Abstract
Six fungal species (Fusarium sp, Exophiala jeanselmei, Penicillium spp, Aspergillus niger, Paecilomyces spp, and Alternaria spp) were used to inoculate soft contact lenses. Four types of soft lenses were used: high-water (58%) and low-water (38%) content lenses and lenses that were unworn or worn for 1 day. The fungi displayed a range of macroscopic and microscopic features that allowed differentiation of species. There was no statistically significant effect of lens water content on growth rate and only Penicillium spp showed significantly higher growth for worn versus unworn lenses. A number of the fungi showed secretions, thought to be enzymes, which potentially aid in the process of lens penetration.

Author Keywords: Soft contact lenses; fungi; microorganism; keratitis

Introduction
Fungal invasion of soft contact lenses is a potentially serious complication of their use. Lenses contaminated with fungi can lead to fungal keratitis, and many cases progress to severe fulminating infections. Although fungal invasion of soft contact lenses has been noted by several investigators, the occurrences have been considered relatively uncommon.[1] Warm weather may favor the dispersal of fungal spores and promote fungal growth as well as enhance the potential for corneal trauma associated with outdoor activities and occupations.

Fungi are primitive nonmotile plant-like structures that grow as unicellular organisms called yeast or multicellular filamentous structures called molds. The true fungi are divided into four major divisions (phyla): Zygomycota, Basidiomycota, Ascomycota, and Deuteromycota.[2] Our study mainly dealt with fungi from the phylum Ascomycota, which includes fungi with septate hyphae and ascospores contained within sacs (asci).

Although simple organisms, fungi are extremely adaptable to diverse environments. The only requirements for growth are a small amount of organic substrate and moisture. More than 60 fungal species have been reported as pathogenic to the cornea, and previous studies have found Aspergillus spp, Penicillium spp, Fusarium spp, and Candida spp to be the most common organisms responsible for fungal keratitis on a worldwide basis.[1, 3 and 4]

Fungal keratitis is rare in normal eyes without predisposing factors, due to the protection afforded by the intact corneal epithelium. Most cases of fungal keratitis are
associated with corneal trauma, in which organic material is inoculated into the cornea. For the past three decades, the incidence of fungal keratitis has increased, and this has been attributed to factors such as the use of topical corticosteroids, contact lens wear, use broad-spectrum antibiotics,[5] and pre-existing ocular and systemic immunosuppressive diseases. [6]

Due to the small-sized pores of hydrophilic contact lenses, they are considered theoretically to be impermeable to microorganisms. However, fungi have demonstrated that they are capable of invading the matrix of a soft contact lens. It is thought that they gain access to the matrix by the action of enzymatic depolymerization of the lens (i.e., they secrete enzymes that "digest" the lens polymer).[7] Kirsch and Brownstein [8] noted the presence of electron-dense substances surrounding fungal hyphae in the lens matrix and proposed that this finding represented physical metabolic degradation of the lens. They also noted the lens matrix adjacent to and between the fungal colonies appeared normal. There is a general consensus that growth of fungi within the lens matrix increases with the water content of the lens. This increased susceptibility to fungal invasion may be due to their larger pore size, which allows easy passage of nutrients into the lens. The high-water medium may aid the excreted enzymes in the degradation of polymer and thus promote colonization of fungi.[7] Yamaguchi and co-workers [9] also found a tendency toward deeper fungus penetration in the matrix of the lenses with higher water content.

It is believed that the reported frequency of fungal infiltration of hydrophilic lenses may be underestimated, as many cases probably are mistaken for nonspecific or mucoprotein deposits.[8] In addition, many lenses with deposits are likely to be discarded without recognition of the possible fungal nature of the accumulation. We devised a classification key based on the morphologic characteristics of seven common fungal specimens as they appear in soft lenses, to aid practitioners in the differentiation of fungal deposits from other harmless deposit formations and to aid in the identification of these common fungal species. We also investigated the effect of water content and the presence of natural lens deposits (pellicle) on the effectiveness of fungal invasion of soft contact.

**Methods**

The fungal species used in this study were chosen because of their prevalence in ocular infections and their availability. Six fungal species (Fusarium spp, Exophiala jeanselmei, Penicillium spp, Aspergillus niger, Paecilomyces spp, and Alternaria spp) were obtained from the Department of Microbiology, Queensland University of Technology. All species were obtained in suspension forms and stored in a refrigerator at 4°C until required. Sabouraud’s dextrose agar plates were used as the culture media.

Seven optometry students who wore contact lens were recruited to participate in the study to supply worn lenses. The students ranged in age from 20 to 30 years (mean 23.4 years). All were free of any ocular pathology and had normal tear function. All
subjects were given two pairs of Acuvue soft lenses (water content 58%) and two pairs of Soflens Medalist lenses (water content 38%) to use for 1 day on a daily-wear basis. Dioptric power of the lenses ranged from −0.75 to −6.00 DS. All subjects were experienced lens wearers and were familiar with care and maintenance regimes. Each subject was instructed to wash and dry his or her hands thoroughly prior to handling lenses and to store the lenses in nonpreserved saline on removal.

After collecting the worn lenses from each subject, an equal number of the same type of unworn lenses was obtained. Each SDA plate was divided into four sections to accommodate a low-water content worn lens, high-water content worn lens, low-water content unworn lens, and high-water content unworn lens. Using sterile forceps, lenses were placed on the SDA plates with convex (anterior) surfaces down. One drop of fungal suspension was placed in the concavities of the lenses using sterile pipettes in a fumehood under aseptic conditions. We tested each of the four lens conditions for the six fungal species for all seven subjects. A series of seven control plates also was tested, which had the four lens conditions but no fungal suspension inoculation. A total of 196 lenses were used (49 SDA plates). An incision at the edge of the lens was made on all lenses to allow it to be flattened onto the underlying agar. After being sealed in plastic bags, the plates were incubated at room temperature (22.4°C) to reduce dehydration of the agar and provide a humid environment.

To quantify the fungal growth, one of the experimenters estimated the relative percentage area of fungal growth covering the lens on a continuous grading scale from 1 to 100. Observation of the lenses and the fungal colonies was made with naked eye inspection and slit-lamp examination. Photographs of the fungal growth were taken using a slit-lamp camera and with a binocular microscope.

**Results**

Fungal growth was observed on all lenses inoculated with the six fungal species. The following is a qualitative analysis of the growth characteristics of the fungal species studied.

*Fusarium* spp showed growth that was observed to be white and pinkish in color, and the fungi were observed to appear in small clumps. White filaments were evident on light microscopy and slit-lamp biomicroscopy.

*Exophiala jeaneselmei*. Gray, white filaments were observed with superficial black granules covering the lens surface. The black granules progressed to become very prominent and covered almost the entire surface. Droplets of brown, oily extracellular enzymatic secretions were observed.

*Penicillium* spp showed pale-green, velvety growth with deep furrows radiating from the center of the lens. Light microscopy revealed filaments protruding from the surface of the deposit, and extracellular enzymatic secretions were noted (Figure 1).
Figure 1. Penicillium spp growing on a soft contact lens surface. The color was pale green and extracellular enzymatic secretions were noted. These enzymes are thought to aid penetration of the lens matrix.  
Aspergillus niger demonstrated two stages of growth. Fungi covered the entire surface on day 2 after inoculation and appeared yellow; however, by day 3 a black superficial layer covered the yellow growth. High magnification showed round reproductive spores and filaments penetrating into the lens surface (Figure 2).

Figure 2. Aspergillus niger on a soft lens showing round, darkly colored reproductive spores and mycelia penetrating into the lens surface. Small dark patches on the lenses were evident with Alternaria spp. The filaments penetrated the lens surface, which was evident on high magnification (Figure 3 and Figure 4). Droplets of brown extracellular enzymes were visible.

Figure 3. Alternaria spp showing mycelia penetrating the lens.

Figure 4. Alternaria spp mycelia within the lens matrix showing darker-colored spore sacks. Paecilomyces spp showed white, yellowish opacities on day 2 of growth. Growth appeared white and fluffy on day 3 and covered the lens completely. High magnification revealed hyphal structures penetrating the matrix of the lens material.
Figure 5. Paecilomyces spp showing hyphal structures penetrating the edge of the soft lens.

Some whitish growth was observed on our control lenses. It later was identified to be a bacterial contaminant. Because there was no fungal growth on these control lenses, it was reasonable to conclude that there was no cross-contamination between the plates used for the different fungal species.

<table>
<thead>
<tr>
<th>Fungal Species</th>
<th>Morphology</th>
<th>Macroscopic (SDA Plate Observations)</th>
<th>Microscopic (Mit-lop Observations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusicoccum spp</td>
<td>Pinkish-white</td>
<td>Vellutoy</td>
<td>Smooth</td>
</tr>
<tr>
<td>Eupenicillium spp</td>
<td>Olive gray</td>
<td>Vellutoy</td>
<td>Wrinkled, convoluted</td>
</tr>
<tr>
<td>Penicillium spp</td>
<td>Pale blue-green</td>
<td>Dense, velvety, powdery</td>
<td>Deep furrows irregularly</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>Brownish-black</td>
<td>Rough, granular</td>
<td>Radiating from the centre</td>
</tr>
<tr>
<td>Paecilomyces spp</td>
<td>Yellowish-tan</td>
<td>Velvety, powdery</td>
<td>Dense and irregular</td>
</tr>
<tr>
<td>Alternaria spp</td>
<td>Blackish</td>
<td>Coarse</td>
<td>Even, regular</td>
</tr>
</tbody>
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Table 1 summarizes the morphologic characteristics of the fungal deposits on the lenses and details the descriptions in terms of color, margin, texture, and topography.

Table 1. Morphologic Characteristics of Various Fungi on Soft Contact Lenses

Most fungal colonies were darkly colored except Fusarium spp, which was distinctly creamy-white in color. Topography was a useful morphologic feature to use as a guide to distinguish between fungal species. Some fungal colonies such as Penicillium spp were clearly verrucose with deep furrows, whereas others such as Fusarium spp and Alternaria spp displayed an even topography.

Growth characteristics of the fungal colonies on the lenses corresponded closely to what has been documented for the same species grown simply on SDA plates without soft lenses present. [2 and 10] Microscopic examination revealed hyphae of varying length and color protruding from the surface of the colonies of each fungal species. These observations again were synonymous descriptions of fungi growing on SDA plates alone. [2 and 10]
Microscopic examination not only revealed the hyphal structures of fungi, it also enabled visualization of lens penetration by hyphae. Lens penetration was evident in the fungal species Aspergillus spp, Alternaria spp, and Paecilomyces spp (Figure 4 and Figure 5, 6d). Penetration through the full-thickness matrix to the posterior surface was not observed with any of these species after 3 days of growth. Although other fungal species did not show lens penetration, Penicillium spp and Exophiala jeaneselmei spp showed oily brown secretions that we assumed to be extracellular enzyme. It is speculated that such enzymes are produced by fungi to help degrade the lens (host) material and hence facilitate the process of penetration.

Statistical analysis of the fungal growth was performed using paired Student t-tests to determine the significance of differences in the extent of growth on different lens types. The analysis was performed on growth data after day 2, because by day 3 the growth was close to 100% surface coverage. The null hypothesis tested was that there was no difference between growth rate for the four lens conditions tested (worn vs unworn, high-water vs low-water content). The mean levels of growth for each of the six fungal species and four lens conditions are presented in Table 2. Table 3 shows the statistical analysis.

Table 2. Mean Levels of Growth for Each Fungal Species (Sample Size = 7)

Table 3. Comparison of Fungal Growth on Different Types of Soft Contact Lenses in Six Fungal Species

The results showed no significant differences between the extent of fungal growth on lenses, whether worn or unworn (p > 0.05). This suggests that the presence of natural lens deposits (pellicle) neither inhibits nor facilitates fungal growth, but it should be acknowledged that the SDA plate medium may have overwhelmed any potential effect of the natural lens deposits.

There also appeared to be no consistent difference between fungal growth on lenses of high-water and low-water content (p > 0.05). However, the exception to this was Penicillium spp, which showed significantly higher growth on worn high-water
content lenses (p < 0.01) and worn low-water content lenses (p < 0.001) compared with identical unworn lenses.

**Discussion**

The fungal species we studied had a wide diversity of macroscopic and microscopic appearances at various stages of their development. It is conceivable that without careful observation, these manifestations of the fungi could be misdiagnosed as various lens deposits such as rust spots, protein deposits, or calcium deposits. However, the defining characteristic for distinguishing fungal growth on a contact lens is the filamentous appearance of the mycelial strands, which we observed with all fungal species.

Fusarium spp has been recorded as having fuzzy-white growth of hyphal elements in an extended-wear contact lens,[1] and this description closely matches our observations. The slimy creamy appearance is related to how this fungus produces its spores. Unlike most other fungi that produce airborne spores, its spores become encased in a slime. [11]

Penicillium spp is well known throughout society because of its historic importance in the development of antibiotics. It is encountered most commonly in temperate regions compared to the other well-known species such as Aspergillus spp, which is more prevalent in tropical regions. We observed the expected bluish color of this species when grown on soft contact lenses.

Aspergillus niger is also a well-known fungal species and is encountered frequently in contact lenses and mycotic keratitis. It is characterized by black filamentous growths, but it should be noted that it has a yellow appearance early in its development. It is the black heads of the conidia reproductive structures that develop later and give it the typical blackish appearance. This is an example of a dimorphic fungus as it exhibits two colors as it matures. This characteristic is common among fungal species and needs to be remembered when considering the hue of a fungal colony. Aspergillus niger deposits have been initially mistaken as crystalline contact lens deposits[12] and have been described as a white, feathery patch in a contact lens. [13]

We found no significant differences in fungal growth between lenses of high-water and low-water content in our study. But the abundance of nutrients provided by the agar plates probably masked the more subtle differences between growth on highwater and low-water content lenses. Simmons and co-workers[14] and Tripathi et al [7] demonstrated that the chance of growth of fungi within a lens matrix increased with increasing water content.

Lens deposits have been suggested to act as adherence sites for the initial penetration process of fungi.[15] Our data suggested that the presence of deposits had no effect on facilitating the growth of fungi, except in the case of Penicillium spp. But again the effect of the deposits may have been minor in comparison to the nutrition provided by the SDA agar plates. We have been more successful in eliciting information with shorter observation intervals (e.g., 6 hourly) and a more diluted nutrient supply.
Several possible mechanisms of lens penetration by fungi have been postulated.[16] The fungi adhere to the lens surface and remain in contact with the surface long enough to digest its way into the lens. Some authors have noted penetration by Aspergillus spp, Alternaria spp, and Fusarium spp to be complete within 96 hours by some fungal species.[14] We also documented lens penetration by the fungal species Aspergillus spp, Paecilomyces spp, and Alternaria spp.

We noted the presence of extracellular enzymes in the colonies of Penicillium spp, Alternaria spp, and Exophiala jeanselmei. These enzymes are thought to facilitate penetration into the lens matrix. Enzymes were not observed on Fusarium spp, Aspergillus niger, and Paecilomyces spp colonies. Simmons and associates[14] examined the ultrastructure of an electron-dense substance surrounding fungal hyphae within the lens matrix and proposed that it represented metabolic degradation of the lens. Yamaguchi and associates [9] also observed similar electron-dense material. Fungi use mycelial strands and polysaccharide adhesions, as well as ionic bridges, to gain a hold on the lens surface.[14] It is thought that deep infiltration into the matrix is accomplished by secretion of enzymes that depolymerize the lens. [7 and 17] Some authors have even suggested that the lens material itself may provide a nutrient source for the proliferation of fungi and that enzymatic activity by the fungus may enhance infiltration of the soft lenses.[8]

After 17 days of growth, we removed the lenses from the SDA plates and manually rubbed the lenses with saline as if performing a daily cleaning procedure. The lenses were very fragile and typically disintegrated, suggesting that the fungi had penetrated deeply into the lens matrix and broken down much of the lens polymer structure. As fungi are not usually part of the ocular flora,[18] fungal contamination of soft contact lenses must occur from other sources. Advising patients to avoid contact lens wear in moldy environments and reinforcement of care and maintenance compliance are important methods for reducing the risk of fungal contamination of soft lenses. Regular replacement of soft lenses also potentially reduces the risk of lens contamination.

**Conclusion**

We inoculated soft contact lenses with six species of fungi and made qualitative and quantitative observations of growth. The fungal species had macroscopic and microscopic characteristics that allow clinical differentiation of species.

**Acknowledgements**

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**References**