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Modernized Management of Ocular Keratitis *via* Nanovesicular Drug Delivery Systems

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ABSTRACT

Objective: Keratitis is considered as one of the major leading causes of blindness internationally. Briefly, it is corneal infection also termed corneal ulcer or corneal opacity. Bacterial keratitis is often a consequence of multidrug-resistant bacterial infections, which becomes unaffected by broad-spectrum antibiotics. Both, Aspergillus *and Fusarium* species, are the most frequent sources for mycotic keratitis. Protozoal keratitis was recently presented where best treatment results were achieved with early diagnosis. **Methods:** Various methods of diagnosis were adopted such as: corneal smear and corneal culture. These methods suffer from diagnostic insensitivity and being time-consuming. The Polymerase Chain Reaction is a newer, matching method used in the identification of keratitis; the findings are sample-dependent. Recently, *In vivo* confocal microscopy (IVCM) is an encouraging diagnostic technique of increasing significance. It is non-invasive real-time direct imagining of the microorganism and displaying infection directly in the patient's cornea. Ocular drug delivery usually suffers from low bioavailability due to various eye barriers which finally led to ineffective treatment. **Results:** Substantial attempts were demonstrated to enhance drug ocular bioavailability via boosting corneal diffusion and enhancing residential time. Different types of nanoparticles, liposomes, and self-emulsifying systems achieved great attention in optimizing ocular drug delivery. Nanovesicles are innovative systems with minute particle size and uniform size distribution revealing extended ocular residential time so confirming satisfactory bioavailability, less irritation, and high tolerance with ocular tissues. **Conclusion:** This review declares the treatment policies and highlights numerous innovative nanovesicles developed with a focus on advanced findings regarding formulation systems.

Keywords: *Keratitis; Nanovesicles; Transferosomes; Novasomes; Collagen crosslinking.*

INTRODUCTION

Eye Anatomy

The eye is considered to be a highly sensitive and the most crucial sensory organ of human body in which any defect or damage would massively affect the quality of life [1]. It converts images into electrical signals. The eye, a spherical shape located in the orbital cavity and constitutes 20% of the orbital volume. The eyeball is held within the orbital cavity by ligaments and muscles [2].

Keratitis

Keratitis is an acute infection of the cornea caused by bacteria, fungi, viruses or amoeba. It can cause serious visual morbidity unless early diagnosed and treated quickly.

Fungal infections are widely distributed worldwide but are more commonly prominent in tropical and subtropical areas where young farmers are the most liable individuals to infection and usually this occurs after minor ocular trauma [3].

Fungal keratitis is more prominent in agricultural and outdoor workers. However, it was found that few cases appeared in children, and most are among adults. It was also reported that ocular surgery, ocular surface disease, contact lens use, previous use of corticosteroids (topical or systemic), and immunosuppressive conditions such as HIV/AIDS are predisposing factors for fungal keratitis. Fungal keratitis is accompanied by ocular pain, hazy vision, redness, increased eye secretions and photalgia. Additionally, ulcers and opacified cornea may also occur. It was noted that corneal perforations are more prominent when compared to other types of keratitis [4].

The majority of the reports noted that 1% w/v voriconazole eye drops is successful for the management of ophthalmic fungal keratitis [5]. Ocular ointments were found to produce outstanding drug bioavailability as they resist nasolacrimal drainage and enhance drug eye contact time, however, they aggravate blurred vision which causes ocular ointments to be limited to only night application [6]. PEGylated micelles were formulated to enhance the natamycin contact time with the mucus layer for treatment of fungal keratitis [7].

Bacterial keratitis is the most frequent type among microbial keratitis in the USA [8]. It was found that bacterial keratitis is caused by Pseudomanas aeruginosa in more than 90 % of patients. These bacteria are resistant to antibiotics and can penetrate through damaged corneal epithelium resulting in accumulation in the corneal stroma. Single treatment using fluoroquinolones is the class of choice for the bacterial keratitis management in the USA. Ciprofloxacin- nanemulsion was developed by [8] to improve drug ocular bioavailability for the treatment of bacterial keratitis. Moreover, poloxamer-based thermoresponsive in situ gel for the delivery of oxytetracyclin loaded nanoparticles were also developed [9].

Ocular inserts have been widely used loaded with different formulation using a variety of polymers to achieve the desired properties. They can produce a prolonged contact time which subsequently reduces the frequency and concentration of dose application and thereby, reduce systemic side effects and improved efficacy for the treatment of eye disorder, including keratitis [10]. Amongst the most innovative methods is the preparation of polymeric ocular insert loaded with nanofibers prepared using electrospinning technique to encapsulate drugs by Singla et al., 2019 [11].

Acanthamoeba keratitis (AK) is a critical inflammation of the cornea, that may lead to loss of sight. It also causes other symptoms including, redness of the eye, foreign body sensation, decreased visual activity, photophobia and tearing. It is caused by a protozoa (amoeba) that lives in many environments including soil, air and water. It was found that most cases of this keratitis are amongst people wearing contact lenses as it may facilitate the direct inoculation of the amoeba. Treatment of Acanthamoeba keratitis is achieved by early diagnosis associated with vigorous therapies. Treatment may involve topical antimicrobial such as chlorohexidine (0.2 %) with polyhexamethylene biguanide (PHMB) [12]. Other antimicrobials including rifampicin, amphotericin B, pentamidine, flucytosine, pyrimethamine and cotrimoxazole are also used. Moreover, antifungal drugs, such as ketoconazole, voriconazole or clotrimazole, as well as nonsteroidal anti-inflammatory (NSAIDS) drugs be used orally or topically. These treatments are encountered by the problem of poor drug penetration at the site of infection. Contact lenses were also used impregnated with a combination of voriconazole as an antifungal and diclofenac as a NSAID were tested [13]

It is divided to 2 main anatomic areas: the anterior segment (adnexa, cornea, anterior chamber and posterior chamber) and the posterior segment (sclera, vitreous chamber, retina and optic nerve) [14]. Yet, it can be divided according to its physiological structure into three concentric parts. The outermost part comprised of sclera and cornea. The mid covering also known as the uvea, is enriched by a high blood flow and constitutes the iris, ciliary body, and choroid. The innermost layer in the eye is the retina. The lens, an elastic organ, permits light to the retina.

The cornea, the finest tissue, covers the foreside of the iris (the colored part of the eye), anterior part of the eye, and the pupil. The retina, which is present in a safe position as it is enclosed by the cornea and sclera found at the posterior region of the eye. This tissue is highly innervated because it is exposed to the external environment. It senses light, changes to signals, and transmits them via the optic nerve to the brain [15]. It is also made of five different layers (epithelium, Bowman's membrane, stroma, Descemet's membrane, and endothelium). All these layers refract light through the lens on the retina [16]**.** The endothelium is a thin layer, which keeps the cornea transparent because cornea as a non-vascularized tissue is supplied with a fluid pump the aqueous humor as well as lacrimal fluids [17]. The aqueous humor and vitreous humor are crucial fluids. The aqueous humor is a clear fluid found in the anterior and posterior eye cavity, whereas the vitreous humor is a transparent jelly-like fluid is only found the posterior eye

region. Its main function is holding the eyeball in place and nourish the cornea [18].

The sclera, is an elastic hard structure made of collagen that maintain the intraocular pressure [2].

There are many barriers in the eye that reduce the administered drug bioavailability. Tear film is a precorneal film that forms the first natural barrier that hinders ocular drug delivery. Anatomically, it has an outer oil layer which prevents evaporation of water and consequently decreases drug absorption into the cornea and sclera. This is followed by a middle aqueous layer, where some proteins such as globulin, albumin, and lactoferrin can bind and metabolize the administered drug, resulting in reduced bioavailability. Finally, the inner layer, the mucus layer, is composed of water, mucins, lipids, salts, enzymes. This layer plays a major role in hindering drug delivery due to its structure which can trap foreign particles. In addition, the retained drug could be also diluted by rapid tear turnover, which decreases the concentration gradient and diffusion rate, resulting in low bioavailability of intraocular drugs in aqueous humor [19].

The cornea represents the most prominent mechanical and chemical barrier to both hydrophobic and hydrophilic molecules. The epithelium, which mainly acts as a barrier for large molecules, is lipophilic that permits lipophilic drugs to penetrate the cornea via the intracellular route. On the other hand, hydrophilic and low molecular weight drugs through paracellular route [14].

The outer corneal stratified epithelium is a hydrophobic layer which produces a great barrier for the hydrophilic drugs that can diffuse through paracellular channels [20]. Also, there are drug degrading enzymes (cytochrome P450) which is responsible for the reduced topical ocular drug bioavailability [21]. Then comes the stroma, which limits the hydrophobic molecules penetration, which are transported by transcellular path. However, the stroma allows hydrophilic molecules to easily pass due to its high hydration degree. This indicates the importance of molecular size and drug nature in corneal permeation. Moreover, the aqueous humor, secreted by ciliary body behind the iris, presents a barrier to drug elimination from the ocular tissues and the concentration of these drugs will decrease by the vast aqueous humor turnover [22].

Additionally, the conjunctiva that covers the eye's surface around the cornea, is 25 times more absorbent than the cornea due to its larger surface area, fewer layers of epithelial cells, with larger paracellular spaces. This causes it to be more permeable, especially for large hydrophilic molecules [30,31]. On the other hand, drugs that penetrate the conjunctiva may enter the general blood circulation via the conjunctival sac or the nasal cavity due to its high blood vessel density [21]. This can cause remarkable drug loss therefore ocular bioavailability is reduced.

Ocular drug delivery is challenging due to presence of various eye barriers which hinder topical drugs' absorption resulting in a short duration of action. There is also a wide range of ocular diseases that require administration of ocular medication either systemically (oral, parenteral), topically intraocular, and ocular injections (subconjunctival, periocular, and intravitreal). Topical application is the most commonly used way to treat ocular diseases, such as eye drops, suspensions, and ointments as they have a good compliance. However, they are subjected to drug loss due to tear film and other factors including anatomical barriers responsible for this loss and only 5% of the drug is absorbed through the ocular surface which causes low drug bioavailability [23]. In addition to systemic administration which include Parenteral and oral dosing, but only 1-2% of the drug administered systemically can reach the retina and vitreous regions due to low ocular blood supply. This requires frequent administration which results in systemic side effects [24].

Factors and challenges affecting ocular drug delivery.

1. Eye related factors/barriers affecting ocular drug delivery

One of the main obstacles in ocular delivery is to achieve effective drug level for an efficient period at the desired action site. Ocular drug delivery can be hindered either by intraocular environment (epithelial and retinal blood aqueous barriers), intraocular metabolism (enzymes such as cytochrome P450, lyosomal enzymes and monoamine oxidases), static barriers (represented by Corneal structural layers of epithelium, sclera and conjunctiva), and dynamic barriers (expressed by naso-lachrymal and tears drainage) [1] as represented in **Figure 1**.

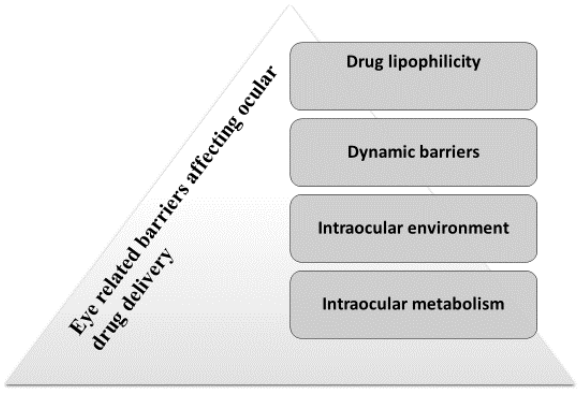

Figure 1: Natural eye barriers ocular affecting drug delivery

2. Formulation and Nanovesicle-related factors affecting ocular drug delivery

Drug lipophilicity, Size of the nanovesicles, surface characteristics, targeting techniques and dosage form types are considered to be the main physicochemical characteristics that affect ocular drug delivery as displayed in **Figure 2.**

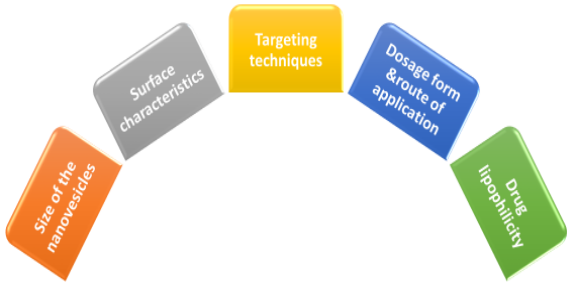

Figure 2: Formulation challenges ocular affecting drug delivery

- ***Drug lipophilicity***

It was reported that lipophilic drugs are less affected by nasolacrimal pathway than hydrophilic drugs. AbdelKader et al, 2021 previously reported that prednisolone in its acetate form showed higher corneal permeation than in the phosphate form due to higher lipophilicity of the acetate form [25]. Also, Kesharwani et al, 2018 stated that increasing ciprofloxacin lipophilicity by ion pairing with potassium sorbate enhanced its precorneal permeability and retention [26].

- ***Size of the nanovesicles***

Vesicular size affects both drug entrapment efficiency and the ocular drug delivery mechanism. Nanovesicles should have the proper size to pass through the ocular barriers and ensure good eye tolerability at the same time. It was previously reported that nanovesicular size of 50-400 nm ensures good mucoadhesion, high ocular penetration and low ocular irritation. With respect to the precorneal segment, topical administration of nanovesicles <200 nm ensures easy corneal and conjunctival absorption [27].

- ***Surface characteristics***

Distribution of nanovesicles in the different ocular tissues is highly determined by nanovesicles surface charge. The high sulfate and sialic acid content in the mucous layer, imparts a negative surface charge to the corneal tissues. Therefore, cationic nanovesicles exhibit higher retention time and deep penetration than that of anionic nanovesicles [26].

- ***Targeting techniques***

Surface targeting of nanovesicles with moieties would increase accumulation of these vesicles into specific tissues [28]. The combination of hyaluronic acid with gold nanoparticles was found to enhance distribution of the carrier particles across ocular tissues [29]. It was found that the usage of microneedles can give targeted drug delivery (ex; chorio-retinal tissues) [30]. Tian et al, 2017 loaded amphotericin B into chitosan-modified nanostructured lipid carriers to target it to the ocular mucosa and this showed a prolonged ocular application [31]. Non-ionisable lipophilic molecules were more localized in the corneal epithelium, while, ionisable lipophilic ones were confined in the aqueous humor [1].

- ***Dosage form and route of application***

Although, eye drops are the most popular ocular dosage forms, most of the drug is exposed to precorneal loss via nasolacrimal drainage, blinking reflex and on the eyelids. the remaining drug fraction should overcome ocular barriers to reach the desired site of action and produce the required therapeutic effect. To enhance topical bioavailability of ocularly administered drugs, various dosage forms such as suspensions, ointments, inserts, and aqueous gels were used, however such dosage forms also may have poor patient compliance due to the resulted blurred vision as in case of ointments or due to difficulty in application as in case of implants [32].

Importance of Nano-medicine in ocular drug delivery systems

The use of nanotechnology in ocular delivery systems has played a crucial role that had the lead over those conventional dosage forms. Conventional ocular dosage forms usually show considerably less bioavailability due to several ocular natural barriers including lacrimation, conjunctival absorption, tear film dilution, short residence time, corneal epithelium impermeability, etc [33]. However, nanovesicles demonstrated superiority owing to their small size, extended residence in the eye [34] increased permeability, chance of drug targeting, reduced unstable drug degradation [35] producing minimal eye irritation and guarantees a satisfactory ocular bioavailability.

Different nanovesicles were developed for drug delivery and/ or diagnostic applications as shown in **Figure 3**. The application of nanovesicles as ocular carriers witnessed incredible progression and presented a paradigm advance in nanomedicine. These different types of nanovesicles were well displayed in **Tables 1,2, and 3**.

Niosomes

Niosomes are nano-sized bilayer vesicles fabricated by non- ionic surfactants and cholesterol self-assembly in an aqueous medium resulting in closed

bilayer structures [15]. The type and proportions of each component was found to control the release profile, size and entrapment efficiency of niosomes. Niosomes are characterized by having the ability of incorporating both lipophilic and hydrophilic entities. Niosomes are classified according to number of layers into small uni-lamellar, large uni-lamellar and multi-lamellar vesicles and submicron size [36]. Elmotasem and Awad studied the ability of enhancing the ocular availability of fluconazole via its incorporation into niosomal vesicles using span 60. Then, coating with chitosan as a cationic polymer and loading in situ gel improved drug flux and a large AUC0-6h was obtained when compared to plain drug. Confocal and histopathological examination proved deep permeation and safety of the fabricated optimized formulation [37]. Soliman et al. also tried to incorporate fluconazole into Niosomal gels using span 60, cholesterol and Carbopol and microemulsions using IPM as oil phase and a 3:1 mixture of polysorbate 80 and PEG 400 were characterized. They found that Niosomal gel showed superior bioavailability over microemulsion where a more sustained release was noticed in niosomes due to incorporation in the in situ gel. Finally, comparing ocular bioavailability of both preparations, revealed the prominence of niosomal gel to microemulsion by ≈2-folds [38] as listed in **Table 1**.

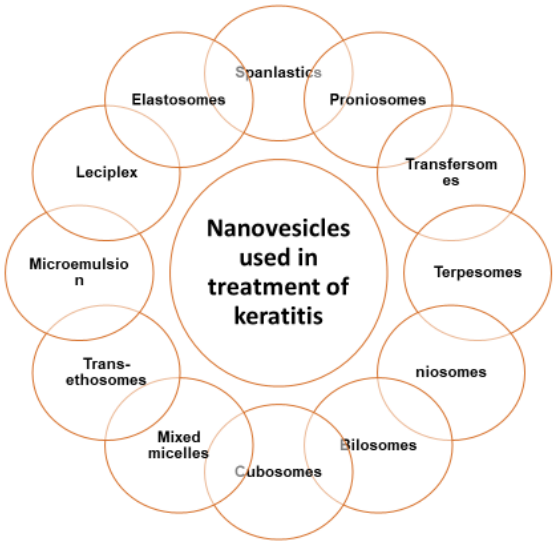

Figure 3: Nanovesicles used in management of keratitis

Mucoadhesive niosomes coated with trimethyl chitosan (TMC) were prepared and investigated by verma et al. who reported superiority of these niosomes in treatment of fungal keratitis due to its mucoadhesiveness and elongated local release of the drug. Coating natamycin loaded niosomes with TMC showed a steady drug release and proved to be safe and effective with significantly increased antifungal activity when compared to marketed preparations [39].

Proniosomes

Proniosomes were mainly designed in order to overcome the stability problems associated with niosomes such as; accumulation, coalition and leaking [40]. This can be achieved via providing a non-aqueous storing environment for lipophilic and photosensitive drugs via incorporating into an internal protected layer. Proniosomes are considered to be a precursor for niosomes and can be produced in two different forms; either dry powder of high flow ability which can be rehydrated into niosomes or gel like formulations of water soluble carriers coated with surfactants which also can be rehydrated to niosomes by shaking at temperature above that of the transition phase temperature of the surfactant. Proniosomal gels are preferred because of their ability to adhere to cornea and conjunctiva and as consequence provide longer retention time. Generally, proniosomes were previously reported to increase bioavailability of ocularly administered drugs [41].

El-Emam et al. studied the possibility of using voriconazole loaded proniosomal ocular inserts to improve its antifungal activity and reported that voriconazole- proniosomes loaded ocusert exhibited a superior antifungal activity compared to free drug or nano-suspension. Further investigation was carried out to assure this antifungal activity when mean inhibition zone was compared to that of 5% natamycin market eye drops. Voriconazole- proniosomes loaded ocuserts proved to be a good candidate for the treatment of fungal infections [40] and [15] as shown in **Table 1.**

Spanlastics

Spanlastics are surfactant-based nanovesicles which are characterized by its elasticity over niosomes because of incorporating edge activator in its composition which gives high elasticity instead of using cholesterol in case of conventional niosomes. Edge activators are single chain surfactants which enhance deformability and decrease surface tension of the vesicles. High elasticity and flexibility of spanlastics provide a range of advantages such as adjustable zeta potential and size as well as improvement of corneal permeability to reach both anterior and posterior segments through ability to enter membrane pores with decreased risk of vesicular leakage. Moreover, spanlastics also showed superior target specificity and stability. Abdelbari et al. studied the effect of using spanlastics to enhance ocular delivery of clotrimazole. They found that Tween 80 spanlastics showed higher properties than other studied edge activators such as Pluronic F12 and Kolliphor RH4. It produced extremely elastic spherical vesicles. In vitro release test showed a sustained release and ex vivo permeation study was

higher through rabbit cornea when compared to its suspension. In vivo histopathological study revealed safety of the optimal formula (S1) when applied ocularly on mature male albino rabbits [42]. Spanlastics were previously reported to increase the possibility of the drug molecules to be delivered to the posterior segment of the eye [43]. Aziz et al. were the first to study the effect of using cosolvent-tailored nanovesicles for enhancing ocular drug absorbtion. They showed that incorporating Tolnaftate into Cosolvent-modified spanlastics of 30% propylene glycol gave superior efficacy [44] as mentioned in **Table 1.**

Elastosomes

Elastosomes are vesicles mainly depend on coupling phospholipids with edge activators. Polyvinyl alcohol and hyaluronic acid, may also be used in order to increase stability and bioadhesion of elastosomes. This structure is very beneficial for penetrating the corneal tissue either in the hydrophilic stroma or the lipophilic epithelium. Elastosomes guarantee safe and sustained corneal drug delivery with chemical and physical stability of the vesicles [45] and [46].

Fahmy et al. previously found that Phosphatidyl choline ultradeformable elastosomes enriched with hyaluronic acid and brij S100 were able to enhance corneal delivery of voriconazole. On The optimal formulation (OE) when compared to conventional liposomes, showed a higher elasticity and didn't cause any ocular irritation or blurred vision. Also, antifungal activity testing against Candida albicans, exhibited a prominent and long lasting growth inhibition ability when compared to voriconazole suspension [46].

Transferosomes

Transferosomes were first proposed by Gregor Cevc in 1991 to be promising way to deliver the drug slowly and safely with the least side effects. Like liposomes, they are made of natural phospholipids, thus they are highly compatible nontoxic carrier systems and enhance the capabilities of the liposomal systems [47]. The word "transfersomes" means "carrying body" and it was originated from the Latin word "transferred" meaning "to carry across" and the Greek word "soma" for a "body" [48].

Transferosomes, like bilosomes, are ultra-deformable carrier systems, made of phospholipid and edge activators (surfactants), which are the main reason for the elasticity of the lipid bilayer of transferosomes. They can also encapsulate both hydrophilic and lipophilic drugs and cross them through ocular barriers to deliver these target drugs to the site of action [49].
Transferosomes were used to deliver the high molecular weight hydrophobic cyclosporine A across corneal epithelium facing the challenges that hinders such peptides permeability. These showed an improved ocular corneal permeability. Linoleic acid (partially substitutes cholesterol), tween 80 and span 80 were used during formulation which exhibited a high compatibility with the ocular tissues. Also, the initial burst of cyclosporine A in the 24 hours and stayed over a week indicated the biocompatibility in the ocular cell line, stability over a period of 4 months at −20 °C [47].

Transethosomes

Transethosomes are lipid nanocarriers characterized by the presence of edge activator with ethanol to combine the characters of transfersomes and ethosomes. Ethanol can easily reduce density of the lipid bilayer and as a result increase its fluidity creating pores through which penetration of the drug loaded vesicles occurs. On the other side, edge activator enhances elasticity of the vesicles itself which also enhance penetration and therapeutic activity as result. It was also reported that transethosomes provides the advantage of easy scaling up [50].

Transethosomes loaded were previously studied by Ahmed et al. 2021 for its effect to enhance ocular permeation of ketoconazole. It showed a significantly improved activity after treatment using the optimized nanovesicles. Then, optimized nanovesicles incorporated in situ gel were safe, non-irritating formulations to the cornea. They also revealed high permeation into the posterior eye segment without any toxic side effects. This Ketoconazole- trans-ethosomes loaded in situ gel formulations can be used as a promising preparation for the management of deep ocular fungal infections [51] as shown in **Table 1.**

Cubosomes

Cubosomes are lipid nanovesicles covered with a polymer outer shell and a cubic structure. It combines the advantages of high fluidity, large surface area increased viscosity and high retention time which positively serve both tissue penetration as well as prolonged drug release. However, Cubosomes were reported to show low mucoadhesion after absorption of the formulation moisture content [52].

Lipid materials used in the preparation of cubosomes are mainly Phytantriol and monoolein. Phytantriol-based cubosomes showed superior characters in ocular drug delivery. Non-ionic surfactants such as pluronics, and stabilizers were previously reported to be used in the preparation of cubosomes. Alhakamy et al. previously found that voriconazole cubusomes loaded into ocular in situ enhanced both transcorneal permeation by 4.5 fold in comparison to voriconazole aqueous dispersion. Also, antifungal activity was evaluated and it was noticed that, voriconazole-cubosomes loaded in situ gel gave a significant increment of 3,89 fold in the fungal growth inhibition zone [29] as demonstrated in **Table 1.**

Table 1. Fungal keratitis

Drug	Best findings	Reference
Clotrimazole	• Clotimazole was loaded in spanlastics prepared using polysorbate 80, poloxamer F127, or Kolliphor RH40 as an edge activator (EA). This was further subjected to statistical optimization and the optimal formula was composed of 20 mg polysorbate 80 as an edge activator and 80 mg of sorbitan monostearate. It produced extremely elastic spherical vesicles that had a particle size of 206.20 ± 4.95 nm, PDI of 0.39, zeta potential of −29.60 ± 0.99 mV and EE% of 66.54 ± 7.57%. • In vitro release test showed a sustained release and ex vivo permeation study was enhanced in comparison to its suspension. Upon testing antifungal activity against Candida albicans, a significantly higher growth inhibition was noticed when compared to suspension. Finally, in vivo histopathological study revealed safety of the optimal formula (S1) when applied ocularly on mature male albino rabbits.	42
Fenticonazole nitrate	• Terpesomes were utilized for loading fenticonazole nitrate and statistical optimization produced an optimal formula composed of 20 mg Eugenol which was then subjected to characterization showing: a particle size = 287.25± 9.55 nm, PDI value = 0.46±0.01 and stable vesicles with zeta potential = 36.15±1.06 mV and a high entrapment efficiency of 79.02±2.35%. • The optimal terpesome formulation was then optimized again using stearylamine as a source of positive charge to increase its adherence to the oppositely charged ocular mucus. This then revealed after performing in vivo study the prominently high ocular retention of selected terpesomes loaded with fenticonazole nitrate. • The tolerance of these terpesomes in the management of ocular diseases with enhanced retention in the eye was proved.	53
Fluconazole	• To enhance fluconazole ocular bioavailability, for the effective management of fungal keratitis, it was loaded in niosomes which were formulated using sorbitan monostearate. Polymeric nanoparticles were fabricated using Eudragit RS100 and RL 100 as cationic polymers. These were all analyzed and revealed proper encapsulation, small size, and acceptable stability. • Factorial design and optimization were applied and the selected formulae in were then used in further loading of fluconazole- HP-β-CD complex. After this complex loading, niosomes were then coated with chitosan as a cationic, bioadhesive polymer. The formulations FL-CD-ERS1 and FL-CD-Nios-ch analysis revealed PS of 151.1 and 392 nm and showed an acceptable zeta potential of +40.1 and +28.5 mV respectively. Afterwards, they were incorporated in situ gel fabricated from poloxamer P407, HPMC and chitosan. • Further in vitro drug release showed a sustained release along 24 hours and ex vivo study on excised rabbit corneas revealed an improved drug flux and a large AUC0-6h. Confocal laser microscopy also was used to show corneal permeation of the selected formulae after labelling with Rhodamine B. Safety and tolerance of the selected formulations were proven after histopathological examination. Finally, effective antifungal activity against Candida albicans was proved by an improved growth inhibition. Accordingly, the resulting selected formulations can be used as good candidates for improved corneal permeation, boosted antifungal activity and sustained action.	37
Fluconazole	• A comparative study was carried out on Fluconazole loaded in niosomal gel and microemulsion. Niosomes were fabricated using sorbitan monostearte and cholesterol followed by loading in carbopol 934 based gel. On the other hand, microemulsion loaded fluconazole was prepared using an oil phase of isopropyl myristate (IPM) of 0.3 % w/v and a mixture of surfactant and co-surfactant composed of tween 80 and PEG 400 in a ratio of 3:1 respectively. • Statistical optimization was done and the optimized formulations in both preparations were assessed. the niosomes produced a particle size range of (63.67–117.13 nm) and microemulsions range was (57.05–59.93 nm), a negative zeta potential was obtained in both niosomes and microemulsions with values range of −45.37 to −61.40 and −20.50 to −31.90 mV respectively. Then, they showed a sustained release profile over 12 hours. • Additionally, loading the niosomes in gel produced a more sustained release when compared to either preparation. Also, stability test was performed and it revealed that the optimized niosomal gel was made of sorbitan monostearate and cholesterol equimolarly and the microemulsion was composed of 45% w/w isoprpylmyristate and 40% w/w of 3:1 polysorbate 80-PEG 400 mixture.	38

	• These two stable formulations significantly improved fluconazole ocular bioavailability. Finally, comparing ocular bioavailability of both preparations, revealed the prominence of niosomal gel to microemulsion by ≈2-folds.	
Ketoconazole	• This was used to formulate ketoconazole trans-ethosomes as a way to improve drug ocular permeability and efficacy. Four factors were studied viz. drug: phospholipid molar ratio (X1), edge activator percentage (X2), ethanol percentage (X3) and stearyl amine percentage (X4). These factors significantly affect the vesicles properties. • Characterization of the optimal nanovesicles revealed spherical vesicles of small stable particles with encapsulation efficiency (EE%) = 94.97 ± 5.41%, and deformability index= 95.44 ± 4.33%. • Further investigation of ketoconazole antifungal activity showed a significantly improved activity after treatment using the optimized nanovesicles. These optimized nanovesicles were then incorporated in situ gel showing safe, non-irritating formulations to the cornea. Therefore, Ketoconazole- trans-ethosomes loaded in situ gel formulations are potential preparations in the management of deep ocular fungal infections.	51
Ketoconazole	• Ketoconazole loaded cubosomes were fabricated, statistically optimized and the optimized formula consisted of 12% GMO, 10% Pluronic-127 and 6% PVA. The optimal cubosomes were then loaded into a gel made of biodegradable polymer to prolong its ocular residence and accordingly effective ocular permeation. • Further examination of antifungal activity proved effectiveness and a decrease in MIC when compared to marketed preparation. This impacted on its efficacy for the treatment of ocular fungal infection.	54
Natamycin	• The study aimed to formulate cationic coated natamycin niosomes using a modified thin film hydration technique. These niosomes were prepared using one step carbodiimide. They were then characterized using 1H-NMR and degree of quaternization. Incubation of TMC in presence of natamycin, cholesterol, span 60 and dicetyl phosphate to formulate the niosomes and get mucoadhesive cationic natamycin loaded niosomes (MCNNs). • The obtained formula was then characterized for their particle size, PDI, Entrapment efficiency % and they exhibited a spherical shape with PS of 1031.12 ± 14.18 nm, PDI less than 0.3, and EE% of 80.23 ± 5.28%. in vitro drug release study revealed a steady natamycin flux from the coated niosomes. Antifungal efficacy of the chitosan coated niosomes was proved using MIC assay and disk diffusion method and it was compared to marketed preparation. • The safety and effectiveness of natamycin was improved after loading in the cationic niosomes. Accordingly, these formulated niosomes proved to be promising method for proper management of ocular fungal keratitis.	39
Natamycin	• Natamycin cubosomes were prepared and optimized at the lowest concentrations of span 80 and poloxamer P-407. The optimal formulation showed PS= 158.2 nm, PDI and ZP values of 0.328 and -40 mV respectively and entrapment efficiency of 99.85%. • Further in vitro study showed that 84.29 % of natamycin was released from the optimal cubosomes after 8 hours, and antifungal activity testing of these cubosomes against Candida albicans and Aspergillus funigatus proved superiority when compared to drug suspension. XRD proved that natamycin was fully encapsulated into the cubosomes. • An improved corneal permeation was also confirmed by ex vivo permeation study. Also, ocular irritation test revealed that they are non- irritant when tried on rabbits.	55
Posaconazole	• Posaconazole was loaded in egg phosphatidylcholine (EPC) based mixed micelles prepared using several surfactants and it was revealed that those prepared using TPGS in combination to EPC in a molar ratio of 30:70 produced proper characteristics such as a small spherical particles of an average size of 58nm, high entrapment efficiency of 80 %, a high stability when stored for a month, an abrupt then sustained drug release and a prominently high antifungal activity. • Therefore, this prepared formula can be used as a good candidate for ocular delivery of posaconazole.	56
Sertaconzole	• Sertaconazole nitrate loaded leciplex were fabricated and subjected to statistical optimization, where the optimal formulation consisted of CTAB (a cationic surfactant) and Soy lecithin in a molar ratio of 1:1. The optimal formula exhibited spherical 39.70 ± 1.35 nm particles with acceptable PDI and zeta potential values = 0.242 ± 0.006 and +54.60 ± 0.24 mV respectively. The permeability coefficient (Kp) of 0.0577 ± 0.0001 cm/h which was revealed by ex vivo study that showed an improved Kp and corneal deposition by 2.78 and 12.49 folds, respectively, in comparison to sertaconazole suspension. • Further in vivo study done by treatment of rats with induced keratomycosis showed prominence of the optimized formula to drug dispersion. Therefore, these sertaconazole loaded leciplex proved to be superior topical candidates for the management of ocular diseases.	57

Terconazole	• TZ-loaded bile-based nanovesicles (BBNV) were prepared. Optimization was adopted and the optimized formulation was prepared using 73.59 mg sorbitan monostearate, 3.11 mg sodium deoxycholate and 1.28 mg cholesterol. • The optimized formulation vesicles adopted a particle size (PS) of 526 nm, and zeta potential (ZP) of − 42.2 mV and encapsulation efficiency of 93.86%. Then a prompt TZ release was necessary to obtain an abrupt antifungal activity, improved ocular residence as well as better permeation in the ocular tissues. This was accomplished through the integration of the optimized TZ- loaded nanovesicles in a self-nanoemulsifying system (SNES). The nanoemulsifying system was prepared using an oily phase composed of Labrafil® M 2125 CS, polysorbate 80 as a surfactant, and Transcutol® HP as co-surfactant/co-solvent in ratios of 20:50:30, respectively incorporating the previously optimized TZ nanovesicles.TZ showed an enhanced release profile from the integrated nano emulsified system. [34] • This integrated preparation revealed a higher in vivo antifungal activity against infection induced by Candida albicans.
Tolnaftate	• A nucleus formula (BS6) was adopted for the formulation and optimization of co-solvent modified spanlastics. This was prepared using 400 mg sorbitan monostearate and 100 mg polysorbate 80. BS6 exhibited a particle size= 349.55 ± 34.44 nm, zeta potential (ZP)= −28.75 ± 2.76 mV and an entrapment efficiency (EE%) of 76.80 ± 7.07%. The obtained optimized formulation (MS6) was formulated using 30 % propylene glycol. Analysis of the optimal formulation showed a PS of 231.20 ± 0.141 nm, ZP of −32.15 ± 0.07 mV and EE% of 66.10 ± 0.57%. [44] • The two formulae BS6 and MS6 showed a significant increment in corneal permeation ability in both nanovesicles when compared to drug suspension. Then further invivo histopathological analysis confirmed the appropriateness of formula MS6 for ocular use. Also, it displayed a more prominent growth inhibition after testing antifungal activity against Aspergillus niger. Thus, MS6 can be used as a promising candidate for an improved Tolnaftate ocular delivery. • The use of polymeric pseudorotaxanes (PSR) to incorporate Tolnaftate was adopted. The selected PSR1 was prepared using 10 mg TOL, poloxamer to HPβCD (1:1) and poloxamer system using F127 and L121 (as independent variables). This optimal formulation showed a particle size of 237.05 ± 12.80 nm, zeta potential of −32.65 ± 0.92 mV and an encapsulation efficiency (EE%) of 71.55 ± 2.90%. [58] • PSR1 as well as conventional PMM were compared. Both revealed a significant rise in drug release resulting in enhanced drug permeation/ cm^2 in 8. PSR1 was further analyzed, and histopathological examination ensured ocular safety of this formula. Antifungal activity testing against Aspergillus niger revealed a sustained growth inhibition activity up to 24 hours. Accordingly, PSR1 was chosen as a potential nanoconstruct for Tolnaftate ocular delivery.
Voriconazole	• Proniosomes (PN) were prepared using Voriconazole, pluronic F127 and cholesterol. The optimized formulation was composed of Pluronic F127: cholesterol weight ratio 1:1 w/w. Further analysis of the optimized voriconazole formulation revealed a suitable nan-osized particles of 209.7±8.13 nm and proper zeta potential (ZP) of −33.5±1.85 mV. This optimal formulation was then integrated into ocusert prepared using 1 % hydroxypropyl methyl cellulose (HPMC) and 0.1% w/w carbopol 940. [40] • Voriconazole- proniosomes loaded ocusert exhibited a superior antifungal activity compared to free drug or nano-suspension. Further investigation was carried out to assure this antifungal activity when mean inhibition zone was compared to that of 5% natamycin market eye drops. Voriconazole- proniosomes loaded ocuserts proved to be a good candidate for the treatment of fungal infections. • Voriconazole loaded cubosomes were formulated and optimized using Poloxamer F127, phytantriol and voriconazole weights as independent variables. Obtained nanovesicles were tested for the dependent variables encapsulation efficiency (EE%), particle size (PS), and transcorneal steady state flux (Jss). • The selected optimal formula, composed of 60 mg F127, 100 mg phytantriol and 21 mg voriconazole, showed small particle size (71 nm). The encapsulation efficiency was 66 %. The steady state flux= 6.5 µg/(cm2·min). Stability index of 94 ± 2% was achieved. This optimal formula was then incorporated in situ gel base as way to improve voriconazole ocular residence. 84 % of voriconazole was released from the in situ gel after 12 hours and an enhanced drug permeation of 4.5 fold in comparison to voriconazole aqueous dispersion. [29] • Then, antifungal activity was evaluated against voriconazole aqueous dispersion, and it was noticed that, voriconazole-cubosomes loaded in situ gel gave a significant increment of 3,89 fold in the fungal growth inhibition zone. Consequently, loading voriconazole –cubosomes in

	situ gel proved to efficient nano- model for management of fungal infections with amended corneal permeation and antifungal activity. • Voriconazole was designed to be incorporated into ultradeformable elastosomes and optimization of design was exhibited and proposed an optimal formulation (OE) consisted of phosphatidyl choline (PC) and brij S100 in a weight ratio of 3.6:1. Hyaluronic acid was also added in a percentage 0.25 %w/v and 5 % polyvinyl alcohol was also used as a percent from PC- brij blend. • The optimized formula demonstrated satisfactory PS and PDI values (362.4 nm and 0.25), respectively, stable vesicles with adequate zeta potential (ZP) of -41.7 mV and a high encapsulation efficiency % (EE) of 72.6 %. On comparison to conventional liposomes, OE showed a higher elasticity and it didn't also cause any ocular irritation or blurred vision. Further histopathological investigation showed a safe ocular formula (OE). Also, antifungal activity testing against Candida albicans, exhibited a prominent and long lasting growth inhibition ability when compared to voriconazole suspension. • Finally, OE (Optimized Voriconazole- elastosomes) proved to be a good candidate for the treatment of ocular fungal keratitis with superior safety and efficacy.	46
	• Ternary micellar system (TMSs) loaded with voriconazole was fabricated for the management of ocular mycosis. These were formulated using water addition/ solvent evaporation technique and a 3- factor design was then used for optimization. This proposed an optimal formulation made up of pluronic to drug in a ratio of 22.89:1 and two pluronics were included P123 and F68 in a ratio of 1:1. Additionally, labrasol was added as 2 % w/v. • The optimal formulation was further analyzed and proved significant solubilization effect of 98 %, a small particle size of 21.8nm, acceptable zeta potential and PDI of -9.0 mV and 0.261, respectively. The formula was safe shown by histopathological examination with no ocular irritation or blurred vision. • Antifungal activity testing was carried out on Candida albicans showing prominence on aqueous voriconazole suspension with a persistant fungal growth inhibition. This proved good candidate for the treatment of ocular mycosis.	59
	• Voriconazole loaded cubosomes were fabricated and subjected to statistical optimization and the optimal formula consisted of 15 % GMO and 1.2 % poloxamer F127. It produced spherical vesicles of 160 nm diameter and elevated drug loading of 0.81 %. These cubosomes were then coated with chitosan to benefit its biocompatibility, safety and mucoadhesive properties and consequently promote its corneal residence time. Further in vivo study exhibited pharmacokinetic parameters and exhibited higher concentration in vitreous humor in comparison to voriconazole suspension. Accordingly, deeper permeation in ocular tissue.	52

Table2. Bacterial Keratitis

Drug	Best findings	Reference
Linezolid	• Linezolid- xanthan gum ophthalmic solution was fabricated and statistically optimized. The optimal formulation F2, composed of 1:1 LZD: Xanthan gum) showed acceptable viscosity, superior content uniformity and a cumulative release rate similar to the linezolid solution. It also revealed accepted in vitro and in vivo eye tolerance. • It was noted that linezolid- xanthan gum solution significantly improved the drug permeation than linezolid solution. Also, antimicrobial assay proved better activity of the optimal formulation than ophthalmic linezolid solution. • Further in vivo study proved the therapeutic efficiency of the optimal formulation against ocular tissues damaged by Staphylococcus aureus, where it decreased colony forming counts and lowered the activity of myeloperoxidase in the cornea.	**60**
Moxifloxacin	• Terpesomes and leciplex loaded moxifloxacin were fabricated, statistically optimized and compared for the effective ocular delivery. The optimal formulae TP4 and LP1 were analyzed producing entrapment efficiency % of 84.14±0.21 and 78.47 ±0.17%, particle size of 578.65±5.65 and 102.41±3.39 nm, PDI of 0.56±0.04 and 0.28±0.01, zeta potential of−12.50±0.30 and 32.50±0.50 mV, respectively. • Further investigation showed that LP1 produced superior features when compared to TP4 and moxifloxacin solution where, it had a better corneal permeation shown by fluoro-labeled LP inside the corneal tissues. In vivo study also revealed the ability of LP1 to stick to mucous membrane and thus biofilm inhibition and treatment of mice that were infected by methicillin resistant Staphylococcus aureus without any inflammatory response. • The safety and biocompatibility of LPs was proved by a histopathological study.	61

	• Moxifloxacin loaded bilosomes were prepared and optimized. The optimized formulation revealed a small particle size = (192 ± 4 nm) as well as great entrapment efficiency (76 ± 1%). This was then loaded in situ gel, which was further subjected to optimization. The optimal in situ gel (MX-BSop-Ig4) was assessed for gelling ability, pH, clearness, drug release, bio-adhesiveness, ex vivo permeation, toxicity, and antimicrobial properties. Moreover, in vitro release study showed a sustained release of 82± 4% in 24 hours. • An increase in corneal permeation was observed by when compared to MX-Ig and a pure MX solution, respectively. No toxicity was observed when tested on ocular tissues. Also, no internal changes in the corneal histopathology. Upon testing antimicrobial activity against gram positive and gram negative, MX-BSop-Ig4 showed a prominently greater antimicrobial activity than Moxifloxacin alone.	62

Table 3. Protozoal Keratitis management

Drug	Best findings	Reference
Curcumin	• In this study, curcumin and curcumin niosomal nanovesicles were tested for their activity against Acanthamoeba castellanii. They both showed effectiveness, however, nanovesicles proved a more enhanced anti-Acanthamoebic effect than curcumin alone. The nanovesicles were fabricated using 30 mg curcumin, 30 mg cholesterol and 60 mg compound KM-38 in the ratio 1:1:2. • Increasing concentration of curcumin either when used alone or inside the prepared niosomal vesicles (FCBR18) showed a decreased viability in a dose dependent manner and the least cytotoxic effects against human cells in all the used concentrations. FCBR18 proved efficient against amoeba excystation in all concentrations used. however, curcumin alone didn't produce significant results against amoeba excystation. • According to these results, the incorporation of curcumin in these niosomal nanovesicles proved promising candidate to be considered in management of Acanthamoeba due to their enhanced efficacy without affecting human cells.	63
Malonic acid and salicylic acid	• This study reported the primary trial on the effects of deep eutectic solvents on amaebae, a main causative agent of protozoal keratitis responsible for fatal infection. Five malonic and salicylic acid- based deep eutectic solvents (DES) on A. castellanii were chosen and investigated. They were formulated as salicylic acid-trioctylphosphine (DES 1), salicylic acid- trihexylamine (DES 2), salicylic acid-trioctylamine (DES 3), malonic acid-trioctylphosphine (DES 4) and malonic acid-trihexylamine (DES 5). Investigations were carried on as amoebicidal, encystment, excystment, cytopathogenicity, and cytotoxicity assays. It was shown that the solvents DES 2 and DES 3 produced at micromolar dosage level a significant amoebicidal effect where encyctement and excystment were suppressed when tested on cytopathogenic effect of A. castellanii as well as human cells where a minimal cytotoxicity was revealed. Also, testing individual components of these eutectic solvents (s salicylic acid, trihexylamine, and trioctylamine) produced least effects. • Therefore, the use of these eutectic solvents proved very promising for use as novel contact lens disinfectants against Acanthamoeba castellanii.	64

Previously reported work by Elfaky et al.2021 showed increased ocular delivery of ketoconazole through its incorporation into cubosomes loaded in biodegradable gel base. The optimal cubosomal gel prolonged its ocular residence and accordingly produced an effective ocular permeation. And enhanced antifungal activity when compared to marketed preparation [54].

Kaul and his coworkers proved the improved ocular residence, decreased dosing frequency, enhanced permeation of vancomycin loaded cubosomes These can be considered as good candidates for improving ocular drug bioavailability in management of bacterial keratitis [65].

Microemulsion

Microemulsions are among the most potential carriers in ocular drug delivery. They can be used for drugs with different polarities (hydrophilic and lipophilic drugs). Microemulsion has proved to be advantageous over conventional eye drops. Microemuslion can be easily scaled up, has a thermodynamic stability, release the drug in a sustained or controlled way, improve the solubility of poorly soluble drugs which is the main target use of microemulsion in ocular delivery in addition to increased permeability in lipid membranes including the cornea, thus allow fast absorption of drugs that struggle to cross it [66]. Moreover, being homogeneous and

transparent systems of oil phase and water with stabilizers such as surfactants and co-surfactants, they reduce the diffusional barriers, increase the amount of drug incorporated due to presence of surfactants that act as permeation enhancers that allow penetration into the deeper layers of the eye and aqueous humor, thus ocular drug concentration is also increased [67] and [68].

A high antifungal activity against Candida albicans was proved by Yousry and her coworkers after fabrication of terconazole loaded bile based nanovesicles and their integration in a self-nanoemulsifying system (SNES). This accomplished an abrupt antifungal activity, improved ocular residence as well as better permeation in the ocular tissues [34].

Mixed micelles

Mixed micelles are new nano-drug delivery systems that have attracted much attention, are usually made from two or more different block copolymers and surfactants, which facilitate nanoaggregate formations above a critical substrate concentration in aqueous solutions. Recently, they have been extensively used to solve many problems in ocular drug delivery. Having small size permits efficient drug into targeted tissues[69]. They have high stability and drug-loading capacity, improved ocular availability of BCS class II and class IV drugs through improvement of their solubilization and permeation[70], *controlled drug releasing abilities* [71]. Highly water-soluble drugs can predominantly solubilize on the micellar surface, while poorly water-soluble ones disperse in the micelle core, moreover, amphiphilic drugs are located at the interface of hydrophilic and lipophilic micelles. Micelles can also escape undesired drug exposure to a variety of biological barriers in the eye, such as corneal or conjunctival barriers [72]. Polymeic micelles were used as a promising system for hampering early disintegration and flux of the targeted APIs [71].

Also, phospholipid-based, mixed micelles (MMs) were prepared to improve the ocular delivery of posaconazole, a broad spectrum antifungal drug, a promising system for fungal keratitis management. Mahraz and his coworkers found that phosphatidylcholine (EPC) based mixed micelles prepared using TPGS produced small spherical particles with high entrapment efficiency of 80 %, a high stability when stored for a month, an abrupt followed by a sustained release and a prominently higher antifungal activity when compared to the drug suspension [56]. A mixed micellar delivery system formulated using chitosan, PLGA, and poloxamers in presence of moxifloxacin as an antimicrobial agent proved efficacy in the treatment of bacterial keratitis (BK). The two selected formulae exhibited high cellular uptake, corneal retention, muco-adhesiveness, and enhanced antibacterial activity *against P. aeruginosa* and *S. aureus*-infected BK mouse model [73] as denoted in **Table 2**.

Bilosomes

Bilosomes, first developed by Conacher and his coauthors in 2001, are closed bilayer nanovesicles made of lipid, surfactants and bile salts[74]. Bilosomes are characterized by nanosized particle size (5–200 nm), high elasticity, and extreme distortion and high stability in contrast to classical liposomes and niosomes [75]. They resemble conventional niosomes but superior, as they contain bile salts as well as edge activators which are embedded in the lipid bilayer made only of nonionic surfactant and cholesterol [76]. This conveys flexibility to their membrane to be easily squeezed into pores that are smaller than their diameters [77]. They can also incorporate hydrophilic and lipophilic drugs and release these drugs in a controlled manner, thus increase their duration of action [78]. They are also safe with no reported toxicity, consequently, they are suitable for drug delivery [79]. Zafar and his co-workers prepared in situ gel based moxifloxacin bilosomes that proved to increase in corneal permeation by 1.2- fold and 2.8- fold when compared to pure MX solution. No toxicity or internal changes were observed when tested on ocular tissues. Also, the optimal formulation (MX-BSop-Ig4) showed a prominently ($p < 0.05$) higher antimicrobial effect than pure moxifloxacin [62].

Terpesomes

Terpesomes are terpenes containing negatively charged vesicles. Terpenes are derived from essential oils, which are well-known penetration enhancers, as well as antimicrobial and antifungal agents. This is due to their ability to reside in the cell lipid bilayer, consequently, they can enter the cell cytoplasm and causes cell death [53]. Al-mahallawi et al, 2023 reported the safety and high ocular retention of fenticonazole loaded terpesomes. This high retention was achieved after using stearylamine as a positive charge inducer to increase its adhesion to the oppositely charged mucus ocular surface [53].

Leciplex

Leciplex, a promising self- assembled nanocarrier system, is composed of a combination of negatively charged lecithin phospholipid, a cationic surfactant and a biocompatible surfactant, namely Transcutol [80]. Leciplex has numerous advantages over other nanovesicular carrier systems. It is easily prepared without including an organic solvent during preparation using a single procedure fabrication technique [57]. It also promotes corneal penetration, which is attributed to the presence of a positive charges, which facilitates the intimate interaction of the nanovesicles with the negatively charged corneal mucus membrane. This leads

to enhanced corneal penetration and residence which is also supported by its small particle size and consequently reduced drug deposition through lachrymal flow and enhanced ocular bioavailability [81].

M. Abdellatif et al, 2022 reported the efficacy of Sertaconazole nitrate loaded leciplex for the management of fungal keratitis, where ex vivo study showed an improved Kp and corneal deposition by 2.78 and 12.49 folds, respectively, in comparison to aqueous sertaconazole dispersion. Also, in vivo study done by treatment of rats with induced keratomycosis showed prominence of the optimized formula to drug dispersion [57].

Prospective for the management of keratitis

New investigations and diagnostic techniques are now carried out to obtain prompt diagnosis before the growth of pathogen as well as identifying new strains causing keratitis. As displayed in **Figure 4**, It has been also noted that using in vivo confocal microscopy (IVCM), a non-invasive model, used for imaging that can produce images of layers through the cornea. This new trend of imaging used for the diagnosis of fungal keratitis develops high resolution photos of the examined ocular tissues. It is also exploited for the management of fungal keratitis as a new promising technique. This mostly allows the identification and diagnosis of the infection before further complications [82]. This method also overcomes the traditional diagnostic methods such as corneal smears and isolation of fungus in culture [83] which have many disadvantages viz long detection time which may take from days to weeks [84] and false negative results. Also among the traditional methods are corneal scrapes which include invasive tissue sampling and PCR which is also used to detect the presence of any fungal infection [85]. PCR has the advantage that it only needs a small clinical sample amount for diagnosis and it doesn't require the long detection time as conventional diagnosis methods, only from 4 to 8 hours [86].

Figure 4: Prospective techniques in management of Keratitis

Recent studies recommend using omics approaches such as genomic, metagenomics and tear proteomic which offers a great hope for the follow up of fungal keratitis [87].

Recently, the use of collagen crosslinking in the treatment of keratitis has been adopted through different mechanisms of actions viz, anti-inflammatory and antimicrobial action which cause damage of DNA/RNA of the pathogen as well as the high resistance of the causing organism to the enzymatic degradation inside the infected tissues [88]. Ocular penetration and in vivo bioavailability of some antifungals was also increased using corneal collagen crosslinking [88] . This was also supported by some case reports [89] and most studies carried out [90] and [91] which showed that corneal crosslinking can be considered as a promising auxiliary management of fungal keratitis.

To improve ocular bioavailability of voriconazole used in the management of fungal keratitis, a molecular imprinting polymerization (MIP) technique was used. Molecular imprinting produces a prominent surge in drug loading and a sustained drug release. Therefore, this technique can be used to overcome complications encountered in ocular transport of targeted drugs. Abouelatta et al, 2021 utilized MIPs based nanoparticles that were then incorporated into collagen shields. This produced better binding affinity of voriconazole to the used monomers, sustained drug release, high stability, and durability against harsh conditions [92].

Microneedle ocular patch (MOP) is new method for the application of poorly water soluble antibiotics which have a problem of accomplishing the required therapeutic concentration in the cornea. This was proposed by Garg et al, 2019 when they loaded either liposomal or free amphotericin B (AmB) in these patches using micro-molding method as a simulation of contact lens. It showed an improved in vivo effectiveness, increased drug permeability as well as corneal retention, significant antifungal activity against Candida albicans. This can be used as a promising technique for corneal drug delivery for the management of fungal keratitis [93].

Mesoporous carbon nanocarriers have been exploited for the first time [94]. This was successfully used to improve therapeutic efficacy and reduced dosing frequency of natamycin due to their large surface area, sustained drug release and increased drug dispersity.

A collaborative antibacterial and wound-healing strategy is suggested to treat bacterial keratitis by using poly (phenylboronic acid-(3,4-dihydropyrimidin-2(1H)-one))-co-(2-lactobionamidoethyl methacrylate) (p(PBA-DHPM-r-LAMA)) glycopolymeric micelles. The phenylboronic acid-(3,4-dihydropyrimidin-2(1H)-one) (PBA-DHPM) groups were active targeting ligands to help epithelial dissemination and bacterial anchorage.

Furthermore, they act as efficient ROS-scavenging mediators to reduce infection. The encapsulation of both levofloxacin (LEV) and chondroitin sulfate (CS) into the antioxidant glycopolymeric micelles, results in nanostructures with multiple functions such as antibacterial activity, antioxidant activity and advanced wound healing [95].

Novasome technology is the patented encapsulation innovative method developed by the IGI laboratories NOVAVAX to solve problems related to efficiency effectiveness of drug delivery systems. These are considered as enhanced liposomal or niosomal structure, which are prepared using cholesterol, free fatty acid (FFA), and monoester of polyoxyethylene fatty acid blend. These systems can be used to deliver many drugs due to its multilayered vesicular structure with a high-capacity core [96].

Another promising technique is the photodynamic therapy (PDT) which can deal with drug resistant bacterial infections. Qu et al, 2022 used oxygen producing cyanobacteria to carry photosensitizer (Ce6), and ultra-small $Cu_{5.4}O$ nanoparticles ($Cu_{5.4}O$ USNPs) with catalase activity for infection and inflammation elimination and rapid tissue repair (CeCycn-$Cu_{5.4}O$). Both rapid sterilization and long term free radical removal through PDT were accomplished. This also revealed enhanced antibacterial activity and promoted tissue repair after in vivo study on periodontitis and refractory keratitis animal models [97].

CONCLUSION

The management of Fungal keratitis approaches challenges at every stage, starting from the diagnosis to selecting the type and nanovesicular dosage form of the anti-fungal drug. Conventional ophthalmic preparations suffer from poor penetration, low residence time, and thus poor bioavailability. That's why nanovesicular structures were adopted to overcome the previously mentioned defects and promote ocular delivery of drugs. Nanotechnology along with novel technology such as IVCM would be the most effective therapy to overcome the challenges of ocular drug delivery.

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Conflict of interest

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