Contents

1

Mycotic Keratitis

An Old Disease with Modern Nanotechnological Solutions

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INTRODUCTION

Mycotic Keratitis (MK) is also known as fungal keratitis and is considered as one of the major causes of corneal blindness, especially in tropical and subtropical environments (Maharana et al. 2016). MK caused by *Aspergillus* sp. was for the first time described in Germany by Leber in 1879 in a 54-year-old farmer, who had a mild corneal injury due to oat chaff while working with a shredder (Dreschmaschine) (Leber 1879). It is a condition which is usually manifested by severe inflammation, the formation of a corneal ulcer, and hypopyon, with the presence of fungal hyphae within the corneal stroma (Thomas and Kaliamurthy 2013, Venkatesh et al. 2018). It is observed that among all the cases of microbial keratitis, MK accounts for about 1–44%, depending upon the geographic conditions (Gower et al. 2010, Garg 2012).

It is proposed that the frequency of MK is more in developing countries as compared to developed countries (Acharya et al. 2017). As far as the statistics are concerned, no information is available about recent MK cases. But, Thomas and Kaliamurthy (2013) presented some analysis based on old information available. According to them a single institution (L.V. Prasad Eye Institute, Hyderabad) in India reported about 1360 cases of MK during February 1991 and June 2001 (Gopinathan et al. 2009) and Shandong Eye Institute,

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Qingdao in Northern China reported 654 MK patients from January 1999 to December 2004 (Xie et al. 2006). On the contrary, Royal Victorian Eye and Ear Hospital, Melbourne, Australia documented only 56 cases of MK between July 1996 to May 2004 (Bhartiya et al. 2007) and according to clinical and microbiology records of the New York Eye and Infirmary, USA only 61 cases were recorded during January 1, 1987 and June 1, 2003 (Ritterband et al. 2006). From the above mentioned data, it is clear that number of MK cases in developing countries are considerably higher than that of developed countries.

The most common fungal genera responsible for mycotic infections include *Fusarium, Aspergillus, Curvularia, Bipolaris* and *Candida* (Gower et al. 2010, Revankar and Sutton 2010, Garg 2012, Paty et al. 2018). Worldwide it was observed that *Aspergillus* species are most frequently associated with MK; however, as mentioned above, it varies greatly depending on geographic regions. In one of the studies performed, it was reported that in India *Aspergillus* species is the most common causative agent (27 to 64%), followed by *Fusarium* (6 to 32%) and *Penicillium* (2 to 29%) recovered from patients suffering from MK. However, in another study performed with 275 patients, 198 patients were diagnosed with MK. From these patients, about 210 fungal isolates were recovered and identification confirmed that these isolates belong to 17 genera and 29 species. Among these isolates, *Fusarium* was found to be most common genus (49.5%), followed by *Aspergillus* (18.6%), *Candida* (12.4%), and other genera (19.5%) such as *Alternaria*, *Acremonium*, *Cladosporium* and *Beauveria* (Al-Hatmi et al. 2018, Castano and Mada 2018). Moreover, other causative agents of MK are listed in Table 1.1.

Although, various approaches such as pharmacological treatment, surgery and corneal crosslinking are investigated for the management of MK, unfortunately none of these approaches are very effective in the management for MK. Pharmacological agents are topical antifungal medications and each of them showed varied corneal penetration activity and effectiveness. Topical use of antifungal agents is still considered as a gold standard treatment protocol because other therapies like the use of intra-stromal injections did not show any proven benefit over topical pharmacological treatment (Acharya et al. 2017). It is postulated that delay in diagnosis and treatment of MK can result in many mild complications like formation of abscess and severe complications like corneal scarring which may lead to visual disability. In addition, such infections may lead to disruption of the anterior segment of the eye with increased intraocular pressure leading to glaucoma and endophthalmitis which is sufficient to make the patient visually handicapped (Acharya et al. 2017). Considering the severity of MK and unavailability of effective treatment strategies, it is necessary to expedite the scientific efforts by the researchers in this particular field, so as to develop effective management strategies with no or negligible side effects.

In this chapter, we have focused on various important topics related to MK, which mainly include worldwide severity and epidemiology of disease, existing methods for diagnosis, various management approaches and toxicological issues.

Epidemiology and Severity of MK

MK is mostly observed in male outdoor workers (Bharathi et al. 2003, Raval et al. 2014). It is proposed that occupation plays an important role in the appearance of infectious keratitis (Raval et al. 2014). Veena et al. (2017) reported keratitis in 380 out of 450 (84.4%) corneal scrapings from patients, which include a vast range of fungi viz. *Asperigillus, Fusarium, Yeast, Paecillomysis, Acremonium, Curvularia* spp. and *Scytidilia* spp. In addition, the studies performed by Leck et al. (2002) and Bajpai et al. (2016) also demonstrated the severity of MK. Apart from these, some other isolates were found to be responsive to topical antifungals but some were responsive to oral administration of drugs along with a topical one.

Table 1.1: Various causative agents in mycotic keratitis (Adapted and modified from Thomas and Kaliamurthy 2013; with a copyright permission from European Society of Clinical Microbiology and Infectious Diseases Published by Elsevier Ltd.).

Genus	Species	Genus	Species
• **Hyaline filamentous fungi**		• **Phaeohyphomycetes**	
Acremonium	*A. atrogriseum*, *A. curvum*, *A. kiliense*, *A. potronii*, *A. recifeib*, *Acremonium* species	*Alternaria*	*A. alternata*, *A. infectoria*, *Alternaria* spp.
Arthrographis	*A. kalrae*	*Aureobasidium*	*A. pullulans*
Aspergillus	*A. clavatus*, *A. fischerianus*, *A. flavipes*, *A. flavus*, *A. glaucus*, *A. fumigatus*, *A. janus*, *A. niger*, *A. terreus*, *A. nidulans*, *A. oryzae*, *A. wentii*	*Bipolaris*	*B. hawaiiensis*, *B. spicifera* (formerly Drechslera)
Beauveria	*B. bassiana*	*Cladosporium*	*C. cladosporioides*
Cephaliophora	*C. irregularis*	*Curvularia*	*C. brachyspora*, *C. geniculata*, *C. lunata*, *C. pallescens*, *C. senegalensis*, *C. verruculosa*
Chrysonilia	*C. sitophila* (formerly *Neurospora sitophila*)	*Dichotomophthoropsis*	*D. nymphearum*, *D. portulacae*
Chrysosporium	*C. parvum*	*Doratomyces*	*D. stemonitis*
Cylindrocarpon	*C. lichenicola* (*C. tonkinense*)	*Exophiala*	*E. jeanselmei* var. *dermatitidis*, *E. jeanselmei* var. *jeanselmei*
Diplosporium	*Diplosporium* species	*Exserohilum*	*E. rostratum*, *E. longirostratum*
Engyodontium	*E. alba* (formerly *Beauveria alba*)	*Fonsecaea*	*F. pedrosoi*
Epidermophyton	*Epidermophyton* species	*Lecytophora*	*L. mutabilis*
Fusarium	*F. aquaeductum*, *F. dimerum*, *F. oxysporum*, *F. solani*, *F. verticilloides* (*F. moniliforme*), *F. nivale*, *F. subglutinans*, *F. ventricosum*	*Phaeoisaria*	*P. clematitidis*
Glenospora	*G. graphii*	*Phaeotrichoconis*	*P. crotalariae*
Metarhizium	*M. anisopliae*	*Phialophora*	*P. bubakii*, *P. verrucosa*
Microsporum	*Microsporum* species, *M. canis*	*Tetraploa*	*T. aristata*
Myrathecum	*Myrathecum* species	• **Phaeoid sphaerosidales**	
Ovadendron	*O. sulphureo-ochraceum*	*Colletotrichum*	*C. capsici*, *C. coccodes*, *C. dematium*, *C. graminicola*, *C. gloenosporioides*, *Colletotrichum* state of *Glomerulla cingulata*
Paecilomyces	*P. farcinosus*, *P. lilacinus*, *P. variotii*	*Lasiodiplodia*	*L. theobromae*
Penicillium	*P. citrinum*, *P. expansum*	*Microsphaeropsis*	*M. olivacea*
Rhizoctonia	*Rhizoctonia* species	*Phoma*	*P. oculo-hominis*, *Phoma* species
Sarcopodium	*S. oculorum*	*Sphaeropsis*	*S. subglobosa*

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Genus	**Species**	**Genus**	**Species**
Scedosporium	*S. apiospermum* (reported as *Pseudallescheria boydii;* previously *Allescheria boydii, Petriellidium boydii, Monosporium apiospermum*)	• **Dimorphic fungi**	
Scopulariopsis	*S. brevicaulis*	*Blastomyces*	*B. dermatitidis*
Tritirachium	*T. oryzae*	*Coccidioides*	*C. immitis*
Ustilago	*Ustilago* species	*Paracoccidioides*	*P. brasiliensis*
Verticillium	*V. searrae, Verticillium* species	*Sporothrix*	*S. schenckii*
• **Other fungi**		• **Yeast and yeast-like fungi**	
Absidia	*A. corymbifera*	*Candida*	*C. albicans, C. famata, C. glabrata, C. uilliermondii, C. krusei, C. parapsilosis, C. tropicalis*
Chlamydoabsidia	*C. padenii*	*Cryptococcus*	*C. laurentii, C. neoformans*
Pythium	*P. insidiosum*	*Geotrichum*	*G. candidum*
Ulocladium	*U. atrum*	*Malassezia*	*M. furfur*
Scytalidium	*Scytalidium* sp.	*Rhodotorula*	*R. glutinis, R. rubra, Rhodotorula* species
Blastoschizomyces	*B. capitatus*	*Rhodosporidum*	*R. toruloides*
• **Newly reported agents**			
Aspergillus viridinutans			
Candida fermentati			
Thelavia subthermophilia			

Fungal keratitis in rigorous conditions penetrate Descemet's membrane and invades the anterior chamber and pupillary spaces (Pleyer et al. 1995) thus showing feathery margins, raised surface, satellite lesions, and non-yellow infiltrate colour along with moderate-to-large ulcers (Thomas et al. 2005, Dalmon et al. 2012, Chidambaram et al. 2018). The severity of fungi causing keratitis is a result of certain characteristics like the adherence capacity of the fungus to cells, enzyme and toxin production which destroys anatomical defence (Gopinath et al. 2009, Nath et al. 2011). MK is sometimes followed by an autoimmune condition known as Sympathetic Ophthalmia (SO), which is a result of retinal antigen reaction to conjunctival or orbital lymphatics. In the period of 15 months, 23 new cases were identified in a study by British Ophthalmic Surveillance Unit with the incidence of SO as 0.3/100000 (Kilmartin et al. 2000, Buller et al. 2006). SO is characterized by the sudden outbreak of immune responsive reaction involving exposure of retinal antigens and the concurrent appearance of corneal infections (Liddy and Stuart 1972).

Wu and colleagues (2004) established a mouse model for the demonstration of corneal fusariosis caused by *F. solani*, permitting assessment of fungal infection and pathogenesis. Topical corneal inoculations of *F. solani* were performed in immunocompetent and cyclophosphamide-treated adult BALB/c (i.e., immunosuppressed) mice and observed daily for 2 weeks. Histopathological examination following quantitative fungal recovery

was carried out at regular intervals. The dose dependent responses were observed in immunosuppressed mice, which resulted in increased disease severity and deferred fungal pathogen clearance. Under severe conditions, fungal hyphae, stromal edema, and inflammatory cells were evident in corneal tissue. Although immunosuppressed mice showed infection after corneal surface scarification which was assessed both *in vivo* and *in vitro* methods (Wu et al. 2004).

In another study, the MK mouse model was generated by intrastromal injections of *A. fumigatus*, which were then divided into different groups on the basis of treatment to be given, such as PBS treated (group I), voriconazole treated (group II), FK506, i.e., tacrolimus treated (group III), and voriconazole and FK506 treated (group IV). After the zymosan stimulation (10 mg/ml for 8 hours) the mRNA and protein expression levels of type I and II INFs were found to be profoundly elevated in macrophages, neutrophils, lymphocytes, and corneal epithelial cells (A6(1) cells). Also, the inflammatory cytokines were quantitatively analyzed at regular time intervals by quantitative real-time PCR (qRT-PCR) and western blotting (Zhong et al. 2018).

Epidemiology refers to the study of the distribution and determinants of a disease in a given population at a given period of time. Whereas prevalence is the rate or frequency with which the disease is found in a group or population under study at a particular point in time, and the incidence is the frequency with which new cases of a disease arise over a defined period of time (Sommer 1980). There are no previous reports on the prevalence of the disease. The prevalence of disease and severity can be estimated on the basis of infection cases presented to the hospitals (Tuft and Tullo 2009).

Microbial keratitis was mainly reported from North America, Australia, the Netherlands and Singapore and MK was largely reported in India (Shah et al. 2011). In Brazil, epidemiological study was performed on the basis of sales distribution of antifungal eye drops, which showed the linear regression relationship between reduction of humidity and antifungal eye drop sales, i.e., more cases of MK observed during the third quarter of the year when the agricultural activities are at the peak (Ibrahim et al. 2012).

Keratitis caused due to filamentous fungi is primarily observed in people continuously working in an outdoor environment like agriculture, where the penetration and invasion by fungal conidia is secondary to trauma (Gopinath et al. 2009, Nath et al. 2011). Fungal conidia and other traumatizing materials from plant and animal origin or the dust particles are responsible for intrastromal and intracameral invasion (Thomas 2007, Arora et al. 2011, Hu et al. 2016, Veena et al. 2017). The fungal species isolated from patients is primarily dependant on environmental factors like wind, humidity and rainfall. For example, *Curvularia* spp. are more frequent along the Gulf of Mexico during hotter and moister summers because of more airborne spores of *Curvularia* spp. in this period (Leck et al. 2002, Thomas 2007). The yeast-like non-filamentous fungi mainly include *Candida albicans* and related fungi which causes keratitis in the situations where there is insufficient tear secretion, defective eyelid closure or systemic causes includes diabetes mellitus and immunosuppression (Sun et al. 2007).

Current methods for diagnosis of MK

The diagnosis has been a vital part in the treatment of the disease, which includes non-invasive methods. In non-invasive *in vivo* diagnosis, confocal microscopy and anterior segment, optical coherence tomography are the main imaging techniques used. On the other hand, direct microbial examination, the culture of corneal scrape or biopsy materials are still the aid of diagnosis (Thomas and Kaliamurthy 2013).

Diagnosis based on clinical presentations

Keratitis caused by filamentous fungi mainly affects the cornea, producing symptoms like firm and dry slough observed in more than 50% cases, extension of hyphae into normal cornea, multifocal feathery grey-white 'satellite' stromal infiltrates cellular infiltration into adjacent stroma and mild iris (Rosa et al. 1994, Srinivasan 2004, Thomas 2007). The keratitis due to yeast-like fungi (*Candida* spp.) is similar to bacterial keratitis involving overlay of defective epithelia, discrete infiltration and gradual progression (Sun et al. 2007). In the case of older diagnostic methods, species-level detection of the aetiological agent was less frequent. So, proper microbiological tests are needed for the diagnosis (Dalmon et al. 2012).

In vivo diagnosis

In vivo diagnosis methods are the non-invasive techniques which include confocal microscopy, anterior segment optical coherence tomography for the real-time detection of aetiological agents of keratitis. Moreover, the responsiveness of an individual to the treatment of MK is also monitored by *in vivo* confocal microscopy and anterior segment optical coherence tomography (Martone et al. 2011, Kurbanyan et al. 2012, Soliman et al. 2012). The first-generation confocal microscopy and Heidelberg Retina Tomograph II-Rostock Cornea Module (HRTII-RCM) are aided for the *in vivo* diagnosis of keratitis due to *Cylindrocarpon lichenicola* and *Colletotrichum gloeosporioides* respectively, showing the branch like white hyphae in infiltrating cornea (Mitani et al. 2009). Two methods also demonstrated the pronounced reduction in inflammatory cells, removal of branching infiltrates and appearance of hyperreflective scar-like tissue representing the complete recovery (Martone et al. 2011). In the microscopic examination, the KOH mounts, lactophenol cotton blue staining, Giemsa, or an optical brightener, and culture from scrapings or biopsies are predominantly observed which are found to be reliable, rapid and inexpensive modality (Avunduk et al. 2003, Chowdhary and Singh 2005, Ansari et al. 2013).

Conventional in vitro diagnosis (microbiological approach)

In this method, the corneal scrape is taken by the help of corneal spatula or blade from the base and edges of the ulcerated part multiple times, which are used for inoculation on to the agar in plates followed by direct microscopic examination. Sometimes, corneal biopsies are required in case of a negative result. For the detection, two agar media used are blood agar and Saboroud glucose-neopeptone agar. In addition, brain heart infusion broth is also used in some cases (Leck et al. 2002, Thomas 2003). This method permits the rapid and presumptive diagnosis of keratitis.

In vitro diagnosis (molecular approach)

As the conventional methods of diagnosis have some drawbacks, which gives only the presumptive idea of an aetiological agent, firm and accurate identification of genus and species of the causative agent is necessary. This has led to the development of molecular tools like Polymerage Chain Reaction (PCR) for keratitis diagnosis (Alfonso 2008). PCR has also been reported previously for diagnosis of *F. solani* mediated keratitis in rabbit models (Alexandrakis et al. 1998). PCR is an ideal tool which uses minute sample quantities for analysis. In first study in 1996, the cutinase gene was targeted primarily; along with 18S

rRNA, 28S rRNA and ITS (Internal Transcribed Spacer Sequences). Mostly the PCR-based amplification is performed using universal primers and is less time consuming (Gaudio et al. 2002, Ferrer and Alio 2011). Usually, positive correlation is seen between the results of the conventional method and PCR-based diagnosis. But, the PCR cannot be used for monitoring the response to treatments (Embong et al. 2008, Kim et al. 2008).

Pharmacological treatments

The effective management of MK is a major concern. Moreover, the delay in diagnosis and unavailability of potential antifungal agents is mostly responsible for the poor outcome. However, in the last decade, considerable research efforts have been made by the scientific community towards the development of new techniques for rapid diagnosis of MK and efficient therapeutic and pharmacological treatments (Maharana et al. 2016). Despite the availability of some topical and systemic antifungal agents and adjuvant surgery like corneal transplantation, it is difficult to treat MK. Thomas and Kaliamurthy (2013) made the Cochrane Database systematic review of medical interventions for MK and analyzed nine randomized controlled trials which were performed on 568 MK patients, who were subjected to randomized treatments of different antifungal agents (i.e., 1% topical itraconazole versus 1% topical itraconazole and oral itraconazole, voriconazole 1% versus natamycin 5%). Further, from the various studies including this report, it was proposed that there is no antifungal drug, or combination of drugs very effective in the management of MK. Moreover, the new triazole, Posaconazole is recently used as active broad-spectrum antifungal agent against various causative agents of MK such as *Candida* species, *Cryptococcus neoformans*, *Aspergillus* species, Zygomycetes, and endemic fungi. Chemically it is a synthetic structural analogue of itraconazole. It helps in the management of MK by blocking of the fungal cell wall ergosterol synthesis (Torres et al. 2005, Prajna et al. 2010).

It is proposed that the ideal and effective antifungal drug used in the treatment of MK should not be irritating and toxic for the eyes. In addition, it must penetrate the eye and exhibit potential efficacy against at least one significant causative agent and should be easily available. Generally, the first line treatment in MK initiated on the basis of direct microscopic examination if the clinical evaluations are consistent, otherwise, it commences after getting results of cultural analysis. Initially, natamycin (5%) as a topical dose is usually recommended for superficial mycotic infection (Castano and Mada 2018). In addition, some other antifungal agents such as amphotericin B, ketoconazole and itraconazole are also prescribed in case of deep corneal infections. Moreover, the treatment also varies depending on the direct microscopic examination, i.e., whether the causative agent is filamentous fungus (showing hyphae in the microscopic examination) or yeast. If the infection is due to filamentous fungus, the treatment preferably includes 5% natamycin as a topical dose or other drugs like amphotericin B (0.15%) or, voriconazole (1%) can also be recommended. On the other hand, if the causative agent is yeast or fungus with pseudohyphae, amphotericin B (0.15%), fluconazole (1%) or voriconazole (1%) as the topical dose is preferred (Arora et al. 2011, Ramakrishnan et al. 2013). Some more details about the routinely used antifungal agents and their concentrations are listed in Table 1.2.

In addition to pharmacological treatments, surgery and corneal crosslinking are considered as effective approaches in the management of MK. Surgery is generally recommended when a response to the pharmacological agent is poor and there are more chances of spreading of infections. In surgery like periodic debridement, the necrotic, infectious and antigenic portion is removed to develop a favourable environment

Table 1.2: Currently available antifungal agents for treatment of mycotic keratitis (Adapted and modified from Maharana et al. 2016; a free open access article).

Antifungal agent	Route of administration	Dose/ Concentration	Spectrum	Major limitation	Current indication	References
Amphotericin B	Topical Intracameral/ Intrastromal	1.5–5 mg/ml 5–10 µg/0.1 ml	Both yeast and filamentous	Preparation and stability	• First choice in the treatment of keratitis by yeasts • Alternative to NTM† in filamentous fungi IC/IS in deep keratitis or endothelial plaque	Kaushik et al. 2001, Lalitha et al. 2007, Yoon et al. 2007, Das et al. 2009
Natamycin	Topical	50 mg/ml	Drug of choice for filamentous fungi Can also be used for yeast	Poor penetration	• First choice in filamentous fungi • Alternative to AMB‡ in keratitis by yeasts	Lalitha et al. 2007, Das et al. 2009
Miconazole	Subconjunctival	1.2–10 mg/ml	Both yeast and filamentous fungi	Less effective than polyenes Limited data	• SC with topical therapy in patients with low compliance	Lalitha et al. 2007, Das et al. 2009
Econazole	Topical	20 mg/ml	Filamentous fungi	Limited data	• Alternative to NTM in filamentous fungi	Lalitha et al. 2007, Das et al. 2009
Ketoconazole	Systemic	100–400 mg every 12 h	Broad spectrum	Systemic toxicity	• Used along with topical therapy in deep fungal keratitis	Lalitha et al. 2007, Das et al. 2009
Itraconazole	Topical Systemic	10% 400 mg/day	Effective as an adjunct in *Candida* spp. Less effective in *Fusarium* spp.	Topical use not as effective as NTM	• Used systemically along with topical therapy in deep keratitis due to yeasts or those affecting intraocular tissues	Lalitha et al. 2007, Das et al. 2009
Fluconazole	Oral Topical Subconjunctival	200–400 mg/day 2 mg/ml 2 mg/ml	Effective for *Candida* species	Narrow antifungal spectrum	• Topical as alternative to polyenes • Oral as adjunct in deep keratitis or those affecting intraocular tissues	Lalitha et al. 2007, Das et al. 2009

The major goals for nanosystems used in drug delivery include enhanced permeation of drug, controlled release, the bioavailability of the drug, and surface modification of nanosystems with specific target moieties. Moreover, the development of nanomaterial-based formulations will have the ability to remain at the site of infection for a long period of time, which ultimately helps in reduction of dose administration leading to minimize the adverse effects (Vandervoort and Ludwig 2007, Mudgil et al. 2012, Ingle et al. 2017).

The use of various nanomaterials such as nanoparticles, liposomes, solid lipid nanoparticles, dendrimers, micelles, polymeric nanoparticles, etc., are reported to enhance the ocular drug delivery at the targeted site (Kumar et al. 2014, Rai et al. 2016, Ingle et al. 2017). Moreover, natural polymers such as gelatin, chitosan, albumin, sodium alginate and biodegradable polymers like poly alkyl cyanoacrylate (PACA), poly lactic acid (PLA), poly lactic-co-glycolic acid (PLGA), poly epsiloncaprol acetone (PCL), etc. (Ingle et al. 2017) can be widely and effectively used for antimycotic drug delivery to the infected site.

Till date, only a few reports are available on the efficacy of nanomaterials mostly nanoparticles against MK causing microorganisms. On the contrary, various studies have been performed on the use of nanomaterials as a drug carrier agent for effective management of eye diseases. The most common fungi causing MK are *Aspergillus* and *Fusarium* sp. and azoles are widely used as first line of treatment therapy for MK. Lachmapure et al. (2017) evaluated antifungal activity of silver nanoparticles alone and in combination with antimycotic drugs including ketoconazole, and amphotericin B against the MK causing fungi in human (see Fig. 1.2). The study indicates that silver nanoparticles in combination with antimycotic drugs showed remarkable inhibitory activity against isolated MK fungi namely *Aspergillus flavus, A. fumigatus, A. niger, Curvularia* sp., *Fusarium* sp. and *Bipolaris* sp. Similarly, Xu et al. (2013) also reported the *in vitro* activity of silver nanoparticles against ocular pathogenic filamentous fungi. The chitosan nanoparticles are commonly used as a carrier for ocular drug delivery by assessment of their interaction with the ocular mucosa and toxicity in conjunctival cells. Various studies revealed that use of chitosan nanoparticles in the delivery of antimycotic drugs is promising and safe because of no relevant toxicity on penetration into corneal and conjunctival cells (Sandri et al. 2017, Kumar and Sinha 2017). Terbinafine hydrochloride encapsulated nanoemulsions based gel was formulated for effective treatment of fungal keratitis by Tayel et al. (2013). The resultant nanoformulation was found to be effective. Therefore, nanoparticles are highly promising materials which can help to treat the mycotic infection in the eye. But with its application, there is also a need to study the toxicity and safety associated with their use for a medical purpose.

Ocular toxicity of nanoparticles

The eye is a structure which contains various tissues in a confined area. However, it is a very sensitive organ in terms of the development of inflammation and response to toxic materials. As mentioned above, during the treatment of fungal infections in the eye, various drugs can be targeted to the specific region of the eye with nanoparticles. Here it is important to note that we do not have any mechanism to remove nanoparticles especially the metallic nanoparticles from tissues. Hence, the antifungal treatment although can remove fungi, nanoparticles used for this treatment purpose can be accumulated, as a result, there are high chances that the nanoparticles can exert the toxic effects. For instance, when silver nanoparticles are used for the treatment of MK, it can reach the ocular mucosa and remain there for a prolonged duration. The repeated administration of nanoparticles can result in the toxicity to the

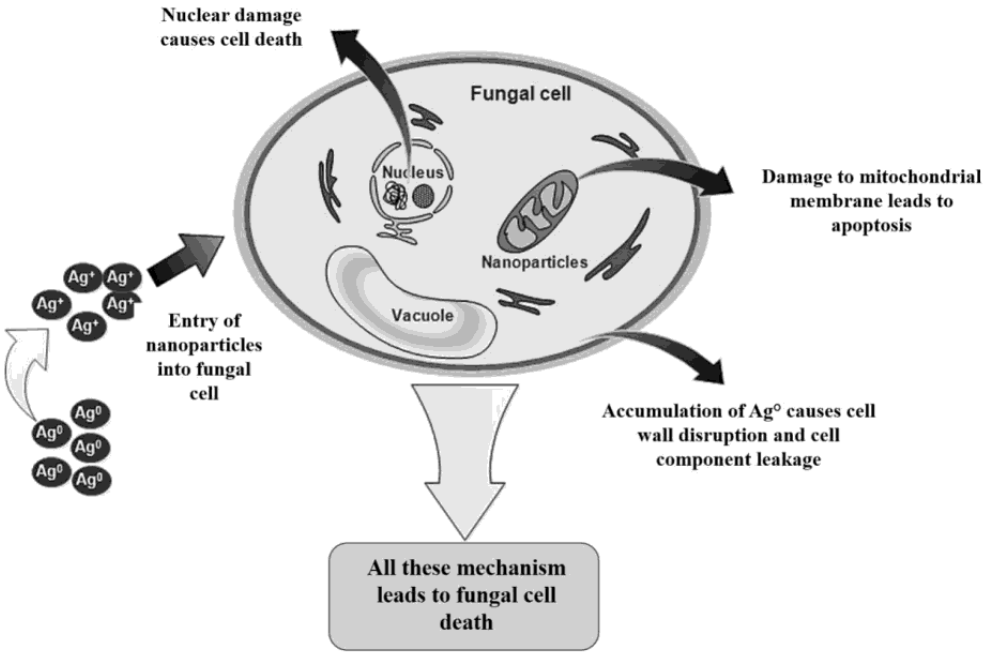

Fig. 1.2: Schematic representation of mode of antifungal action of silver nanoparticles.

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ocular mucosa (de Campos et al. 2004). However, chitosan nanoparticles are biodegradable and hence, they are advantageous over other nanoparticles with no or negligible toxicity. Their biodegradability therefore, minimizes the side effect of frequent administration to the ocular region. Additionally, chitosan nanoparticles can lead to sustained release of drugs, thus reducing frequent dosing. They also protect the drug from various enzymes, thereby increasing their half-life (Tsai et al. 2018).

The toxicity of nanoparticles used in the treatment of ocular disease is size dependent. The smaller the nanoparticle size, the greater will be its penetration, leading to higher toxicity (Iswarya et al. 2016). This is because smaller nanoparticles can easily cross the cell membrane and thus interacts with the intracellular components. Whereas, large particles get accumulated outside the cell and thus it can cause the local toxicity. Magnetic nanoparticles of 50 nm diameter were found to be less toxic to corneal endothelial cells than its larger counterparts of the size 4 µm. This is due to the fact that smaller nanoparticles rapidly drained into the systemic circulation whereas bigger nanoparticles remained at the site of the injection. However, it is a matter of further research to know about the fate of smaller nanoparticles which flow into the systemic circulation. Gold nanoparticles (AuNPs) having a diameter less than 30 nm were found to be highly internalized by adult retinal pigment epithelial cell line 19 (ARPE19) as compared to the bigger nanoparticles suggesting that the surface area of AuNPs plays a key role in exerting its toxic effects (Karakocak et al. 2016).

The toxic effect of nanoparticles is due to its presence or due to the release of ions by their dissolution is still a matter of debate (Bianco et al. 2016). It has been found that silver gets deposited in the cornea and conjunctiva (Hadrup et al. 2018). Therefore, it offers a high chance of disturbance to the function of the eye. Nanoparticles toxicity is also being defined by their surface chemistry. Many nanoparticles have been shown to be capped