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In vitro colonization of hydrophilic contact lenses by *Aspergillus niger*

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***In vitro* colonization of hydrophilic contact lenses by *Aspergillus niger* was investigated. Five strains of the fungus, four polymers, two culture media and four incubation periods were considered for analysis. Only the 2700 strain colonized the lenses. The degrees of adhesion and invasion varied significantly according to the characteristics of the culture under investigation.**
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Keywords: hydrophilic contact lenses; fungal adhesion; fungal invasion; colonization; *Aspergillus niger*

Introduction

Colonization of contact lenses by fungi causes several health problems, both indirectly through the deterioration of the lenses and directly to the users' eyes [2,7–9,13,14,17,18]. The colonizing fungi come into contact with the lens surface, stick to it (adhesion) and release enzymes that degrade the polymers (invasion) [13,17]. The resulting assimilable products facilitate the growth of microorganisms. *In vitro* studies of the fungal colonization of contact lenses are relatively scarce. Simitzis-Le Flohic *et al* [12] and de Gioia *et al* [6] indicated that yeasts do not invade the lenses as some filamentous fungi do. Bisignano *et al* [3] noted that to colonize contact lenses, large numbers of *Aspergillus niger* conidia are needed over 15 days of incubation with a variable concentration of peptides and carbohydrates as the culture medium. Therefore, inoculation in saline solution prevents penetration of the mycelium into the lenses. Further studies of this kind that expand on these initial findings and increase our understanding of the conditions under which fungi colonize contact lenses would clearly be valuable. With this in mind, I initiated this study with the aim of determining the *in vitro* colonizing potential of the filamentous fungus *A. niger* towards hydrophilic contact lenses. Specifically, the colonization process was analysed by considering the fungal strain, the type of lens material, the culture medium and the incubation period.

Materials and methods

The following strains of *A. niger* were investigated: CECT 2574, CECT 2700 (equivalent to ATCC 9642), 22 MR-AN, 24 MR-AN and 25 MR-AN. The first two came from the *Colección Española de Cultivos Tipo* and the others from the *Instituto Municipal de Investigaciones Médicas de Barcelona* (Spain). Each strain was cultured on 2% Sabouraud's dextrose agar slants (Bio-Mérieux, Marcy L'Etoile, France) and incubated for 24 h at 25°C. The conidia were then washed and the inocula of the different strains were obtained. The final concentration of the conidia varied between 3.5 and 4.4×10^6 UFC/ml.

Four different hydrophilic polymers were used in the analyses. Each belongs to one of the groups established by the US FDA [19]. Since these polymers are commercially available and the chemical composition is frequently not indicated, the identification of the functional groups was determined in the laboratory. To this end, a lens of each polymer was placed in a desiccator containing anhydrous calcium chloride for 3 days. The samples were then ground and mixed with potassium bromide in a weight ratio of 1:10. Infrared spectra were recorded at room temperature on a FT-IR Perkin-Elmer 2000 instrument. The number of accumulated scans was 64.

Forty lenses were colonized for each fungal strain, lens material, culture medium and incubation period, and the percentages of lenses with fungal adhesion and invasion were calculated. Each lens was placed in a 4.5-cm-diameter glass Petri dish. Each plate contained 8 ml of saline solution or Sabouraud's broth with gentamicin sulfate, together with a known concentration of conidia [5,13]. The lenses were incubated at 25°C and examined on days 3, 7, 14 and 21. In order to avoid the interruption of growth of the fungus and its potential adhesion or invasion, new lenses were examined on each of these days, i.e., they did not come from the previous incubation period.

In order to determine the possible colonization of the matrix of the lenses by *A. niger*, they were observed by means of optical scanning electron microscope (SEM) and confocal scanning laser microscope (CSLM). Optical microscopy allowed the observation of any hyphae adhering to the lens surfaces and the determination, together with a calibrated ocular micrometer, of their densities. Four degrees of adhesion were established: 0 (*no adhesion*); 1 (*low density*), hyphae covered up to 25% of the lens surface; 2 (*medium density*), hyphae covered between 25% and 50% of the lens surface; 3 (*high density*), hyphae covered more than 50% of the lens surface. Subsequently, each lens was washed to remove the adhered hyphae and was observed with an optical microscope to determine the extent of the fungal invasion. The same ranges as those used in the adhesion process were used to quantify the internal colonization.

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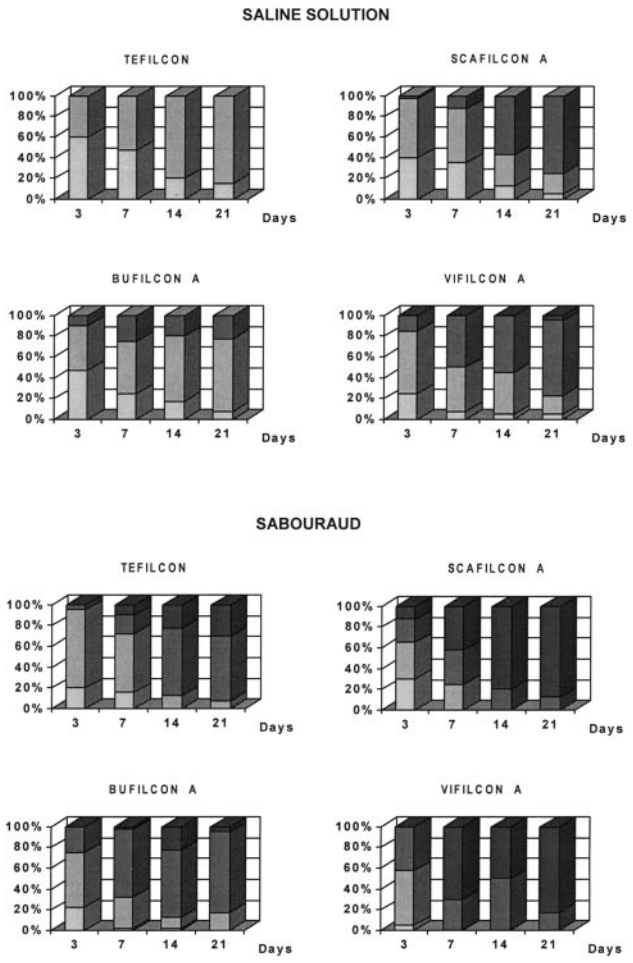

Figure 1 Percentages of adhesion of *A. niger* 2700 to hydrophilic contact lenses according to culture medium, lens material and length of incubation. Different shadings indicate different degrees of adhesion (0, lightest; 3, darkest).

The sizes of all colonies and their internal hyphae were quantified with a calibrated ocular micrometer. Four vifilcon A lenses invaded by *A. niger* were observed by SEM [5,8,12] in order to analyse the deterioration of the lenses. Likewise, four scafilcon A lenses colonized by the fungus were selected for CSLM analysis; they were observed by light transmission with a Leica TCS 4D microscope (Heerbrugg, Switzerland) and a krypton–argon ion laser that emits at 568 nm.

The relationship between the degrees of adhesion or invasion and several parameters (including the type of material, culture medium and period of incubation of the polymers) was calculated from the corresponding absolute frequencies of each degree. The significance of the differences observed in each comparison was tested by means of contingence tables [20]. This analysis was performed using SPSS programs [10].

Cluster analysis (using the square root of the square of the sum of the Euclidean distances as a proximity measure) was used to determine the relationships between the polymers and the frequencies of fungal adhesion or invasion during the periods under consideration for all the lenses, calculating each as the sum of the squares of the differences between the relative frequencies [4]. Phenograms were constructed by the unweighted pair-group method using arithmetic averages (UPGMA) [15]. Cluster analysis was performed using NTSYS-pc routines [11].

Results

The qualitative characterization of the functional groups of tefilcon, bufilcon A and vifilcon A was determined. Although, according to the available information, scafilcon A has no 2-hydroxyethyl methacrylate (HEMA), analysis revealed a high number of hydroxyl groups.

Of the five strains of *A. niger* investigated, only the 2700 strain colonized the contact lenses *in vitro*. Figures 1 and 2 show the percentages of fungal adhesion and invasion, respectively, according to lens material, culture medium and days of incubation.

With respect to both fungal adhesion and invasion, differences among the four hydrophilic contact lens materials were significant for the two media and for all incubation periods ($P<0.001$, except $P<0.05$ in the saline solution culture at 3 days of incubation). Therefore, it can be assumed that the type of polymer influences the degree of adhesion and invasion of the *A. niger* hyphae, regardless of the culture medium and incubation period. The affinities between

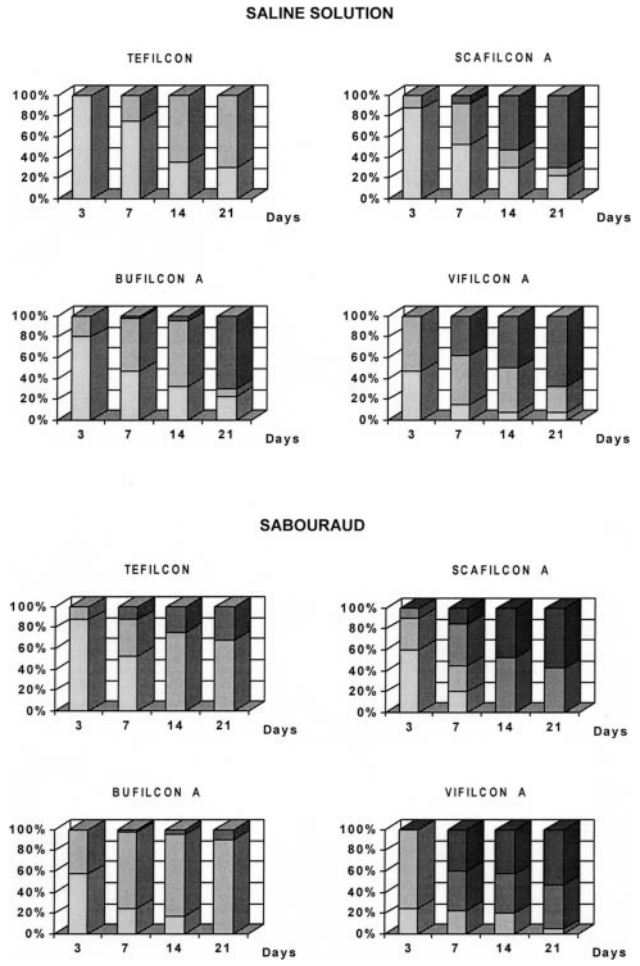

Figure 2 Percentages of invasion of *A. niger* 2700 into hydrophilic contact lenses according to culture medium, lens material and length of incubation. Different shadings indicate different degrees of invasion (0, lightest; 3, darkest).

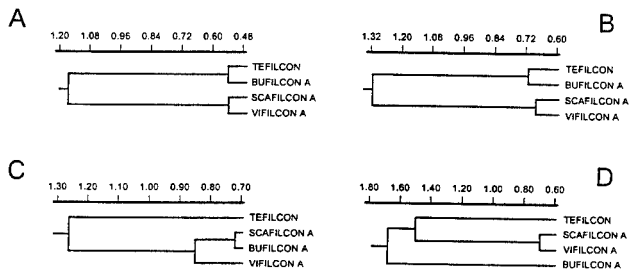

Figure 3 Distance phenogram showing the relationship between hydrophilic contact lens materials and the degrees of adhesion and invasion by *A. niger*. (A) Adhesion in saline solution. (B) Adhesion in Sabouraud's medium. (C) Invasion in saline solution. (D) Invasion in Sabouraud's medium.

the contact lens materials established from the percentages of adhesion and invasion are shown in Figure 3.

With respect to the degree of adhesion and invasion, the statistical comparisons between the two culture media generally showed significant differences regardless of the polymer and period of incubation (adhesion: $P<0.001$, except $P<0.05$ in the culture of bufilcon A at 3 days of incubation and $P<0.01$ in vifilcon A at 3 days; invasion: $P<0.001$, except $P<0.05$ in all polymers at 3 days and tefilcon at 7 days, and no significant differences in bufilcon A at 7 and 14 days). The results indicate that the culture medium influences adhesion to, and invasion of, the contact lenses by the fungus. Both processes are significantly increased in the Sabouraud's medium cultures compared to those in saline solution. Likewise, the percentages of lenses showing adhesion to and invasion of the different materials by the *A. niger* mycelium (Figures 1 and 2) significantly increased during the course of the incubation period, regardless of the type of polymer and culture medium ($P<0.001$ in all comparisons).

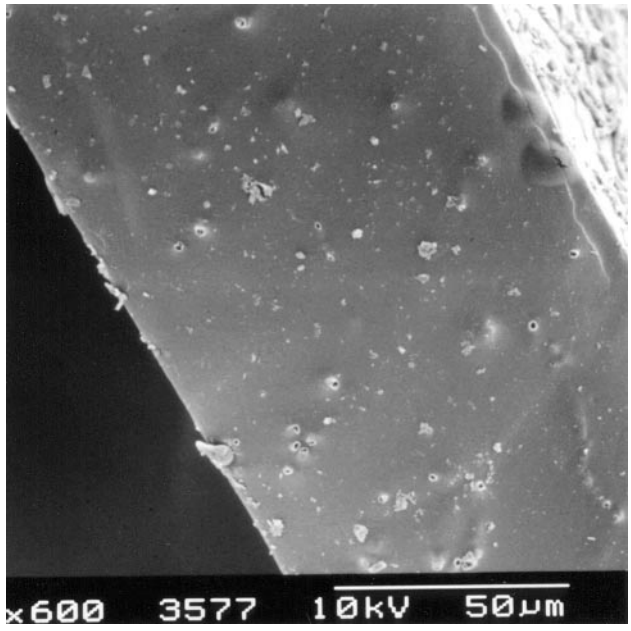

Figure 4 SEM micrograph showing the thickness of a contact lens colonized by *A. niger* 2700 and sectioned inner hyphae.

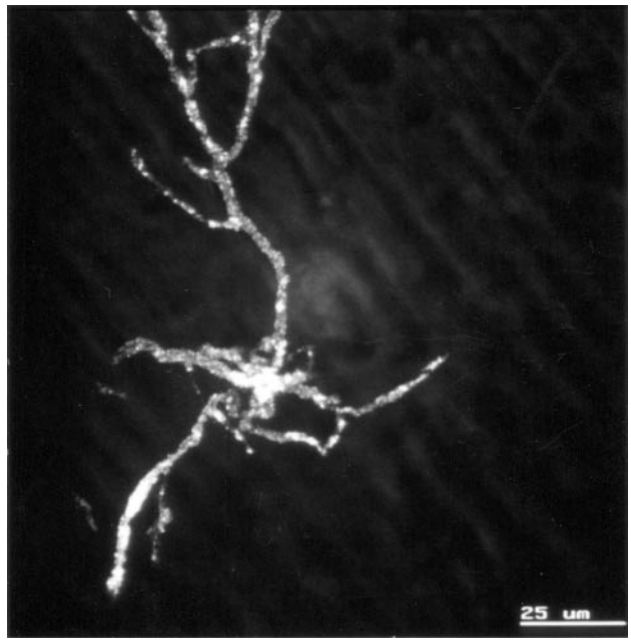

Figure 5 Reconstructed three-dimensional image of an *A. niger* 2700 colony in a scafilcon A lens after 14 days in Sabouraud's medium.

Observation of the lenses using light microscopy indicated that the size of the colonies and their internal hyphae was always greater in Sabouraud's cultures. After 21 days of incubation in this medium, the largest colonies had diameters of 200 μm in the more hydrated polymers and their hyphae had a maximum diameter of 3.5 μm. The colonies in the polymers with lower water content had a maximum diameter of 100 μm and their internal hyphae 3 μm. All hyphae always showed a coiled disposition in the matrix of the polymer [5].

Observation of the lenses by SEM made it possible to determine the deterioration of their surfaces. Internal hyphae were detected in the lens sections (Figure 4). Figure 5 shows a reconstruction of the sections obtained by CSLM into a three-dimensional image of one colony. This image was constructed from 25 optical sections in the horizontal $x-y$ plane, with a 1.2-μm interval between planes. The colony penetrated 28.8 μm into the lens matrix.

Discussion

Only one of the five strains of *A. niger* colonized the contact lens materials analysed. This low incidence was probably related to genetic factors and/or culture conditions since the latter determine the chemical structure of the fungal wall and the synthesis of hydrolytic enzymes responsible for colonization of the lenses. The fungal wall plays a very important role in the process of adhesion to a surface as it is the first point of interaction between the fungal cells and the substrate to be colonized [1]. Glucose causes a high synthesis of manoproteins in the outermost layer of the fungal wall, increasing adhesion [16]. Therefore, the glucose and peptone in Sabouraud's medium provided *A. niger* 2700 with the necessary source of carbon and nitrogen to facilitate colonization of the lenses. My results coincide only partially with those of Batellier *et al* [1], who stated that contamination of the contact lenses requires a nutritive contribution, particularly of peptides and carbohydrates, without which the fungus does not develop.

Since most of the hydrophilic polymers studied here contain *N*-vinylpyrrolidone (NVP), it can be assumed that its presence aids colonization by *A. niger*. Nitrogen present in the pyrrolidone group is arranged lateral to the hydrocarbon chain. The results suggest that the nitrogen in this monomer is easier for the microorganism to use, as it is more vulnerable to enzyme attack than nitrogen in acrylamide diacetone (AAD) present in the bufilcon A amide groups. Likewise, other components (probably the methacrylic acid, MA) must influence fungal colonization of hydrophilic contact lenses. Therefore, lenses made with less hydrophilic (not NVP) and nonionic (not MA) materials, such as tefilcon, yield lower frequencies and degrees of colonization.

According to my observations, if the growing conditions, the chemical composition of the polymer and the type of fungus are all suitable for colonizing a material, invasion will occur within 3 days. These results differ from those published by Bisignano *et al* [3], which indicate that incubation periods between 15 and 20 days are needed for an invasion of HEMA hydrophilic contact lenses with a 38% water content.

In conclusion, this study corroborates the fact that increased polymer water content enhances fungal growth in hydrophilic contact lenses, and it demonstrates that *in vitro* fungal colonization of contact lenses depends on (i) the strain of filamentous fungus, (ii) the culture medium and (iii) the incubation period. Likewise, and contrary to the results obtained by Bisignano *et al* [3], the present study demonstrates that it is possible to colonize polymers using a conidial suspension of *A. niger* in saline solution after short periods of incubation (3 days). Finally, it is worth mentioning that occasionally, fungal colonization of contact lenses has been observed using different species of fungi and different culture media [5,12,13].

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References

1 Batellier L, C Chaumeil and S Liotet. 1992. Champignons et lentilles de contact. *J Mycol Med* 2: 132–139.
2 Berger RO and BW Streeten. 1981. Fungal growth in aphakic soft contact lenses. *Am J Ophthalmol* 91: 630–633.
3 Bisignano G, G Ferreri, CA Squerri and S Ragusa. 1988. Colonisation de *Pseudomonas* et *Aspergillus* sur des lentilles de contacte souples. *Contactologia* 10F: 159–164.
4 Bisquerra R. 1989. Introducción Conceptual al Análisis Multivariante. Vol. 1 Promociones y Publicaciones Universitarias, Barcelona.
5 Buffington JR, RB Simmons, LA Wilson and DG Ahearn. 1988. Effect of polymer differences on fungal penetration of hydrogel lenses. *J Ind Microbiol* 3: 29–32.
6 de Gioia E, MC Ligorio, I di Carlo, O Sobrero, M Lasini and FM Grignolo. 1987. Studi sperimentali sulla contaminazione delle lenti corneali da parte di colonie fongique *Penicillium islandicum*. *Bull Ocul* 66: 305–315.
7 Hurtado I, C Hartung, BL Magran and J Alson. 1995. Identification of fungi growing within soft contact lenses: a report from the neotropics. *CLAO J* 21: 35–36.
8 Jongebloed WL, D Humalda and JFG Worst. 1987. Fungal growth on soft contact lenses: a SEM-study. *Doc Ophthalmol* 67: 119–128.
9 Liotet S and VN Warnet. 1981. Contamination fongique des lentilles molles. *J Fr Ophthalmol* 4: 819–823.
10 Norusis MJ. 1988. SPSS/PC+ Advanced Statistics. SPSS, Chicago, IL.
11 Rohlf FJ. 1994. NTSYS-pc. *Numerical Taxonomy and Multivariate Analysis System Version 1.80 Exeter Software, New York.*
12 Simitzis-Le Flohic AM, P Huguet, J Colin and C Chastel. 1983. Un millieu de culture inattendu pour des champignons microscopiques: les lentilles hydrophiles précornéennes. *J Fr Ophtalmol* 6: 69–74.
13 Simmons RB, JR Buffington, M Ward, LA Wilson and DG Ahearn. 1986. Morphology and ultrastructure of fungi in extended-wear soft contact lenses. *J Clin Microbiol* 24: 21–25.
14 Smolin G, M Okumoto and R Nozik. 1979. The microbial flora in extended wear soft contact lens wearers. *Am J Ophthalmol* 88: 543–547.
15 Sneath PHA and RR Sokal. 1973. Numerical Taxonomy. Freeman, San Francisco.
16 Torres-Rodríguez JM and A Carceller. 1993. Factores de patogenicidad en *Candida*. *Rev Iberoam Mycol* 10: S2–S7.
17 Wilhelmus KR, NM Robinson, RA Font, MB Hamill and DB Jones. 1988. Fungal keratitis in contact lens wearers. *Am J Ophthalmol* 106: 708–714.
18 Wilson LA and DG Ahearn. 1986. Association of fungi with extended-wear soft contact lenses. *Am J Ophthalmol* 101: 434–436.
19 Withe P and C Scott. 1993. Contact lens and solutions summary. *Contact Lens Spectrum* 1–24.
20 Zar JH. 1996. Biostatistical Analysis. Prentice-Hall, Upper Saddle River.