Biofilm Formation by *Candida* Species on the Surface of Catheter Materials In Vitro

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A model system for studying *Candida* biofilms growing on the surface of small discs of catheter material is described. Biofilm formation was determined quantitatively by a colorimetric assay involving reduction of a tetrazolium salt or by *[^3]H*leucine incorporation; both methods gave excellent correlation with biofilm dry weight (r = 0.997 and 0.945, respectively). Growth of *Candida albicans* biofilms in medium containing 500 mM galactose or 50 mM glucose reached a maximum after 48 h and then declined; however, the cell yield was lower in low-glucose medium. Comparison of biofilm formation by 15 different isolates of *C. albicans* failed to reveal any correlation with pathogenicity within this group, but there was some correlation with pathogenicity when different *Candida* species were tested. Isolates of *C. parapsilosis* (Glasgow), *C. pseudotropicalis*, and *C. glabrata* all gave significantly less biofilm growth (P < 0.001) than the more pathogenic *C. albicans*. Evaluation of various catheter materials showed that biofilm formation by *C. albicans* was slightly increased on latex or silicone elastomer (P < 0.05), compared with polyvinyl chloride, but substantially decreased on polyurethane or 100% silicone (P < 0.001). Scanning electron microscopy demonstrated that after 48 h, *C. albicans* biofilms consisted of a dense network of yeasts, germ tubes, pseudohyphae, and hyphae: extracellular polymeric material was visible on the surfaces of some of these morphological forms. Our model system is a simple and convenient method for studying *Candida* biofilms and could be used for testing the efficacy of antifungal agents against biofilm cells.

In recent years, the use of medical implants such as catheters, pacemakers, prosthetic heart valves, and joint replacements has increased dramatically. These devices can become colonized by microorganisms which form a biofilm consisting of a mono- or multilayer of cells embedded within a matrix of extracellular polymeric material (3, 4, 9, 11, 14, 15, 22). Release of microorganisms from the biofilm may initiate an acute disseminated infection. Implant-associated infections are difficult to resolve, because biofilm microorganisms are resistant both to host defense mechanisms and antibiotic therapy (1, 4, 15). Frequently, the implant must be removed.

Although the majority of implant infections are caused by gram-positive bacteria, notably staphylococci, infections due to gram-negative bacteria and fungi tend to be more serious (9). Fungal infections are most commonly caused by the pathogenic *Candida* species, particularly *C. albicans*, *C. tropicalis*, and *C. parapsilosis* (5). These organisms are regarded as increasingly important nosocomial pathogens. A survey carried out between 1983 and 1986 established that *Candida* bloodstream infections represented 10% of all nosocomial bloodstream infections recorded in a university hospital (31). The attributable mortality rate was 38%. The majority of nosocomial septicemias caused by *Candida* species derive from intravascular catheters; more than 40% of all nosocomial septicemias occurring in intensive care unit patients are infusion related (14, 18). Urinary catheters, prosthetic heart valves, cardiac pacemakers, silicone voice prostheses, endotracheal tubes, and cerebrospinal fluid shunts are also strongly associated with *Candida* infections (25).

Bacterial biofilms have received increasing attention over the last decade (3, 22), and a number of model systems have been devised for studying the colonization of various solid surfaces by bacteria. These include the use of agar surfaces (24), submerged test pieces (8), the tubular Robbins device (21), and perfused biofilm fermenters (13). Investigations with pathogenic bacteria have shown that biofilm bacteria in vitro have a substantially reduced sensitivity to clinically important antibiotics compared with cells of the same organism in the dispersed form (4). With the aminoglycoside antibiotic tetracycline, for example, sensitivities of biofilm bacteria (strains of *Pseudomonas aeruginosa*) were decreased at least 1,000-fold (24). There is now direct evidence that bacterial growth rate, nutrient limitation, and reduced drug penetration into biofilms all play a role in this resistance to antibiotics (1).

Formation of a biofilm on an implant surface depends initially on the ability of the microorganism to adhere. Adhesion of pathogenic *Candida* species to a variety of host surfaces, including inert materials, has received considerable attention (2, 10). However, there have been no reports to date of studies with *Candida* biofilms. In this investigation, we have devised a simple model system for *Candida* species by adapting the method described by Prosser et al. (27), previously used for *Escherichia coli* and other gram-negative bacteria. This involved forming biofilms on the surfaces of small discs cut from different types of catheters. Growth of the biofilms was monitored quantitatively by using dry weight, colorimetric, and radioisotope assays and was visualized by scanning electron microscopy (SEM).

**MATERIALS AND METHODS**

**Organisms.** Fifteen isolates of *C. albicans* were used in this study. The origin of six (GRI 681, GRI 682, GDH 2346, GDH 2023, NCPF 3153, and Outbreak) is described elsewhere (29). Isolates GRI 272, GRI 922, and GRI 2773 were obtained from women with vaginal candidiasis at Glasgow Royal Infirmary; isolates AAHB 59, AAHB 72, and AAHB 92 were obtained...
from leukemic patients with line infections or patients infected with aortic grafts at Crosshouse Hospital, Kilmarnock, Scotland. C. albicans 1001 (26) and two mutants derived from it unable to form yeasts (strain 1001-FR) or hyphae (strain 1001-92') were kindly supplied by C. Gil, Universidade Complutense, Madrid, Spain.

C. parapsilosis AAHB 4479, C. tropicalis AAHB 73, and C. glabrata AAHB 12 were isolated from patients with line infections at Crosshouse Hospital. C. tropicalis (London strain), C. krusei (Glasgow strain), C. parapsilosis (Glasgow strain), and C. pseudotropicalis (Glasgow strain) were from clinical specimens and came from the Regional Mycology Reference Laboratory, Glasgow, Scotland. All strains were maintained on slopes of Sabouraud dextrose agar (Difco) and subcultured monthly. Every 2 months, cultures were replaced by new ones freshly grown from freeze-dried stocks.

Medium and culture conditions. All strains were grown in yeast nitrogen base medium (Difco) containing either 50 mM glucose or 500 mM galactose as described previously (19). Batches of medium (20 ml, in 100-ml Erlenmeyers flasks) were inoculated from fresh culture slopes and incubated at 37°C in an orbital shaker at 60 rpm. All strains and species, with the exception of the C. tropicalis (London isolate), grew exclusively in the budding-yeast phase under these conditions. In contrast, the C. tropicalis (London isolate) grew predominantly in the pseudohyphal phase. Cells were harvested after 24 h and washed twice with 0.5 M phosphate-buffered saline (PBS) pH 7.2. Before use in biofilm experiments, all washed cell suspensions were adjusted to an optical density of 0.8 at 520 nm.

Biofilm formation. Discs of catheter material (surface area, 0.5 cm²) were cut from catheters, sterilized with ethylene oxide (Caledonian Medical Ltd., Glasgow, United Kingdom) and placed in wells of 24-well Nunclon tissue culture plates. Standardized cell suspension (80 µl) was applied to the surface of each disc, and the discs were incubated for 1 h at 37°C (adhesion period). Nonadherent organisms were removed by gentle washing with 0.15 M PBS (5 ml), and the discs were then incubated for up to 72 h at 37°C, submerged in 1 ml of yeast nitrogen base medium containing either 50 mM glucose or 500 mM galactose (biofilm formation). In control experiments, discs without organisms were incubated in medium containing either 50 mM glucose or 500 mM galactose. All biofilm and control assays were carried out three times in triplicate.

Quantitative measurement of biofilm growth. (i) Determination of dry weights. After biofilm formation, the discs were removed from the medium and gently washed with 0.15 M PBS (5 ml) to remove nonbiofilm cells. Biofilm organisms were scraped from the discs with a sterile scalpel and vortexed gently for 3 min. The discs were washed in 0.15 M PBS (5 ml) to remove any remaining cells. All organisms were collected on preweighed cellulose nitrate filters (0.45-µm pore size; 25-mm diameter) and given three washes with water (5 ml). The filters were dried to constant weight at 80°C, and the dry weights of cells per disc were calculated. Dry-weights were determined three times in triplicate.

(ii) Incorporation of [3H]leucine. The method used for measuring [3H]leucine incorporation was a modification of one used for labelling biofilms of E. coli (8). After biofilm formation, l-[3H]leucine in PBS (final concentration, 1 µCi/ml; 120 Ci/mmol; Amersham) was added to each well, and the plates were incubated for a further 4 h at 37°C. Separate experiments showed that the uptake of radioactive label by biofilm cells was linear during this time. After labelling, organisms were removed from the discs by incubating the discs in wells containing 0.5 ml of 5% (wt/vol) sodium dodecyl sulfate (SDS) for 3 h at 37°C. The SDS solution with the cells was transferred to tubes containing 0.5 ml of 10% (wt/vol) trichloroacetic acid supplemented with 1% (wt/vol) Casamino Acids (Difco). Each trichloroacetic acid precipitate was filtered through a prewetted cellulose nitrate filter (0.45-µm pore size; 25-mm diameter). The filters were washed three times with 5 ml of 5% (wt/vol) trichloroacetic acid containing 1% (wt/vol) Casamino Acids and were dried, and radioactivity was counted in 4 ml of Opti-Fluor O scintillant (Packard). Without cells were used to determine background counts. All [3H]leucine incorporation assays were carried out three times in triplicate.

(iii) Tetrazolium reduction assay. The tetrazolium salt 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma) was used in an assay modified from that described by Mosmann (23). After biofilm formation, 50 µl of MTT solution (a stock solution containing 5 mg of MTT per ml of PBS, diluted 1:5 in prewarmed 0.15 M PBS prior to application) was added to each well. After incubation for 5 h at 37°C, the medium plus MTT was removed, and the wells were washed three times with 0.15 M PBS (2 ml) to remove all traces of MTT. Dimethyl sulfoxide (1 ml) was then added to solubilize the MTT formazan product. MTT formazan formation was measured at 540 nm by using a Shimadzu spectrophotometer. Control wells contained medium plus MTT to determine background formazan values. All assays were carried out three times in triplicate.

Biofilms of C. albicans GDH 2346 formed on polyvinyl chloride (PVC) catheter discs were fixed with 2.5% (vol/vol) glutaraldehyde in 0.15 M PBS for 1 h at room temperature. They were then treated with 1% (wt/vol) osmium tetroxide for 1 h, washed three times with distilled water (3 ml), treated with 1% (wt/vol) uranyl acetate for 1 h, and washed again with distilled water (3 ml). The samples were dehydrated with a series of ethanol solutions which ranged, in 10% increments, from 30% (vol/vol) ethanol in distilled water to 100% ethanol. All samples were dried to critical point by using a Polaron critical point drier, coated with gold with a Polaron coater, and viewed under a Philips 500 scanning electron microscope.

Catheter materials. Different types of catheter were kindly donated by a number of manufacturers. Latex urinary Foley catheters and PVC central venous catheters were from Rüscher UK Ltd., High Wycombe, United Kingdom; silicone elastomer-coated latex urinary Foley catheters were provided by Simpia Plastics Ltd., Cardiff, United Kingdom; and silicone urinary Foley catheters and polyurethane central venous catheters were from Vygon (UK) Ltd., Cirencester, United Kingdom.

RESULTS

Measurement of biofilm growth. Initially, biofilm growth was monitored by using a viable count procedure first described for bacterial biofilms (27). However, this method failed to give reproducible results when it was applied to C. albicans (data not shown). Two alternative assays were therefore used; they involved (i) measuring the uptake of [3H]leucine by biofilm cells, and (ii) determining the ability of the cells to reduce a tetrazolium salt, MTT. Both assays appeared satisfactory, since there was a strong correlation between biofilm dry weight and either [3H]leucine incorporation (r = 0.945) or MTT formazan formation (r = 0.997) (Fig. 1). By either assay method, growth of biofilms of C. albicans GDH 2346 in galactose-containing medium reached a maximum after 48 h. Thereafter, cell numbers appeared to decline (Fig. 1).

Comparison of galactose-grown and glucose-grown biofilms.
Adhesion of C. albicans GDH 2346 to inert surfaces is significantly promoted by growth in medium containing a high concentration of galactose (19). Biofilm formation by this strain in high-galactose medium was therefore compared with biofilm formation in a low-glucose (50 mM) medium which does not produce enhanced yeast adhesion. The pattern of biofilm growth in glucose medium (Fig. 2) closely resembled that observed in galactose medium (Fig. 1), reaching a maximum after 48 h and then declining. There was again a close correlation between biofilm dry weight and either [3H]leucine incorporation (r = 0.965) or MTT formazan formation (r = 0.995) (Fig. 2), depending on the assay used. However, maximum values (after 48 h) for all three parameters were significantly lower (P < 0.001) than the corresponding values for galactose-grown biofilms (Fig. 1 and 2).

**Biofilm formation by different C. albicans isolates**. A total of 15 isolates of C. albicans were assayed for biofilm formation after incubation for 48 h in medium containing either 500 mM galactose or 50 mM glucose (Table 1). These test organisms consisted of isolates from active infections, including line infections, carrier strains, and mutant strains. There was a strong overall correlation between biofilm dry weight and [3H]leucine incorporation (r = 0.930) or MTT formazan formation (r = 0.965). However, several isolates produced results which were significantly different from those obtained with C. albicans GDH 2346. Paradoxically, some recent isolates from implant infections appeared to form less extensive biofilms in this system. For example, isolate AAHB 92 incubated in galactose medium gave significantly lower values (P < 0.001) for dry weight, [3H]leucine incorporation, and MTT formazan formation, as did isolate AAHB 72 when grown in either galactose or glucose medium (Table 1). On the other hand, biofilm formation by two carrier strains (GRI 681 and GRI 682) was similar to that of strain GDH 2346 when it was assessed by any of the three parameters. Interestingly, a mutant of C. albicans 1001 unable to grow in the hyphal phase (strain 1001-92') appeared to have a decreased capacity for biofilm formation, whereas a related mutant unable to grow in

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**TABLE 1. Quantitative measurement of 48-h biofilms of C. albicans isolates grown on the surfaces of PVC catheter discs**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Dry wt (mg)</th>
<th>[3H]Leucine incorporation (cpm)</th>
<th>MTT formazan formation (A540)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GLC⁺</td>
<td>GAL⁺</td>
<td>GLC⁺</td>
</tr>
<tr>
<td>GDH 2346</td>
<td>1.04</td>
<td>1.96</td>
<td>5.640</td>
</tr>
<tr>
<td>GDH 2023</td>
<td>1.00</td>
<td>1.86</td>
<td>5.140</td>
</tr>
<tr>
<td>GRI 681</td>
<td>1.10</td>
<td>1.89</td>
<td>5.885</td>
</tr>
<tr>
<td>GRI 682</td>
<td>0.96</td>
<td>1.93</td>
<td>5.675</td>
</tr>
<tr>
<td>GRI 272</td>
<td>0.72</td>
<td>1.88</td>
<td>3.696</td>
</tr>
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<td>GRI 922</td>
<td>0.74</td>
<td>1.92</td>
<td>3.775</td>
</tr>
<tr>
<td>GRI 2773</td>
<td>0.52*</td>
<td>1.82</td>
<td>3.900</td>
</tr>
<tr>
<td>AAHB 59</td>
<td>0.63</td>
<td>1.48</td>
<td>4.130</td>
</tr>
<tr>
<td>AAHB 72</td>
<td>0.44*</td>
<td>0.51*</td>
<td>2.550*</td>
</tr>
<tr>
<td>AAHB 92</td>
<td>0.75</td>
<td>0.86*</td>
<td>3.445</td>
</tr>
<tr>
<td>NCPF 3153</td>
<td>0.96</td>
<td>1.99</td>
<td>5.447</td>
</tr>
<tr>
<td>Outbreak</td>
<td>0.87</td>
<td>1.68</td>
<td>3.765</td>
</tr>
<tr>
<td>1001</td>
<td>0.89</td>
<td>1.91</td>
<td>5.152</td>
</tr>
<tr>
<td>1001-92'</td>
<td>0.54*</td>
<td>0.62*</td>
<td>3.007</td>
</tr>
<tr>
<td>1001-FR</td>
<td>1.25</td>
<td>1.96</td>
<td>5.205</td>
</tr>
</tbody>
</table>

* Each value is the mean of three independent experiments carried out in triplicate.

⁺ Biofilms grown in medium containing 50 mM glucose.

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FIG. 1. Biofilm formation by C. albicans GDH 2346 growing on PVC catheter discs in medium containing 500 mM galactose. Assays consisted of measurements of dry weights of biofilms (○) and [3H]leucine incorporation (●) (a) and of dry weights of biofilms (瞑) and MTT formazan formation (■) (b). Data are means ± standard errors of three independent experiments done in triplicate. Error bars are omitted when standard errors of the mean do not exceed the dimensions of the symbols.

FIG. 2. Biofilm formation by C. albicans GDH 2346 growing on PVC catheter discs in medium containing 50 mM glucose. Assays consisted of measurements of dry weights of biofilms (○) and [3H]leucine incorporation (●) (a) and of dry weights of biofilms (瞑) and MTT formazan formation ( ■) (b). Data are means ± standard errors of three independent experiments done in triplicate.
the yeast phase (1001-FR) gave values for biofilm formation similar to those of the wild type (Table 1).

For glucose-grown biofilms, the correlation between dry weight and [3H]leucine incorporation (r = 0.825) or MTT formazan formation (r = 0.800) was less good than that for galactose-grown biofilms. This corresponded with a greater amount of variation in biofilm formation within the 15 strains tested. Typically, glucose-grown biofilms gave values for dry weight, [3H]leucine incorporation, and MTT formazan formation which were 35 to 55% lower (P < 0.001) than those recorded for galactose-grown biofilms (Table 1).

**Biofilm formation by different Candida species.** Biofilm formation by five other Candida species was compared with that of *C. albicans* (Table 2). With galactose-grown cells, the correlation between biofilm dry weight and [3H]leucine incorporation (r = 0.845) or MTT formazan formation (r = 0.815) was lower than that observed for galactose-grown strains of *C. albicans*. This finding presumably reflects the greater physiological differences between the species. Isolates of *C. parapsilosis* (Glasgow, *C. pseudotropicalis*, and *C. glabrata* showed significantly less biofilm growth (P < 0.001) in medium containing either galactose or glucose than did *C. albicans* GDH 2346 (Table 2). However, *C. krusei* which, like these species, is also considered less pathogenic than *C. albicans*, exhibited good biofilm growth.

For glucose-grown Candida species, the correlation between biofilm dry weight and [3H]leucine incorporation (r = 0.920) or MTT formazan formation (r = 0.910) was greater than that obtained with galactose-grown cells. Glucose-grown biofilms of *C. glabrata* AAHB 12 gave increased values (P < 0.001) for dry weight, [3H]leucine incorporation, and MTT formazan formation compared with galactose-grown biofilms of the same organism (Table 2).

**Biofilm formation by *C. albicans* on different catheter materials.** Biofilm formation by *C. albicans* GDH 2346 on discs prepared from a range of different catheter materials was examined (Table 3). Almost identical results were obtained with PVC catheters supplied by two separate manufacturers. By comparison with biofilm formation on PVC, biofilm formation on latex was slightly increased (P < 0.05), whereas that on polyurethane was substantially decreased (P < 0.001). Two types of silicone catheter material produced different results; discs prepared from silicone elastomer catheters (which have a latex core) showed slightly increased biofilm formation (P < 0.05) compared with PVC discs, while discs from 100% silicone catheters were more resistant (P < 0.001) to fungal colonisation (Table 3). SEM has revealed that the surface topographies of these two types of silicone catheter are quite distinct (results not shown).

**SEM. Biofilm formation by *C. albicans* GDH 2346 on PVC catheter discs was monitored by SEM. Replicate discs were removed from galactose medium for processing at intervals of up to 48 h. After 1 h of incubation, the cell population attached to the discs consisted exclusively of cells in the budding-yeast phase of growth (Fig. 3). However, after 3 to 6 h, some of the cells had produced germ tubes, and by 18 to 24 h, the population had increased and consisted of a mixture of yeasts, germ tubes, and young hyphae (Fig. 3). The biofilms had matured by 48 h and comprised a dense network of yeasts, germ tubes, pseudohyphae, and hyphae. Extracellular polymeric material (indicated by the arrows in Fig. 3) was visible on the surfaces of some of these morphological forms.

**DISCUSSION**

Although a number of experimental model systems have been designed to study bacterial biofilms, this is the first report of a biofilm system for pathogenic fungi. We have developed two assays for biofilm growth of *Candida* species on the surfaces of small discs of catheter material. In the first assay, which was adapted from a procedure employed originally with *E. coli* (8), preformed *Candida* biofilms are labelled with radioactive leucine. The second assay depends on the conversion of the yellow tetrazolium salt MTT to a dark-blue product, MTT formazan, as a result of dehydrogenase activity by biofilm cells. This reaction has been used previously to measure the proliferation of various mammalian cell types (23) and to determine the viability of some microorganisms, including fungal species such as *C. albicans* (17). Both assay methods showed an excellent correlation between biofilm dry weight and the parameter being measured, and both are rapid and convenient to carry out.

Growth of biofilms of *C. albicans* GDH 2346 in high-galactose medium reached a maximum after 48 h and then declined, possibly because of depletion of oxygen or nutrients within the biofilm. The growth pattern in low-glucose medium

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### TABLE 2. Quantitative measurement of 48-h biofilms of *Candida* species grown on the surfaces of PVC catheter discs*

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Dry wt (mg)</th>
<th>[3H]leucine incorporation (cpm)</th>
<th>MTT formazan formation (A&lt;sub&gt;490&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GLC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>GAL&lt;sup&gt;a&lt;/sup&gt;</td>
<td>GLC&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>C. albicans</em> (GDH 2346)</td>
<td>1.04</td>
<td>1.96</td>
<td>5.64</td>
</tr>
<tr>
<td><em>C. parapsilosis</em> (AAHB 4479)</td>
<td>0.74</td>
<td>1.35</td>
<td>5.425</td>
</tr>
<tr>
<td><em>C. parapsilosis</em> (Glasgow)</td>
<td>0.41&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.66&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.865</td>
</tr>
<tr>
<td><em>C. tropicalis</em> (AAHB 73)</td>
<td>1.10</td>
<td>2.05</td>
<td>6.990</td>
</tr>
<tr>
<td><em>C. tropicalis</em> (London)</td>
<td>0.85</td>
<td>1.74</td>
<td>4.790</td>
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<tr>
<td><em>C. pseudotropicalis</em> (Glasgow)</td>
<td>0.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.89&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.45&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td><em>C. krusei</em> (Glasgow)</td>
<td>1.30</td>
<td>2.17</td>
<td>6.630</td>
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<td><em>C. glabrata</em> (AAHB 12)</td>
<td>0.54&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.35&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.332</td>
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</table>

* Each value is the mean of three independent experiments carried out in triplicate.

### TABLE 3. Effect of different catheter materials on the formation of biofilms by *C. albicans* GDH 2346<sup>d</sup>

<table>
<thead>
<tr>
<th>Catheter material</th>
<th>Dry wt (mg)</th>
<th>[3H]leucine incorporation (cpm)</th>
<th>MTT formazan formation (A&lt;sub&gt;490&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GLC&lt;sup&gt;d&lt;/sup&gt;</td>
<td>GAL&lt;sup&gt;d&lt;/sup&gt;</td>
<td>GLC&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>PVC (Rüsch)</td>
<td>1.04</td>
<td>1.96</td>
<td>5.640</td>
</tr>
<tr>
<td>PVC (VYGON)</td>
<td>1.06</td>
<td>2.05</td>
<td>4.995</td>
</tr>
<tr>
<td>Latex (Rüsch)</td>
<td>1.22&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.36&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.160&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>Polyurethane (VYGON)</td>
<td>0.72&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.53&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.550&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Silicone elastomer (Simpla)</td>
<td>1.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.050&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Silicone (VYGON)</td>
<td>0.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.52&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.488&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>d</sup> Each value is the mean of three independent experiments carried out in triplicate.

<sup>c</sup> Biofilms grown in medium containing 50 mM glucose.

<sup>b</sup> Biofilms grown in medium containing 500 mM galactose.

<sup>a</sup> Value significantly different at P < 0.05 from that obtained with PVC (Rüsch) material.

<sup>d</sup> Value significantly different at P < 0.001 from that obtained with PVC (Rüsch) material.
FIG. 3. Scanning electron micrographs of biofilm formation by *C. albicans* GDH 2346 on PVC catheter discs in medium containing 500 mM galactose at 1 (A), 3 (B), 6 (C), 18 (D), 24 (E), and 48 (F) h. Arrows indicate the presence of extracellular polymeric material. Bar, 10 μm.

was similar, although the extent of biofilm formation was considerably decreased. This result was not unexpected, since the overall cell yield of planktonic organisms is also lower in low-glucose medium. However, by analogy with bacterial systems, biofilm formation will depend on the ability of the yeasts initially to attach to the solid surface and on the production of extracellular polymeric material in which the cells become embedded. In *C. albicans* GDH 2346, both of these properties are affected by the nature and concentration of the available carbon source. Growth in high-galactose medium promotes the synthesis of a fibrillar mannoprotein adhesin (10, 20), resulting in increased yeast adhesion to epithelial cells and inert surfaces such as acrylic (19). Prolonged incubation in this medium causes release of the fibrils, and extracellular polymeric material can be isolated from culture supernatant fluid (20). Presumably, this extracellular material could function as a matrix...
substance in biofilms. Our scanning electron micrographs provide some evidence for such a matrix material, although it was not detected in abundant quantities (Fig. 3).

Comparison of biofilm formation by 15 different isolates of C. albicans failed to reveal any correlation with pathogenicity within this group. On the contrary, biofilm formation by two carrier strains (GRI 681 and GRI 682) was similar to that of strains isolated originally from active infections, such as GDH 2346 and GDH 2348. Moreover, two recent isolates from implant infections (AAHB 72 and AAHB 92) formed less-extensive biofilms than some of the other isolates tested here. This unexpected result may be explained by the incubation conditions used in the present assay. Preliminary experiments indicate that gentle shaking to produce a flow of liquid over the catheter disc increases the synthesis of extracellular matrix material by biofilm cells. Such conditions may approximate those found in vivo more than does static incubation and may, in consequence, favor biofilm formation by certain strains. We are currently investigating this possibility.

There was some correlation with pathogenicity when different Candida species were tested for biofilm formation. Isolates of C. parapsilosis (Glasgow), C. pseudotropicalis, and C. glabrata gave significantly less biofilm formation than the more-patogenic C. albicans. However, a second isolate of C. parapsilosis (AAHB 4479), recently obtained from a line infection, showed a much greater propensity for biofilm formation than the first strain. Infections due to C. parapsilosis are frequently associated with indwelling catheters or other prosthetic devices (30). Both C. glabrata and C. krusei grew poorly in shake cultures on high-galactose medium, reaching optical densities which were 26 and 35%, respectively, of that for C. albicans GDH 2346 (results not shown). A limited ability to assimilate galactose may also partly account for the relatively poor biofilm formation by C. glabrata in this medium. By contrast, C. krusei gave better biofilm growth than C. albicans in both low-glucose and high-galactose medium, an anomalous result that remains unexplained. Like C. parapsilosis, C. krusei is now emerging as a significant nosocomial pathogen (5).

There is increasing evidence that the chemical nature of catheter material plays an important role in the pathogenesis of device-related infections. Central venous catheters are the intravascular devices most likely to cause iatrogenic septicemia; most of these are made of PVC, although various studies indicate that Teflon or polyurethane catheters are less likely to become colonized by bacteria (18). C. albicans is known to adhere to PVC more readily than to Teflon (28). Our results now demonstrate that PVC catheters are more susceptible to biofilm formation by this yeast than are polyurethane ones. The most extensive biofilm formation by C. albicans was observed on latex urinary catheter material. Most Candida urinary tract infections are nosocomial and occur in patients with urinary bladder catheters. Duration of catheter placement is directly related to the incidence of candiduria (12). A 100% silicone catheter produced significantly less biofilm formation than either of the other urinary catheter materials. This may be related to its surface topography, which appeared exceptionally smooth when viewed by SEM. However, pure silicone catheters are not ideally flexible and afford a lower degree of patient comfort than silicone elastomer-coated latex catheters. In vivo, catheters and other prosthetic devices are rapidly coated with host proteins such as albumin, fibrin, fibrinogen, and fibronectin. Some of these proteins are known to affect bacterial adhesion and biofilm formation (9). The biofilm system described here should facilitate analogous studies with Candida species.

A number of reports suggest that certain bacterial genes are switched on in the presence of a surface. In a study by Dagostino et al. (6), for example, transposon mutagenesis was used to insert promoterless lacZ genes into appropriate recipient bacteria, giving some the ability to express β-galactosidase at a polystyrene surface but not in liquid or on agar medium. Recently, Davies et al. (7) described activation of the aigC promoter, which is required for the production of the exopolysaccharide alginate, by biofilms of P. aeruginosa. Activation resulted from association of the bacteria with a Teflon substrate. In the present study of Candida biofilms, we observed that biofilm cells growing on discs of PVC catheter material exhibited mixed yeast and hyphal morphologies, whereas on an agar surface of the same medium (or in liquid culture), only the yeast form was found. The processes by which morphogenesis in C. albicans is regulated are poorly understood at the molecular level; our SEM studies now suggest the possible involvement of some form of contact-induced gene expression.

One of the most significant features of bacterial biofilms is their resistance to antimicrobial agents (1, 4). Investigations into the mechanisms of resistance indicate that reduced drug penetration into biofilms, nutrient limitation, and a low bacterial growth rate are all contributory factors (1). Preliminary experiments carried out in this laboratory have shown that Candida biofilm cells are similarly less susceptible to a number of antifungal agents than are planktonic yeasts (16). An important application of our model biofilm system will, therefore, be to characterize the susceptibilities of different Candida biofilms to the various antifungal agents in current clinical use and possibly also to others in development.

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