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Effect of polymer differences on fungal penetration of hydrogel lenses

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SUMMARY

Two chemically distinct types of hydrogel lenses, vifilcon A and bufilcon A, each with a water content of 55%, were challenged in a balanced salts solution with *Aspergillus fumigatus, Cladosporium cladosporioides, Curvularia lunata* and *Fusarium solani*. The lenses were cleaned, disinfected and stained after varying periods of incubation and examined with light microscopy and scanning electron microscopy. For three of the four fungi, the bufilcon A lens was more susceptible to fungal attack than the vifilcon A lens. *Curv. lunata* produced the greatest number of penetration pegs within 72 h for both lens types. Etching of lens surfaces was observed with *C. cladosporioides*. In general, the susceptibility of a hydrogel lens to penetration with a fungus appeared to vary with the species of fungus and the chemical composition of the lens.

INTRODUCTION

The penetration of hydrogel lenses by fungi is not uncommon [2,6]. The process is considered irreversible and the replacement of invaded lenses is of economic importance [3,4]. Lens invasion occasionally has been associated with ocular infections [6,8]. Fungi penetrate lenses via finely tapered coiled hyphae that may be surrounded by electron-dense material suggesting metabolic alteration of the lens [3,7]. The higher-water-content lenses used for extended wear have been noted to be most susceptible, but differences in invasiveness between fungi or in the recalcitrance of various hydrogel polymers have not been defined.

MATERIALS AND METHODS

Cultures

Four fungi, *Aspergillus fumigatus* ATCC 10894, *Cladosporium cladosporioides* LW, *Curvularia lunata* DAM and *Fusarium solani* G, the latter three initially from soft contact lenses or corneal ulcers [6], were used in the challenge studies. All cultures were lyophilized and working stocks were main-

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tained on Mycological agar (Difco) at room temperature. The fungi were grown on Potato-Dextrose Agar (Difco) in 1 liter Erlenmeyer flasks at room temperature for 5–14 days. Conidia were collected by flooding the surface with phosphate-buffered saline (PBS) (NaCl 8.0 g, KCl 0.20 g, KH_2PO_4 0.12 g and Na_2HPO_4 0.91 g in 1000 ml deionized H_2O; pH 7.2). Conidia were concentrated by centrifugation, washed twice and resuspended in PBS. The conidial suspensions were stored at 2–5°C for up to 14 days.

Lenses

Thirty-six each of bufilcon A and vifilcon A 55%-water-content lenses, placed singly in sterile vials, were obtained from commercial sources. The bufilcon lens is composed of poly[2-hydroxyethyl methacrylate plus *N*-(1,1-dimethyl-3-oxybutyl) acrylamide and methacrylic acid]. The vifilcon A lens contains poly(2-hydroxyethyl methacrylate-coethylene dimethacrylic acid-g-povidone).

Soft lens inoculation

Conidia were added to 10 ml of a balanced salt solution (BSS; NaCl 0.49 g, KCl 1.075 g, $CaCl_2$ 0.048 g, $MgCl_2$ 0.03 g, $C_2H_3NaO_2$ 0.39 g, $C_6H_6Na_2O_7$ 0.17 g in 100 ml deionized H_2O) in a screw-cap test tube to give a final concentration of 10^2 to 10^4 conidia per ml. Each soft lens was aseptically removed from its storage vial with sterile forceps and placed into the test tube with the suspension of conidia. The culture systems were incubated on a roller drum (15–20 rpm) at room temperature usually for 48–96 h.

Disinfection and staining

Lenses were processed through the AOSEPT Catalytic Disinfection System (American Optical Corporation, Framingham, MA). This system includes the application of three drops of Allergan LC-65 Daily Contact Lens Cleaner (Allergan American, Hormiguerous, Puerto Rico) to each lens surface and rubbing of the lens between the thumb and finger for 10 s followed by a 20 s rinse with Allergan Sorbi-Care Saline Solution (Allergan Pharmaceuticals, Inc., Irvine, CA). The disinfection medium was 3% H_2O_2 used according to the manufacturer's instructions. After cleaning, the lenses for light microscopy were stained with a 1:3 dilution of lacto-phenol cotton blue (20.0 ml lactic acid, 40.0 ml glycerol, 18.8 ml phenol, 0.07 g cotton blue, 20.0 ml deionized water; filter-sterilized in BSS). Lenses for scanning electron microscopy (SEM) were processed as described previously [3].

RESULTS

In preliminary experiments, the BSS was supplemented with 0.01 and 0.001% serum albumin. Increased protein content resulted in a more rapid (within 72 h) and more uniform covering of 55%-water-content lenses by *C. cladosporioides*. Light microscopic and SEM examination of the lenses, however, showed that penetration of the lens by the fungus decreased as the protein content was increased. Therefore, the BSS without serum albumin was used for all subsequent tests.

Lacto-phenol cotton blue, diluted 1:3 with BSS, was found to stain most hyphal elements in cleaned and disinfected lenses within 10–20 min. Excess stain within the lenses could be removed by rinsing in saline for 10–15 s followed by immersion for 10 min in 10 ml of saline. During the latter phases of this study, cleaned lenses were placed directly into the lacto-phenol stain without the H_2O_2 disinfection. Viable fungi were not recoverable from these lenses.

The relative degree of lens penetration by the various fungi could be quantitated by counting the coiled penetration pegs under light microscopy (400 ×) (Fig. 1). Results of a representative experiment after 72 h incubation are presented in Table 1. Additional experiments incubated for 120–168 h and 192–240 h gave relatively the same type of data except that hyphae of several species exited and reentered the lens so that the increased numbers of penetration pegs were difficult to count. The bufilcon lens as compared to the vifilcon lens was twice as susceptible to penetration by *Fusarium* and *Curvularia* (99% confidence limit). Only in the case of *C. cladosporioides* by 240 h incubation did the num-

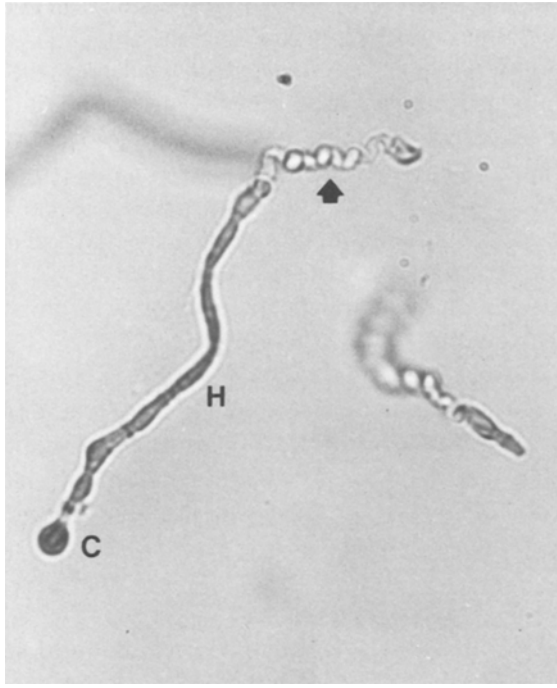

Fig. 1. Penetration of the surface of a vifilcon A lens (55% water content) by *C. cladosporioides*. Conidium (C), hypha (H), tightly coiled penetration peg (arrow). (× 620).

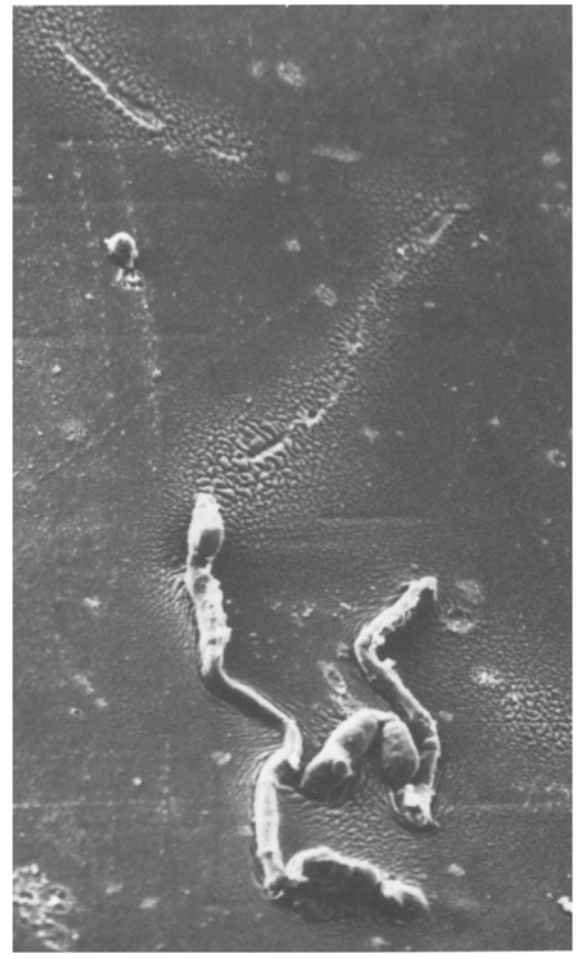

Fig. 2. Scanning electron micrograph of *C. cladosporioides* remaining on the surface of a vifilcon A (55% water content) hydrogel lens after cleaning. Rippling and etching of the lens surface are evident. (× 1700).

bers of penetration pegs in the vifilcon A lens exceed those observed in the bufilcon A lens. *Curv. lunata* had a much higher rate of penetration of lenses within 72 h than the other fungi. This high penetration rate, coupled with the larger coiled penetration pegs, made quantitative observations of this fungus easy. *Aspergillus* and *Fusarium* spp. penetra-

Table 1

Penetration of hydrogel lenses (55% water content) by fungi[a]

	Source code	Bufilcon A		Vifilcon A	
		average	range	average	range
A. fumigatus	ATCC 10894	28[b]	0–65	8	5–12
C. cladosporioides	LW	2	0–5	<1	0–1
Curv. lunata	DAM	1117	976–1226	490	291–771
F. solani	G	197	59–384		6–169

[a] Inoculum: 10^3 conidia/lens in 10 ml BSS.

[b] Average number of penetration pegs in three lenses at 72 h.

tion pegs were less than 1 μm in width, whereas the penetration pegs of *Curvularia* and *Cladosporium* were typically 1–2 μm in width. Surfaces of lenses incubated with *C. cladosporioides* for 72 h or longer were often etched and rippled whether or not penetration occurred (Fig. 2).

DISCUSSION

Simple overgrowth of a lens and susceptibility of a lens to fungal penetration were distinguished by the procedure of cleaning, disinfecting and staining challenged lenses with a lacto-phenol cotton blue preparation followed by microscopic examination. The disinfection and staining could be combined into a single-step procedure since the lacto-phenol preparation was fungicidal. Microscopic observations of the coiled penetration pegs then permitted semiquantitative evaluation of lens susceptibilities to various fungi. More valid quantitative evaluations of lens penetration by fungi would require an increased number of lenses and equivalent handling, packaging, sterilization and storage procedures for the different lenses. The lenses were received from several sources and their histories as to the above factors are unknown. Oil from hand manipulation of the lenses, organics from waters, etc., could markedly influence the growth of a fungus on a given lens. These types of influences were suggested by the range of penetrations by several fungi on multiple challenges of one lens type. Etching of lenses by *C. cladosporioides* further suggested that lens damage may occur without penetration of the hydrogel matrix. Isolates of *F. solani* and *Curv. lunata* penetrated lenses more extensively than *A. fumigatus* or *C. cladosporioides*.

Liesegang and Forster [1] demonstrated *Fusarium* to be the most common etiological agent causing fungal keratitis in the southeastern United States. *Fusarium*, mainly *F. solani*, was isolated from 61% of 134 fungal corneal ulcers diagnosed between 1 January 1969 and 31 December 1977. *Curvularia* was the most common dematiacious agent isolated (5.9%). *F. solani* may be more suitable than *Curv. lunata* for examining lens resistance to fungal penetration because of the greater ease in preparing conidial suspensions for lens challenge and because of its greater role in keratitis.

Both the vifilcon A and bufilcon A lenses contain bound nitrogen within the polymers. The vifilcon A lens, which appeared more resistant to penetration, had nitrogen bound in a carbon ring structure. In the bufilcon A lens, the nitrogen is contained in an amide group, which may have been more accessible. Nevertheless, while a lens is worn, enough required nutrients for fungal growth probably would be provided by the wearer.

Previous studies have shown that fungal growth in soft contact lenses is enhanced in lenses with increased water content [3,8]. This study demonstrates that the type of hydrogel polymer and the species of fungus also affect the degree of fungal penetration.

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