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ORIGINAL PAPER

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Colonization of hydrophilic contact lenses by yeast

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Abstract The growth of six strains of yeast was analyzed in vitro in order to assess their capacity for colonizing (adhesion and invasion) hydrophilic contact lenses. Lenses with different water content were cultured in two culture media for 3, 7, 14, and 21 days. Only strain 93150 of *Candida albicans* could adhere to and invade the polymers. Specifically, fungal growth was observed in cultures with Sabouraud's broth. The degree of yeast colonization of contact lenses was significantly related to the species, the strain, and the culture medium in which the yeast and lenses were cultured. The results here obtained were compared with those reported for the filamentous fungus *Aspergillus niger* 2700. For both microorganisms, the strain and the medium in which the lenses and microorganism were cultured influenced the colonization, but the percentage of colonized lenses, the degree of colonization, and the density and size of the internalized colonies were always noticeably lower for *C. albicans* 93150. Colonization by *A. niger* 2700 was also related to the type of material of the lenses and the incubation period. For both microorganisms, when the strain is right and the growth and development are correct, colonization of hydrophilic contact lenses occurs.

Keywords Hydrophilic contact lenses · Fungal adhesion · Fungal invasion · Colonization · Yeast

Introduction

Fungi are microorganisms that are able to colonize and degrade a great variety of substrates. Specifically, filamentous fungi such as *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Acremonium*, *Cladosporium*, *Scopulariopsis*, and *Paecilomyces*, can invade discarded contact lenses [4, 11–13, 16, 23, 25, 26]. Moreover, yeasts such as *Candida*, *Rhodotorula*, *Torulopsis*, and *Cryptococcus* have also been isolated from contact lenses, although the number of species that can colonize them is lower than that of filamentous fungi [12, 13, 15, 16, 19, 24–26]. Since filamentous fungi and yeasts can produce ocular infections, the colonization of contact lenses by fungi has been studied under an ophthalmological perspective [1, 2, 14, 19, 25, 26]. Other subjects of analysis concerning the fungal colonization of contact lenses have been the determination of the factors involved in the adhesion, invasion, and deterioration of several lens materials [3, 6, 17, 22, 23]. Specifically, the colonization characteristics of *Aspergillus niger* on different contact lens materials cultured in vitro was analyzed to determine the effect of the fungal strain, type of polymer, culture medium, and incubation period [17]. To my knowledge, there is no similar study concerning yeast and there is little and only contradictory information on their adhesion and invasion characteristics on contact lenses. According to Simmons et al. [23], after the adherence of *Candida albicans* to hydrophilic polymers, fungal enzymes were apparently able to degrade them, thus allowing the invasion of the lens matrix. Conversely, Simitzis Le-Flohic et al. [22] indicate that neither *C. albicans* nor *Rhodotorula* were able to colonize hydrophilic materials of contact lenses, since the fungal hyphae were only apparent on the surface of the lenses and did not penetrate their matrix.

The main goal of this study was to determine the effect of several factors on the colonization of four different contact lens materials (tefilcon, scafilcon A, bufilcon A, vifilcon A) by yeast. Specifically, the yeast strain, the types of lens material, the media in which the lenses and yeast were cultured and the number of days of lens incubation were analyzed for their effect on colonization. The results were compared with those previously obtained on the colonization of the same polymers by the filamentous fungus *A. niger* [17].

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Materials and methods

Microorganisms

Strains of *C. albicans* (9200, 93150), *C. tropicalis* (13/2, 92077), and *Torulopsis glabrata* (93189, 93370) were analyzed. All strains came from the Instituto Municipal de Investigaciones Médicas de Barcelona (Spain) and were selected by their great capacity to adhere to intravenous catheters.

Cell growth

Strains were cultured on 2% Sabouraud's agar slants (Bio-Mérieux, Marcy L'Etoile, France) for 24 h at 37°C and subsequently cultured in 2% Sabouraud's broth with agitation for 24 h at 37°C. The cells were recovered by centrifugation and resuspended in saline solution or Sabouraud's broth to obtain a final concentration of 10^6 colony-forming units/ml to use as inocula. In both media, gentamicin sulfate (Bio-Mérieux) was used to avoid bacterial contamination.

Lenses

The following lens polymers were used: tefilcon, scafilcon A, bufilcon A, and vifilcon A. Each of these materials belongs to one of the four groups established by the United States FDA [27]. Tefilcon belongs to FDA group I (nonionic materials, water content lower than 50%), scafilcon A belongs to FDA group II (nonionic materials, water content higher than 50%), bufilcon A belongs to FDA group III (ionic materials, water content lower than 50%), and vifilcon A belongs to FDA group IV (ionic materials, water content higher than 50%). The identification of the functional groups was determined in the laboratory following the procedures indicated in a previous study [17].

Culture conditions

Forty lenses were used for each combination of the following items: fungal strain (*C. albicans* 9200, 93150, *C. tropicalis* 13/2, 92077, *T. glabrata* 93189, 93370), lens material (tefilcon, scafilcon A, bufilcon A, vifilcon A), culture medium in which the lenses and yeast were cultured (saline solution, Sabouraud's broth), and incubation period (3, 7, 14, 21 days). Therefore, 192 combinations (without considering replicates) were analyzed. Each lens was incubated in a Petri dish with inoculum at 37°C. The lenses and the extent of cell growth during the assay were examined on different days, with an optical microscope [17].

Colonization

The possible colonization of lenses by yeast and the morphology of invading colonies were considered. Fungal adhesion to and invasion of the lenses were scored as follows [17]: 0 no adhesion, 1 low density (hyphae/pseudohyphae covering $<25\%$ of the lens surface), 2 medium density (hyphae/pseudohyphae covering 25–50% of the lens surface). Loosely adhered hyphae on the polymers have little importance in the colonization process because the attack by enzymes is more difficult than in closely adhered hyphae. Therefore, each lens was vortexed in sterile saline solution for 1 min to release loosely adhered hyphae from the surface of the polymers and facilitate the subsequent research of potential invading hyphae. For each combination of fungal strain, lens material, culture medium in which the lenses and yeast were cultured, and incubation period, the percentage of lenses with fungal adhesion and invasion (scoring 1 or 2) was determined and the size of the internalizing colonies and hyphae was quantified.

In order to perform a more detailed analysis of the yeast adhesion and the deterioration of the surface of the lenses, two lenses of vifilcon A were randomly selected and observed, using a scanning electron microscope (SEM) [6, 13, 22]. Additionally, two more lenses of vifilcon A were analyzed with a confocal scanning laser microscope (CSLM), in order to observe the morphology of the inner colonies and to quantify their penetration into the matrix.

Statistics

The independence 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 evaluated from the corresponding absolute frequencies of each degree. The significance of the differences observed in each comparison was tested by means of contingence tables. Relationships between the polymers regarding the frequency of fungal adhesion or invasion during the periods considered were determined for all lenses by cluster analyses, using as a proximity measure the square root of the square of the sum of the Euclidean distances, calculating each as the sum of the squares of the differences between the relative frequencies. Phenograms were constructed by the unweighted pair-group method, using arithmetic averages (see [17] for details).

Results and discussion

It is well known that the capacity of fungi to invade substrates depends on, among other factors, the type and amount of enzymes they release and the possibility of developing hyphae [9]. As for this latter factor, it is worth mentioning that only a few yeast species can

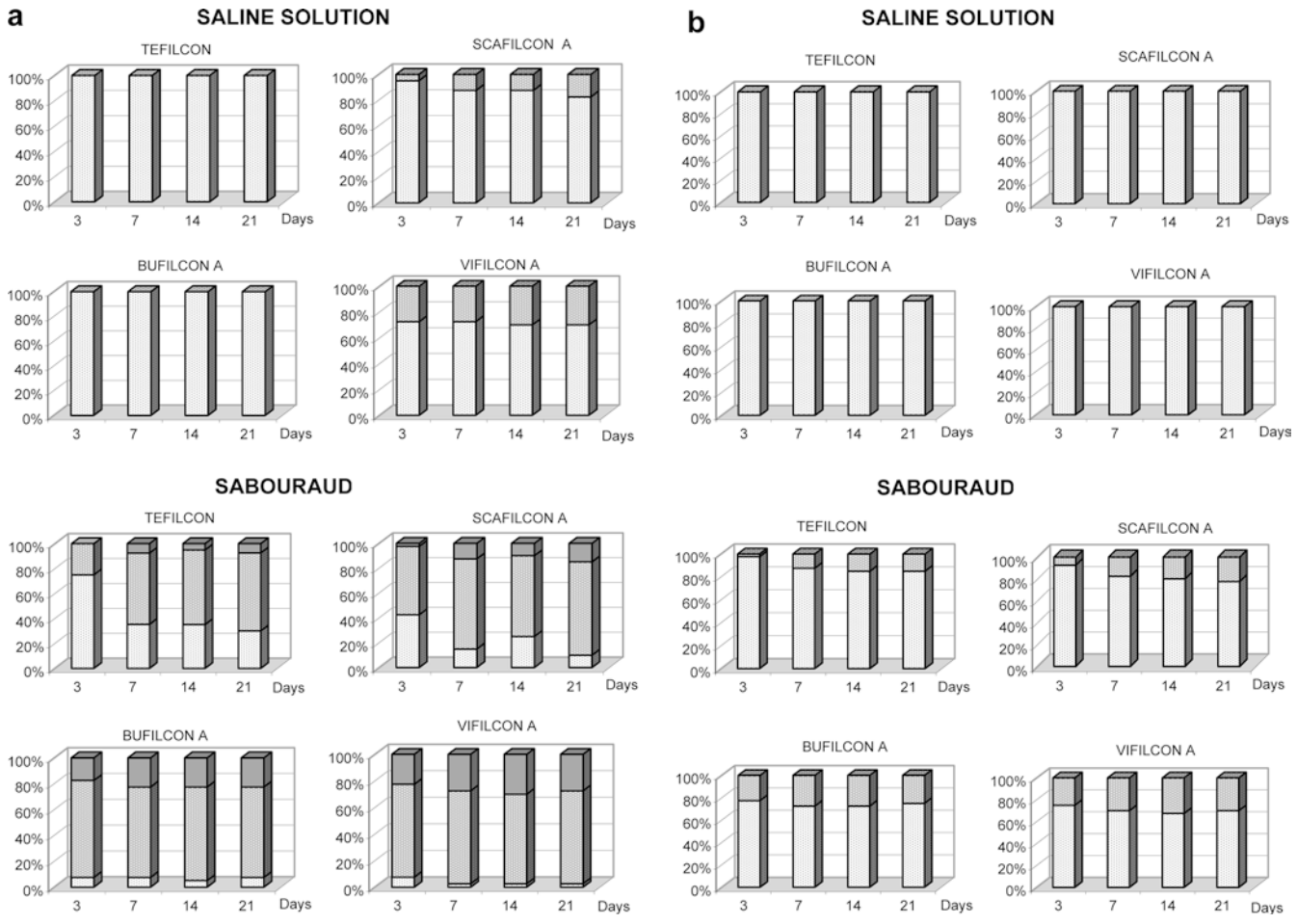

Fig. 1 Comparison of the percentages of adhesion to (**a**) and invasion of (**b**) hydrophilic contact lenses by *C. albicans* 93150, related to culture medium, lens material, and period of incubation. Different degrees of adhesion and invasion are indicated by different *degrees of shading* (*0* lightest, *2* darkest)

produce hyphae and release hydrolytic enzymes to degrade polymers and only in very specific culture conditions [10]. Therefore, the capacity of fungi to colonize substrates and the culture conditions are related to colonization.

The results demonstrate the poor ability of the different yeast strains to colonize the polymers analyzed. *C. albicans* 9200, *C. tropicalis*, and *T. glabrata* were unable to adhere to the lenses and therefore invade them. *C. albicans* 93150 was the only yeast strain that colonized the contact lenses. The percentages of adhesion to and invasion of the lenses by this strain are shown in Fig. 1. The main factor influencing colonization was the medium in which the lenses and yeast were cultured: adhesion (Fig. 1a) and invasion (Fig. 1b) were significantly higher in Sabouraud's medium than in saline solution. Statistical comparisons between the two media showed significant differences in adhesion, regardless of the polymer and period of incubation (Fig. 1a; $P < 0.001$). With respect to the degree of invasion, in general the percentages obtained in both media showed significant differences, irrespective of the material and period of incubation (Fig. 1b; $P < 0.001$ for all periods of incubation, except $P < 0.05$ in the culture of tefilcon, $P < 0.01$ in the culture of scafilcon A). In no cases were invading hyphae found in saline solution cultures. These results indicate that the medium in which the lenses and yeast were cultured influences the degree of adhesion of the yeast to the surface of contact lenses and the subsequent invasion. They also indicate that there is a nutritional stress on the colonization of contact lenses by a specific strain of yeast. Fungal colonization only occurred when glucose and peptone were in the culture medium. Thus, in Sabouraud's medium, yeast synthesized the chemical compounds that were qualitatively and quantitatively required to adhere to the surface of the analyzed polymers. Likewise, Sabouraud's medium allowed the fungus to synthesize and release the hydrolytic enzymes required to invade the lenses. Conversely, none of the yeasts analyzed was able to use the materials of the lenses as a source of carbon and nitrogen when cultured in saline solution. A medium with only sodium and chloride ions was unsuitable to allow the colonization, although some thin hyphae were present in cultures.

Differences in the percentage of adhesion for the four hydrophilic materials were significant in both media and

for all periods of incubation (Fig. 1a; $P < 0.001$). This indicates that the type of polymer influences adhesion by *C. albicans* 93150, regardless of the culture medium and period of incubation. Affinities between the contact lens materials, as indicated by the percentage of adhesion, are shown in Fig. 2. Culturing the lenses in saline solution revealed affinities between the lower hydrated materials, tefilcon and bufilcon A, since no yeast adhesion occurred in either polymer. Culturing the lenses in Sabouraud's broth showed a cluster between two ionic materials, bufilcon A and vifilcon A. In these polymers, the percentage of lenses with a medium density of adhered hyphae/pseudohyphae was similar (Fig. 1a). These results suggest a different influence of the two culture media on the synthesis of chemical compounds in the fungal wall responsible for adhesion to the lenses. Nevertheless, the information available does not allow the exact reasons for this to be determined. In general, statistical comparisons between the percentages of the four materials analyzed did not show significant differences (Fig. 1b). Only differences during the culturing of lenses in Sabouraud's broth at 3 days were significant ($P < 0.01$). This indicates that the type of material did not influence the invasion of the lenses and, consequently, their colonization. Thus, the chemical composition of the four materials was not the limiting factor for the colonization of the lenses. Specifically, neither the percentage of methacrylic acid in the materials (which determines their ionicity) nor the percentage of *N*-vinylpyrrolidone (which conditions their water content) affected the yeast's colonization of the lenses.

The percentage of adhesion of *C. albicans 93150* to the lenses in saline solution did not increase significantly with incubation period (Fig. 1a). In Sabouraud's broth and only in cultures of nonionic polymers, tefilcon ($P < 0.01$) and scafilcon A ($P < 0.05$) significantly enhanced the frequency of adhesion during the incubation period, specifically between day 3 and day 7 of incubation. Consequently, no significant differences were found between the degree of invasion of the lenses and the incubation time (Fig. 1b). When the strain of yeast and its growing conditions were suitable for colonizing a specific material, invasion occurred within a short period of incubation (3 days at the most). Nevertheless, the colonization did not progress significantly over the following days of incubation. It is likely that the yeast's invasion of the lenses was hindered by nutritional stress caused by the diminution of nutrients during the incubation period and the accumulation of cellular metabolic compounds that modified the original good culture conditions. No specific information concerning the minimum incubation periods needed for colonizing contact lenses by yeast was found in the literature. This parameter has only been determined in cultures with filamentous fungi [5, 17].

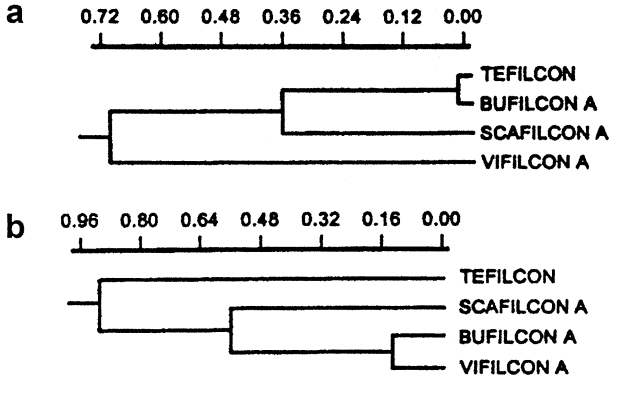

Fig. 2a, b Relationship between hydrophilic contact lens materials and the degree of adhesion by *C. albicans* 93150. **a** Adhesion in saline solution. **b** Adhesion in Sabouraud's broth

Adhesion to and invasion of the contact lenses was distinguished using an optical microscope. When adhesion occurred, several rectilineal hyphae on the surface of the materials were observed in the same focal plane. In the invaded lenses, a few light coiled invasive hyphae were detected; and these hyphae were always observed in different focal planes. Coiled hyphae were lacking when adhesion but not invasion occurred. The size of the invasive colonies and the size of their internal hyphae varied according the type of material. Both characters were always greater in the most hydrated polymers (scafilcon A, vifilcon A). These results agree with those obtained with filamentous fungi [3, 17, 23]. In the more hydrated materials, after 21 days of incubation in Sabouraud's medium, the largest colonies showed a maximum diameter of 30 μm and the internal hyphae showed a maximum width of 2.0 μm. The colonies that grew in lenses with lower water content (tefilcon, bufilcon A) showed a maximum diameter of 20 μm. Internal hyphae in these lenses showed a maximum width of 1.5 μm. Observation of the lenses by SEM determined the deterioration of their surfaces and the adhesion of the yeast cells. Figure 3 shows nine optical sections taken at 0.79-μm intervals of an invasive colony obtained by CSLM. This colony, one of the largest observed, penetrated the lens to a depth of 6.32 μm.

Comparisons between the colonization of hydrophilic contact lenses by *C. albicans* and *A. niger* [17] revealed that, for both species, the strain and the medium in which the lenses and microorganism were cultured influenced the colonization. Likewise, the percentage of colonized lenses and the degree of colonization were higher in Sabouraud's culture medium. Furthermore, the largest invasive colonies were present in polymers with a high water content. Conversely, there were several differences in the colonization of the polymers between the yeast and the filamentous fungus. Thus, the percentage of cell adhesion and invasion of lenses and the density and size of the inner colonies were always noticeably lower in *C. albicans* 93150. Furthermore, the type of lens material and the incubation period influenced only the colonization by *A. niger*. Specifically, the high water content of the lenses of scafilcon A and vifilcon A aided the colonization of *A. niger* in cultures with Sabouraud's broth. This did not occur in the

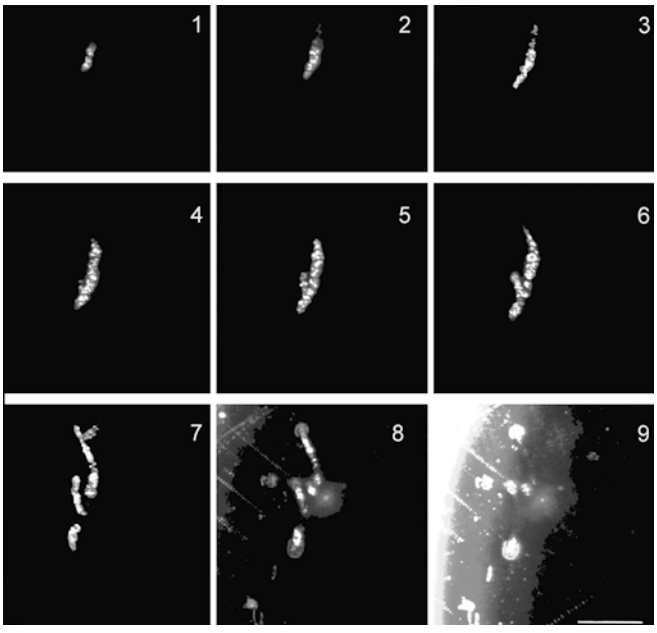

Fig. 3 Serial CSLM optical sections at different levels of an internal colony of *C. albicans* 93150. *Image 1* innermost zone of the colony, *image 9* surface of lens. Bar 25 μm

cultures of *C. albicans* 93150. The results on yeast colonization differed from those reported in the literature, which indicate that, in general, fungi have more ability to adhere and invade hydrophilic lenses with a high water content [3, 25]. This is probably only true for filamentous fungi, since they show a greater invasion capacity and have been more widely analyzed than yeast.

There are some previous studies in the literature about the adhesion of bacteria and *Acanthamoeba* on contact lenses. As for bacteria, generally, nonionic polymers demonstrate higher attachment numbers with *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* at both low and high hydration levels, in comparison with ionic polymers. Adhesion decreases with increasing water content, but there is no strict correlation between adhesion and the percentage of hydration. These data suggest that materials composition and surface hydrophilicity can mask the effects of hydrogel water content [7, 8, 18]. Conversely, results on the adhesion of *Acanthamoeba* to unworn low and high water hydrophilic contact lenses are contradictory. Ramachandran et al. [21] indicated that the adhesion of *Acanthamoeba* cysts and trophozoites was greater with high water content lenses. It is not clear whether the type of lens polymer, water content or the charge on the lenses has any role in the adherence of *Acanthamoeba*. Results here obtained on *C. albicans* adhesion to contact lenses agree with those reported for *Acanthamoeba* [21], since the effect of surface hydrophilicity seems to be, in these microorganisms, less important than in *Pseudomonas* and *Staphylococcus*. Nevertheless, a more recent study to determine the extent of adhesion of cysts and trophozoites of *Acanthamoeba* to hydrophilic contact lenses [20] concluded that the microorganism adhered with equal affinity to different unworn lens materials. These contradictory results clearly reveal the need for further detailed studies that determine the factors involved in the adhesion and invasion processes of the different microorganisms on contact lenses.

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