://doi.org/10.1089/jop.2012.0155.
85. Geißel B, Loiko V, Klugherz I, Zhu Z, Wagener N, Kurzai O, van den Hondel CAMJJ, Wagener J. Azole-Induced Cell Wall Carbohydrate patches kill aspergillus Fumigatus. Nat Commun. 2018;9(1):3098. https://doi.org/10.1038/s41467-018-05497-7.
86. Singh RB, Das S, Chodosh J, Sharma N, Zegans ME, Kowalski RP, Jhanji V. Paradox of Complex Diversity: challenges in the diagnosis and management of bacterial keratitis. Prog Retin Eye Res. 2022;88:101028. https://doi.org/10.1016/j.preteyeres.2021.101028.
87. Tuft S, Somerville TF, Li J-PO, Neal T, De S, Horsburgh MJ, Fothergill JL, Foulkes D, Kaye S. Bacterial keratitis: identifying the areas of clinical uncertainty. Prog Retin Eye Res. 2022;89:101031. https://doi.org/10.1016/j.preteyeres.2021.101031.
88. Cai R, Zhang L, Chi H. Recent development of Polymer Nanomicelles in the treatment of Eye diseases. Front Bioeng Biotechnol. 2023;11. https://doi.org/10.3389/fbioe.2023.1246974.
89. Polat HK, Kurt N, Aytekin E, Bozdağ Pehlivan S, Çalış S. Novel Drug Delivery systems to improve the Treatment of Keratitis. J Ocul Pharmacol Ther. 2022;38(6):376–95. https://doi.org/10.1089/jop.2021.0127.
90. Das S, D'Souza S, Gorimanipalli B, Shetty R, Ghosh A, Deshpande V. Ocular surface infection mediated molecular stress responses: a review. Int J Mol Sci. 2022;23(6):3111. https://doi.org/10.3390/ijms23063111.
91. Semir Yarımada ÖBS. *Kültür Pozitif ve Negatif Mikrobiyal Keratitlerin Karşılaştırılması*. https://doi.org/10.4274/tjo.galenos.2021.98046
92. André C, Van Camp AG, Ung L, Gilmore MS, Bispo PJM. Characterization of the Resistome and Predominant Genetic lineages of Gram-positive Bacteria causing Keratitis. Antimicrob Agents Chemother. 2024;68(3):e01247–23. https://doi.org/10.1128/aac.01247-23.
93. Marquart ME. Animal Models of Bacterial Keratitis. BioMed Res. Int. 2011;2011(1):680642. https://doi.org/10.1155/2011/680642
94. Asbell PA, Sanfilippo CM, DeCory HH. Antibiotic resistance of bacterial pathogens isolated from the Conjunctiva in the Antibiotic Resistance monitoring in ocular micRoorganisms (ARMOR) Surveillance Study (2009–2021). Diagn Microbiol Infect Dis. 2024;108(1):116069. https://doi.org/10.1016/j.diagmicrobio.2023.116069.
95. Durrani AF, Atta S, Bhat AK, Mammen A, Dhaliwal D, Kowalski RP, Jhanji V. Methicillin-Resistant *Staphylococcus Aureus* Keratitis: initial treatment, risk factors, clinical features, and treatment outcomes. Am J Ophthalmol. 2020;214:119–26. https://doi.org/10.1016/j.ajo.2020.03.017.
96. Asbell PA, Sanfilippo CM, Sahm DF, DeCory HH. Trends in Antibiotic Resistance among Ocular microorganisms in the United States from 2009 to 2018. JAMA Ophthalmol. 2020;138(5):439–50. https://doi.org/10.1001/jamaophthalmol.2020.0155.
97. Polat HK, Bozdağ Pehlivan S, Özkul C, Çalamak S, Öztürk N, Aytekin E, Fırat A, Ulubayram K, Kocabeyoğlu S, İrkeç M, Çalış S. Development of Besifloxacin HCl loaded nanofibrous ocular inserts for the treatment of bacterial keratitis: *in Vitro, Ex vivo and in vivo* evaluation. Int J Pharm. 2020;585:119552. https://doi.org/10.1016/j.ijpharm.2020.119552.
98. Jian H-J, Anand A, Lai J-Y, Unnikrishnan B, Chang H-T, Harroun SG, Huang C-C. In situ hybridization of Polymeric Curcumin to Arginine-Derived Carbon Quantum Dots for synergistic treatment of bacterial infections. ACS Appl Mater Interfaces. 2023;15(22):26457–71. https://doi.org/10.1021/acsami.3c04316.
99. Mani H, Sidhu GS, Kumari R, Gaddipati JP, Seth P, Maheshwari RK. Curcumin differentially regulates TGF-B1, its receptors and nitric oxide synthase during impaired Wound Healing. BioFactors. 2002;16(1–2):29–43.
100. Ahmad B, Gurnani B, Patel BC. Herpes Simplex Keratitis. In *StatPearls*; StatPearls Publishing: Treasure Island (FL), 2024.
101. Shivaji S. Viruses of the Human Eye. In *Human Ocular Microbiome: Bacteria, Fungi and Viruses in the Human Eye*; Shivaji, S., Ed.; Springer Nature: Singapore, 2022; pp 199–224. https://doi.org/10.1007/978-981-19-1754-7_7
102. Poon SHL, Wong WHL, Lo ACY, Yuan H, Chen C-F, Jhanji V, Chan YK, Shih KC. A systematic review on advances in Diagnostics for Herpes Simplex Keratitis. Surv Ophthalmol. 2021;66(3):514–30. https://doi.org/10.1016/j.survophthal.2020.09.008.
103. Labib BA, Chigbu DI. Clinical management of herpes Simplex Virus Keratitis. Diagnostics. 2022;12(10):2368. https://doi.org/10.3390/diagnostics12102368.

104. Miller KE, Cassady KA, Roth JC, Clements J, Schieffer KM, Leraas K, Miller AR, Prasad N, Leavenworth JW, Aban IB, Whitley RJ, Gillespie GY, Mardis ER, Markert JM. Immune Activity and Response Differences of Oncolytic Viral Therapy in recurrent glioblastoma: gene expression analyses of a phase IB Study. Clin Cancer Res. 2022;28(3):498–506. https://doi.org/10.1158/1078-0432.CCR-21-2636.
105. Harrison KS, Jones C. Wnt antagonists suppress herpes simplex virus type 1 productive infection. Antiviral Res. 2021;191:105082. https://doi.org/10.1016/j.antiviral.2021.105082.
106. Koujah L, Madavaraju K, Agelidis AM, Patil CD, Shukla D. Heparanase-Induced Activation of AKT Stabilizes β-Catenin and Modulates Wnt/β-Catenin Signaling during Herpes Simplex Virus 1 Infection. mBio. 2021;12(6):e02792-21. https://doi.org/10.1128/mBio.02792-21
107. Spear G P. Opportunities, Technology, and the Joy of Discovery. Annu Rev Virol. 2022;9(9, 2022):1–17. https://doi.org/10.1146/annurev-virology-100520-012840.
108. Xu J, Zhang X, Jin A, Pan Y, Li Z, Meng X, Wang Y. Trends and Risk factors Associated with Stroke recurrence in China, 2007–2018. JAMA Netw Open. 2022;5(6):e2216341. https://doi.org/10.1001/jamanetworkopen.2022.16341.
109. Sarangi SN, Behera BC, Sahoo NK, Tripathy SK. Schottky Junction devices by using Bio-molecule DNA template-based one dimensional CdS-Nanostructures. Biosens Bioelectron. 2021;190:113402. https://doi.org/10.1016/j.bios.2021.113402.
110. Koganti R, Yadavalli T, Naqvi RA, Shukla D, Naqvi AR. Pathobiology and treatment of viral keratitis. Exp Eye Res. 2021;205:108483. https://doi.org/10.1016/j.exer.2021.108483.
111. Labetoulle M, Boutolleau D, Burrel S, Haigh O, Rousseau A. Herpes Simplex Virus, Varicella-Zoster Virus and Cytomegalovirus Keratitis: facts for the Clinician. Ocul Surf. 2023;28:336–50. https://doi.org/10.1016/j.jtos.2021.07.002.
112. Arshad S, Petsoglou C, Lee T, Al-Tamimi A, Carnt NA. Twenty years since the Herpetic Eye Disease Study: lessons, developments and applications to clinical practice. Clin Exp Optom. 2021;104(3):396–405. https://doi.org/10.1080/08164622.2021.1877531.
113. Pandey M, Choudhury H, Abdul-Aziz A, Bhattamisra SK, Gorain B, Su JST, Tan CL, Chin WY, Yip KY. Advancement on sustained antiviral ocular drug delivery for herpes Simplex Virus Keratitis: recent update on potential investigation. Pharmaceutics. 2021;13(1):1. https://doi.org/10.3390/pharmaceutics13010001.
114. Abdallah HM, Wintermans BB, Reuland EA, Koek A, Naiemi Nal, Ammar AM, Mohamed AA, Vandenbroucke-Grauls CMJE. Extended-spectrum β-Lactamase- and carbapenemase-producing Enterobacteriaceae isolated from Egyptian patients with suspected blood Stream infection. PLoS ONE. 2015;10(5):e0128120. https://doi.org/10.1371/journal.pone.0128120.
115. Munita JM, Arias CA. Mechanisms of Antibiotic Resistance. Virulence mechanisms of bacterial pathogens. John Wiley & Sons, Ltd; 2016. pp. 481–511. https://doi.org/10.1128/9781555819286.ch17.
116. Egrilmez S, Yildirim-Theveny Ş. Treatment-resistant bacterial keratitis: challenges and solutions. Clin Ophthalmol. 2020;14:287–97. https://doi.org/10.2147/OPTH.S181997.
117. Ung L, Chodosh J. Foundational concepts in the Biology of bacterial keratitis. Exp Eye Res. 2021;209:108647. https://doi.org/10.1016/j.exer.2021.108647.
118. Khan SA, Lee C-S. Recent progress and strategies to develop antimicrobial contact lenses and Lens cases for different types of Microbial Keratitis. Acta Biomater. 2020;113:101–18. https://doi.org/10.1016/j.actbio.2020.06.039.
119. Vaneev A, Tikhomirova V, Chesnokova N, Popova E, Beznos O, Kost O, Klyachko N. Nanotechnology for Topical Drug Delivery to the Anterior segment of the Eye. Int J Mol Sci. 2021;22(22):12368. https://doi.org/10.3390/ijms222212368.
120. Tsung T-H, Tsai Y-C, Lee H-P, Chen Y-H, Lu D-W. Biodegradable polymer-based drug-delivery systems for Ocular diseases. Int J Mol Sci. 2023;24(16):12976. https://doi.org/10.3390/ijms241612976.
121. Luhar M, Viradiya R, Panjabi S, Patel G. Nanotechnology in Ocular Drug Delivery: the potential of polymeric micelles as a drug delivery vehicle. J Ocul Pharmacol Ther. 2024. https://doi.org/10.1089/jop.2024.0060.
122. Zhang H, Li D, Ren H, Ma Z, Meng S, Qiao Y, Yang J, Wang Y, Zhou Q, Xie LA. Bioinspired Virus-Like Mechano–Bactericidal Nanomotor for Ocular Multidrug-Resistant Bacterial Infection Treatment. *Adv. Mater. n/a* (n/a), 2408221. https://doi.org/10.1002/adma.202408221
123. Vazirani J, Wurity S, Ali MH, Multidrug-Resistant, Keratitis. Ophthalmology. 2015;122(10):2110–2114. https://doi.org/10.1016/j.ophtha.2015.06.007
124. Chatterjee S, Agrawal D. Multi-drug Resistant Pseudomonas Aeruginosa Keratitis and its effective treatment with topical colistimethate. Indian J Ophthalmol. 2016;64(2):153. https://doi.org/10.4103/0301-4738.179721.
125. Zhu Y, Wu S, Sun Y, Zou X, Zheng L, Duan S, Wang J, Yu B, Sui R, Xu F-J. Bacteria-targeting photodynamic nanoassemblies for efficient treatment of Multidrug-Resistant Biofilm infected Keratitis. Adv Funct Mater. 2022;32(14):2111066. https://doi.org/10.1002/adfm.202111066.
126. Abla KK, Mehanna MM. Lipid-based Nanocarriers Challenging the Ocular Biological barriers: current paradigm and future perspectives. J Controlled Release. 2023;362:70–96. https://doi.org/10.1016/j.jconrel.2023.08.018.
127. Fernandes M, Vira D, Medikonda R, Kumar N. Extensively and Pan-drug Resistant Pseudomonas Aeruginosa Keratitis: clinical features, risk factors, and Outcome. Graefes Arch Clin Exp Ophthalmol. 2016;254(2):315–22. https://doi.org/10.1007/s00417-015-3208-7.
128. Tajima K, Miyake T, Koike N, Hattori T, Kumakura S, Yamaguchi T, Matsumoto T, Fujita K, Kuroda M, Ito N, Goto H. *Vivo* Challenging of Polymyxins and Levofloxacin Eye Drop against Multidrug-Resistant *Pseudomonas Aeruginosa* Keratitis. J Infect Chemother. 2014;20(6):343–9. https://doi.org/10.1016/j.jiac.2013.10.015.
129. J. NM. H.n., M. Unraveling Genomic and phenotypic nature of Multidrug-Resistant (MDR) *Pseudomonas Aeruginosa* VRFPA04 isolated from Keratitis Patient. Microbiol Res. 2016;193:140–9. https://doi.org/10.1016/j.micres.2016.10.002.
130. Adrianto MF, Annuryanti F, Wilson CG, Sheshala R, Thakur RRS. In Vitro Dissolution Testing models of ocular implants for posterior segment drug delivery. Drug Deliv Transl Res. 2022;12(6):1355–75. https://doi.org/10.1007/s13346-021-01043-z.
131. Li Q, Wong HL, Ip YL, Chu WY, Li MS, Saha C, Shih KC, Chan YK. Current Microfluidic platforms for Reverse Engineering of Cornea. Mater Today Bio. 2023;20:100634. https://doi.org/10.1016/j.mtbio.2023.100634.
132. Yang S, Wang Y, Liang X. Piezoelectric nanomaterials activated by Ultrasound in Disease Treatment. Pharmaceutics. 2023;15(5):1338. https://doi.org/10.3390/pharmaceutics15051338.
133. Izhiman Y, Esfandiari L. Emerging role of Extracellular vesicles and exogenous Stimuli in Molecular mechanisms of Peripheral nerve regeneration. Front Cell Neurosci. 2024;18. https://doi.org/10.3389/fncel.2024.1368630.
134. Peng C, Sun W, Zhou C, Qiang S, Jiang M, Lam JWY, Zhao Z, Kwok RTK, Cai W, Tang BZ. Vision redemption: self-reporting AIEgens for combined treatment of bacterial keratitis. Biomaterials. 2021;279:121227. https://doi.org/10.1016/j.biomaterials.2021.121227.
135. Chen F, Le P, Fernandes-Cunha GM, Heilshorn SC, Myung D. Bio-orthogonally Crosslinked Hyaluronate-Collagen Hydrogel for suture-free corneal defect repair. Biomaterials. 2020;255:120176. https://doi.org/10.1016/j.biomaterials.2020.120176.
136. Yazdanpanah G, Shen X, Nguyen T, Anwar KN, Jeon O, Jiang Y, Pachenari M, Pan Y, Shokuhfar T, Rosenblatt MI, Alsberg E, Djalilian AR. A light-curable and tunable Extracellular Matrix Hydrogel for in situ suture-free corneal repair. Adv Funct Mater. 2022;32(24):2113383. https://doi.org/10.1002/adfm.202113383.
137. Shen X, Li S, Zhao X, Han J, Chen J, Rao Z, Zhang K, Quan D, Yuan J, Bai Y. Dual-crosslinked regenerative hydrogel for Sutureless Long-Term repair of corneal defect. Bioact Mater. 2023;20:434–48. https://doi.org/10.1016/j.bioactmat.2022.06.006.
138. Shi H, Zhou J, Wang Y, Zhu Y, Lin D, Lei L, Vakal S, Wang J, Li XA. Rapid corneal Healing Microneedle for efficient ocular drug delivery. Small. 2022;18(4):2104657. https://doi.org/10.1002/smll.202104657.
139. He B, Wang J, Xie M, Xu M, Zhang Y, Hao H, Xing X, Lu W, Han Q, Liu W. 3D printed biomimetic Epithelium/Stroma Bilayer Hydrogel Implant for corneal regeneration. Bioact Mater. 2022;17:234–47. https://doi.org/10.1016/j.bioactmat.2022.01.034.
140. Xu Y, Liu J, Song W, Wang Q, Sun X, Zhao Q, Huang Y, Li H, Peng Y, Yuan J, Ji B, Ren L. Biomimetic Convex Implant for corneal regeneration through 3D Printing. Adv Sci. 2023;10(11):2205878. https://doi.org/10.1002/advs.202205878.
141. Kong B, Chen Y, Liu R, Liu X, Liu C, Shao Z, Xiong L, Liu X, Sun W, Mi S. Fiber Reinforced GelMA Hydrogel to induce the regeneration of corneal stroma. Nat Commun. 2020;11(1):1435. https://doi.org/10.1038/s41467-020-14887-9.
142. Zhu P, Chen Y, Shi J. Piezocatalytic tumor therapy by Ultrasound-Triggered and BaTiO3-Mediated piezoelectricity. Adv Mater. 2020;32(29):2001976. https://doi.org/10.1002/adma.202001976.
143. Kong B, Liu R, Hu X, Li M, Zhou X, Zhao Y, Kong T. Cornea-inspired ultrasound-responsive Adhesive Hydrogel patches for Keratitis Treatment. Adv Funct Mater. 2024;34(12):2310544. https://doi.org/10.1002/adfm.202310544.

144. Han H, Li S, Zhong Y, Huang Y, Wang K, Jin Q, Ji J, Yao K. Emerging Pro-drug and Nano-Drug Strategies for Gemcitabine-Based Cancer Therapy. Asian J Pharm Sci. 2022;17(1):35–52. https://doi.org/10.1016/j.ajps.2021.06.001.
145. Kim HS, Lee DY. Nanomedicine in Clinical Photodynamic Therapy for the treatment of brain tumors. Biomedicines. 2022;10(1):96. https://doi.org/10.3390/biomedicines10010096.
146. Ren B, Li K, Liu Z, Liu G, Wang H. White Light-Triggered Zwitterionic Polymer nanoparticles based on an AIE-Active photosensitizer for photodynamic antimicrobial therapy. J Mater Chem B. 2020;8(47):10754–63. https://doi.org/10.1039/D0TB02272A.
147. Mylona V, Anagnostaki E, Parker S, Cronshaw M, Lynch E, Grootveld M. Laser-assisted aPDT protocols in Randomized Controlled clinical trials in Dentistry: a systematic review. Dent J. 2020;8(3):107. https://doi.org/10.3390/dj8030107.
148. Muehler D, Rupp CM, Keceli S, Brochhausen C, Siegmund H, Maisch T, Hiller K-A, Buchalla W, Cieplik F. Insights Into Mechanisms of Antimicrobial Photodynamic Action Toward Biofilms Using Phenalen-1-One Derivatives as Photosensitizers. Front. Microbiol. 2020:11. https://doi.org/10.3389/fmicb.2020.589364
149. Jin Y, Wang Y, Yang J, Zhang H, Yang Y-W, Chen W, Jiang W, Qu J, Guo Y, Wang B. An Integrated Theranostic Nanomaterial for targeted photodynamic therapy of infectious endophthalmitis. Cell Rep Phys Sci. 2020;1(8). https://doi.org/10.1016/j.xcrp.2020.100173.
150. Su G, Wei Z, Wang L, Shen J, Baudouin C, Labbé A, Liang Q. Evaluation of Toluidine Blue-mediated photodynamic therapy for experimental bacterial keratitis in rabbits. Transl Vis Sci Technol. 2020;9(3):13. https://doi.org/10.1167/tvst.9.3.13.
151. Chen H, Yang J, Sun L, Zhang H, Guo Y, Qu J, Jiang W, Chen W, Ji J, Yang Y-W, Wang B. Synergistic chemotherapy and photodynamic therapy of Endophthalmitis mediated by Zeolitic Imidazolate Framework-based drug Delivery systems. Small. 2019;15(47):1903880. https://doi.org/10.1002/smll.201903880.
152. Walvekar S, Anwar A, Anwar A, Sridewi N, Khalid M, Yow YY, Khan NA. Anti-amoebic potential of azole scaffolds and nanoparticles against Pathogenic *Acanthamoeba*. Acta Trop. 2020;211:105618. https://doi.org/10.1016/j.actatropica.2020.105618.
153. Han H, Gao Y, Chai M, Zhang X, Liu S, Huang Y, Jin Q, Grzybowski A, Ji J, Yao K. Biofilm Microenvironment activated Supramolecular nanoparticles for enhanced photodynamic therapy of bacterial keratitis. J Controlled Release. 2020;327:676–87. https://doi.org/10.1016/j.jconrel.2020.09.014.
154. Anwar A, Khan NA, Alharbi AM, Alhazmi A, Siddiqui R. Applications of photodynamic therapy in Keratitis. Int Ophthalmol. 2024;44(1):140. https://doi.org/10.1007/s10792-024-03062-4.
155. Zou Y, Zhang H, Zhang Y, Wu Y, Cheng J, Jia D, Liu C, Chen H, Zhang Y, Yu QA. Activation of dispersing and killing functions by Generating nitric oxide and Peroxynitrite via Cascade reactions. Acta Biomater. 2023;170:389–400. https://doi.org/10.1016/j.actbio.2023.08.038. Near-Infrared Light-Triggered Nano-Domino System for Efficient Biofilm Eradication.
156. Li L, Han X, Wang M, Li C, Jia T, Zhao X. Recent advances in the development of Near-Infrared Organic Photothermal agents. Chem Eng J. 2021;417:128844. https://doi.org/10.1016/j.cej.2021.128844.
157. Zhang W, Hu E, Wang Y, Miao S, Liu Y, Hu Y III, Liu J, Xu B, Chen D, Shen Y. Emerging antibacterial strategies with application of Targeting Drug Delivery System and Combined Treatment. Int J Nanomed. 2021;16:6141–56. https://doi.org/10.2147/IJN.S311248.
158. Ran C, Mansfield JR, Bai M, Viola NT, Mahajan A, Delikatny EJ. Practical Guidance for developing small-molecule Optical Probes for in vivo imaging. Mol Imaging Biol. 2023;25(1):240–64. https://doi.org/10.1007/s11307-023-01800-1.
159. Eckhart KE, Schmidt SJ, Starvaggi FA, Wolf ME, Vickery WM, Sydlik SA. Peptide- and protein-graphene oxide conjugate materials for Controlling Mesenchymal Stem Cell Fate. Regen Eng Transl Med. 2021;7(4):460–84. https://doi.org/10.1007/s40883-020-00182-y.
160. Fresno F, Iglesias-Juez A, Coronado JM, Photothermal Catalytic CO. 2 Conversion: Beyond Catalysis and Photocatalysis. Top Curr Chem. 2023;381(4):21. https://doi.org/10.1007/s41061-023-00430-z.
161. Wei G, Yang G, Wang Y, Jiang H, Fu Y, Yue G, Ju R. Phototherapy-based combination strategies for bacterial infection treatment. Theranostics. 2020;10(26):12241–62. https://doi.org/10.7150/thno.52729.
162. Man J, Zhu J, Weng G, Li J, Zhao J. Using gold-based nanomaterials for fighting pathogenic Bacteria: from detection to Therapy. Microchim Acta. 2024;191(10):627. https://doi.org/10.1007/s00604-024-06713-6.
163. Ling C, Wang X, Shen Y. Advances in Hollow Inorganic nanomedicines for Photothermal-based therapies. Int J Nanomed. 2021;16:493–513. https://doi.org/10.2147/IJN.S285115.
164. Li B, Mao J, Wu J, Mao K, Jia Y, Chen F, Liu J. Nano–Bio interactions: Biofilm-targeted antibacterial nanomaterials. Small. 2024;20(7):2306135. https://doi.org/10.1002/smll.202306135.
165. Doveri L, Diaz Fernandez YA, Dacarro G. Nanomaterials for Photothermal Antimicrobial surfaces. ACS Omega. 2024;9(24):25575–90. https://doi.org/10.1021/acsomega.4c01449.
166. Balitskii OA. Recent energy targeted applications of localized Surface Plasmon Resonance Semiconductor nanocrystals: a Mini-review. Mater Today Energy. 2021;20:100629. https://doi.org/10.1016/j.mtener.2020.100629.
167. Hou Y, Yang X, Liu R, Zhao D, Guo C, Zhu A, Wen M, Liu Z, Qu G, Meng H. Pathological mechanism of photodynamic therapy and Photothermal Therapy based on nanoparticles. Int J Nanomed. 2020;15:6827–38. https://doi.org/10.2147/IJN.S269321.
168. Zhang Z-C, Gu Z-G, Zhang J. Host–Guest Metal–Organic frameworks-based long-afterglow luminescence materials. Molecules. 2024;29(13):2989. https://doi.org/10.3390/molecules29132989.
169. Qiao Y, He J, Chen W, Yu Y, Li W, Du Z, Xie T, Ye Y, Hua SY, Zhong D, Yao K, Zhou M. Light-activatable synergistic therapy of drug-resistant Bacteria-infected cutaneous chronic wounds and Nonhealing Keratitis by Cupriferous Hollow nanoshells. ACS Nano. 2020;14(3):3299–315. https://doi.org/10.1021/acsnano.9b08930.
170. Huang Y, Ren J, Qu X, Nanozymes. Classification, Catalytic mechanisms, Activity Regulation, and applications. Chem Rev. 2019;119(6):4357–412. https://doi.org/10.1021/acs.chemrev.8b00672.
171. Zhang R, Xue B, Tao Y, Zhao H, Zhang Z, Wang X, Zhou X, Jiang B, Yang Z, Yan X, Fan K. Edge-Site Engineering of defective Fe–N4 nanozymes with boosted catalase-like performance for retinal vasculopathies. Adv Mater. 2022;34(39):2205324. https://doi.org/10.1002/adma.202205324.
172. Nguyen DD, Luo L-J, Yang C-J, Lai J-Y. Highly retina-permeating and Long-Acting Resveratrol/Metformin Nanotherapeutics for Enhanced Treatment of Macular Degeneration. ACS Nano. 2023;17(1):168–83. https://doi.org/10.1021/acsnano.2c05824.
173. Sheng J, Wu Y, Ding H, Feng K, Shen Y, Zhang Y, Gu N. Multienzyme-like Nanozymes: Regulation, Rational Design, and application. Adv Mater. 2024;36(10):2211210. https://doi.org/10.1002/adma.202211210.
174. Than A, Liang K, Xu S, Sun L, Duan H, Xi F, Xu C, Chen P. Transdermal Delivery of Anti-obesity compounds to Subcutaneous Adipose tissue with polymeric microneedle patches. Small Methods. 2017;1(11):1700269. https://doi.org/10.1002/smtd.201700269.
175. Kong B, Liu R, Shan J, Li M, Zhou X, Zhao Y. Frozen Reinforced Microneedles loaded with NIR-Photothermal Nanozyme for Keratitis Treatment. Nano Today. 2023;52:102000. https://doi.org/10.1016/j.nantod.2023.102000.
176. Qian X, Han X, Yu L, Xu T, Chen Y. Manganese-based functional nanoplatforms: Nanosynthetic Construction, Physiochemical Property, and Theranostic Applicability. Adv Funct Mater. 2020;30(3):1907066. https://doi.org/10.1002/adfm.201907066.
177. Li Y, Xu L, Liu H, Li Y. Graphdiyne and Graphyne: from theoretical predictions to practical construction. Chem Soc Rev. 2014;43(8):2572–86. https://doi.org/10.1039/C3CS60388A.
178. Saha I, Rai VK. Hyaluronic Acid based microneedle array: recent applications in Drug Delivery and Cosmetology. Carbohydr Polym. 2021;267:118168. https://doi.org/10.1016/j.carbpol.2021.118168.
179. Behlau I, Mukherjee K, Todani A, Tisdale AS, Cade F, Wang L, Leonard EM, Zakka FR, Gilmore MS, Jakobiec FA, Dohlman CH, Klibanov AM. Biocompatibility and Biofilm Inhibition of *N,N*-Hexyl,Methyl-Polyethylenimine Bonded to Boston Keratoprosthesis materials. Biomaterials. 2011;32(34):8783–96. https://doi.org/10.1016/j.biomaterials.2011.08.010.
180. Liu S, Bai Q, Jiang Y, Gao Y, Chen Z, Shang L, Zhang S, Yu L, Yang D, Sui N, Zhu Z. Multienzyme-like Nanozyme Encapsulated Ocular microneedles for Keratitis Treatment. Small. 2024;20(21):2308403. https://doi.org/10.1002/smll.202308403.
181. Cronholm P, Karlsson HL, Hedberg J, Lowe TA, Winnberg L, Elihn K, Wallinder IO, Möller L. Intracellular uptake and toxicity of Ag and CuO nanoparticles: a comparison between nanoparticles and their corresponding metal ions. Small. 2013;9(7):970–82. https://doi.org/10.1002/smll.201201069.
182. Lin Y, Betts H, Keller S, Cariou K, Gasser G. Recent developments of metal-based compounds against fungal pathogens. Chem Soc Rev. 2021;50(18):10346–402. https://doi.org/10.1039/D0CS00945H.

183. Gao SS, Zhao IS, Duffin S, Duangthip D, Lo ECM, Chu CH. Revitalising Silver Nitrate for Caries Management. Int J Environ Res Public Health. 2018;15(1):80. https://doi.org/10.3390/ijerph15010080.
184. He J, Ye Y, Zhang D, Yao K, Zhou M. Visualized Gallium/Lyticase-Integrated Antifungal Strategy for Fungal Keratitis Treatment. Adv Mater. 2022;34(49):2206437. https://doi.org/10.1002/adma.202206437.
185. Soleimani M, Cheraqpour K, Sadeghi R, Pezeshgi S, Koganti R, Djalilian AR. Artificial Intelligence and Infectious Keratitis: Where Are We Now? Life. 2023;13(11):2117. https://doi.org/10.3390/life13112117
186. Li Z, Jiang J, Chen K, Chen Q, Zheng Q, Liu X, Weng H, Wu S, Chen W. Preventing corneal blindness caused by Keratitis using Artificial Intelligence. Nat Commun. 2021;12(1):3738. https://doi.org/10.1038/s41467-021-24116-6.
187. Alquran H, Al-Issa Y, Alsalatie M, Mustafa WA, Qasmieh IA, Zyout A. Intelligent diagnosis and classification of Keratitis. Diagnostics. 2022;12(6):1344. https://doi.org/10.3390/diagnostics12061344.

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