BACTERIAL AND FUNGAL PATHOGENICITY

Adhesion and colonisation of *Candida krusei* on host surfaces

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Summary. *Candida krusei* is an emerging pathogen, especially in immunocompromised hosts. As the adherence of this organism both to host epithelial surfaces and to catheter and prosthetic surfaces appears to be important in the pathogenesis of superficial as well as systemic candidoses, the adhesion of 20 oral isolates of *C. krusei* and five oral isolates of *C. albicans* was compared with the following substrates: cultured (HeLa) epithelial cells, buccal epithelial cells (BEC) from healthy adults and bone marrow transplant patients, and acrylic (polymethylmethacrylate) surfaces. Animal experiments in Sprague Dawley rats were also conducted to evaluate the relative oral carriage rate of the two *Candida* spp. *C. krusei* isolates adhered in far greater numbers to acrylic surfaces than to either of the cell surfaces. Significant intra-species differences in *C. krusei* adhesion for acrylic surfaces were noted between 74 (39 %) of 190 pair comparisons in contrast to 18 (9.5 %) of 190 with HeLa surfaces ($p < 0.05$). A positive correlation was also observed between the adhesion of *C. krusei* isolates to HeLa cells and acrylic surfaces. Five isolates of *C. albicans* showed very low adherence to HeLa surfaces when compared with BEC obtained from either healthy individuals or bone marrow transplant patients. The adherence of *C. albicans* to BEC from the healthy individuals was *c.* 12-fold greater than that of *C. krusei*, a figure similar to the relative murine oral carriage rate of the two *Candida* spp. However, the adhesion of *C. albicans* to BEC from bone marrow transplant patients was three-fold less than to BEC of healthy individuals whilst *C. krusei* adhesion remained the same, reflecting a possible selective colonisation process which may operate in these patient groups, possibly as a result of drug therapy. The current data, while confirming the inter- and intra-species differences in adherence of *Candida* spp. to host surfaces, illustrate that adherence-related factors may operate during colonisation of *C. krusei* on mucosal, catheter and prosthetic surfaces, *in vivo* in both health and disease.

Introduction

Adhesion to host surfaces is a prerequisite in the pathogenesis of many microbial infections.¹ Despite the extensive data now available on bacterial adhesion, relatively few studies have focused on yeast adhesion, and most of these relate to *Candida albicans*.¹ The adhesion of other *Candida* spp. has been little studied.

In recent years, infections with species other than *C. albicans*, such as *C. krusei*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata* and *C. guilliermondii*, have been described increasingly both in compromised and non-compromised hosts.² The increasing incidence of *C. krusei* infections, in particular, has been documented

clearly.^{3,4} One article, for instance, described systemic infection with fungaemia due to *C. krusei* in seven neonates in a department of neonatology.⁵ *C. krusei* has been reported as a pathogen in an intra-abdominal abscess,⁶ endocarditis,⁷ infective arthritis,⁸ ureteral obstruction,⁹ oesophagitis,¹⁰ disseminated disease,^{11–15} ocular infection,¹⁶ bone marrow transplant recipients¹⁷ and immunocompromised patients with cancer.¹⁸

Although *C. krusei* has been regarded traditionally as an organism of low virulence, this ever increasing incidence of *C. krusei* infections warrants further studies of its pathogenic potential. There is only a handful of investigations that evaluate the adherence potential of *C. krusei*,^{19–21} these describe the adherence of the organism to buccal epithelial cells (BEC) and denture acrylic surfaces and a maximum of seven isolates was investigated. However, such studies may

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not reveal clearly the entire spectrum of this phenomenon, as there are differences in adherence amongst *Candida* isolates belonging to the same species.²² Furthermore, there are no studies to date on adhesion of *C. krusei* to BEC from healthy and diseased individuals or on its oral carriage in animal models.

Therefore, the present study was designed to compare the in-vitro adhesion of 20 oral isolates of *C. krusei* and five isolates of *C. albicans*, both to epithelial cells and to acrylic surfaces. *C. albicans* was chosen as the reference species as it is regarded as the most virulent medically important *Candida* sp. The epithelial surfaces studied included cultured HeLa epithelial cells, BEC obtained from healthy adults and bone marrow transplant patients. Acrylic was included as the inanimate surface representative of prosthodontic appliances or catheter surfaces. In-vivo studies in Sprague Dawley rats were also conducted to evaluate the oral carriage rate of the two *Candida* spp. in an attempt to relate these results to the in-vitro findings.

Materials and methods

Candida isolates and growth conditions

A total of 20 oral isolates of *C. krusei* and five of *C. albicans* was used in this study. All were from patients attending the Oral Medicine Clinic of the Glasgow Dental Hospital and School and were speciated by the germ tube test²³ and API 20C identification test kits (API Laboratory Products Ltd, Basingstoke, Hants). They were stored in distilled water at -70°C .²⁴ All isolates were subcultured monthly on to Sabouraud's Dextrose Agar (SDA; Gibco Limited, Paisley) and maintained at 4°C during the experimental period. Purity of cultures was ensured by regular identification by standard techniques.

Preparation of yeast inoculum

A loopful from the stock culture of the test isolate was streaked on to SDA and incubated at 37°C for 18–20 h. A loopful of this fresh yeast growth was then transferred into glucose-supplemented (27 mM) Yeast Nitrogen Base (YNB; Difco, USA) and incubated at 37°C for 18–20 h. The culture was then centrifuged at 3500 g for 10 min and the deposit was washed twice with phosphate-buffered saline (PBS; pH 7.2, 0.1 M). A final candidal suspension of $c. 1.0 \times 10^7$ yeasts/ml was prepared by appropriate dilution and the number of yeasts/ml was monitored by microscopical counting in an improved Neubauer haemocytometer chamber (Hawksley Ltd, London).

Preparation of HeLa epithelial cells

HeLa cells (American Type Culture Collection, CCC2; Flow Laboratories, Irvine) were maintained in

tissue-culture flasks as monolayers in RPM1-1640 (with L-glutamine and 25 mM HEPES buffer without sodium bicarbonate) supplemented with fetal bovine serum 10% v/v, sodium bicarbonate 0.2% w/v, penicillin G 100 units/ml and streptomycin 100 units/ml (all from Sigma).²⁵

The nutrient medium was discarded from the monolayer and the cells were washed once with sterile PBS and treated with 1 ml of trypsin 0.15% solution until the cells detached. The resulting cell pellet was suspended in 2–3 ml of warm RPM1-1640 working medium and 150–200 μl of this cell suspension was seeded aseptically into each well (35 mm diameter) of a multi-well tissue-culture tray (Sterilin, Middlesex) containing a sterile coverslip (22 $\times$ 22 mm) and 3 ml of working medium. The cells were then incubated at 37°C in an atmosphere of air 95% and CO_2 5% for 2–3 days until confluent monolayers formed on the glass coverslips. Before the adhesion assay, the nutrient medium was discarded and the monolayers were washed once with PBS.

Preparation of buccal epithelial cells

BEC were collected by gently rubbing the cheek mucosa of 16 healthy adult volunteers (eight males, eight females) with sterile swabs and then rotating the swabs in 10 ml of PBS. The cells were washed three times in PBS by centrifugation, pooled and resuspended to a concentration of 1×10^5 cells/ml by haemocytometer counting.

BEC from bone marrow transplant patients were used for some experiments. These were obtained from patients at the Queen Mary Hospital, Hong Kong. The patients were receiving a regimen of regular chlorhexidine gluconate 0.2% mouthwashes and the triazole antifungal fluconazole (800 mg/day by oral or intravenous route) during the cell collection period. Five or six adult patients (two females, four males) were sampled every 2 weeks for a period of 6 weeks by gently rubbing the cheek mucosa of each patient with two sterile cotton wool swabs. The BEC were then collected in 10 ml of PBS by manual agitation of the swabs, washed three times in PBS by centrifugation, pooled and adjusted to 1.0×10^5 cells/ml by haemocytometer counting. The same cohorts of patients and healthy individuals (controls) were used throughout the adhesion assay, conducted as described below.

All BEC from both groups were screened microscopically before the assay for attached yeasts. If adherent yeasts were present the cell suspension was discarded.

Adhesion assays

HeLa cells. The adhesion of yeasts to cultured epithelial cells was determined according to the method of Samaranayake and MacFarlane.²⁵ Briefly, washed HeLa cell monolayers on glass coverslips were

incubated with 1 ml of *C. krusei* or *C. albicans* suspension (1×10^7 yeasts/ml) in PBS at 37°C for 1 h in a shaking water bath at 40 rpm. The suspension was then removed aseptically and the monolayers with the adherent yeasts were washed twice with sterile PBS for 30 s by gentle manual agitation and fixed in formaldehyde 10% for 5 h. The monolayers were subsequently air-dried, gram-stained and mounted on glass microscope slides. All samples were set up in duplicate for each experiment and the yeasts attached to the monolayers were quantified as described below. Each experiment was repeated on three separate occasions.

Buccal epithelial cells. The assay was performed according to the method of Kimura and Pearsall.²⁶ Briefly, equal volumes (0.5 ml) of BEC (1.0×10^5 cells/ml) and *Candida* suspensions (1.0×10^7 cells/ml) were mixed and incubated at 37°C for 1 h in a shaking water bath at 40 rpm. The cells were then harvested on 12- μ m pore polycarbonate filters (Costar, MA, USA) and washed with 30 ml of PBS to remove unattached yeasts. The washed cells were fixed with methanol, air-dried and stained with crystal violet for 30 s, washed with tap water and air-dried once more. These filters were mounted on glass slides and the number of adherent yeasts quantified as described below. This assay was performed on three separate occasions.

Experiments with BEC from bone marrow transplant patients were limited to only single isolates of *C. krusei* (CK9) and *C. albicans* (CA2) because of the practical difficulties in harvesting large quantities of cells from these individuals. The adhesion of these two *Candida* isolates to pooled BEC from five or six bone marrow transplant patients and 16 healthy individuals was compared. This assay was performed in exactly the same manner as above.

Acrylic. Acrylic strips for the assay were prepared according to the method of Samaranayake and MacFarlane.²⁷ Transparent self-polymerising acrylic powder (polymethyl methacrylate powder; Dental Fillings Ltd., London) 1.5 g was spread on a glass slide covered with aluminium foil and the monomer liquid was poured on to the surface; immediately, a second slide was placed on top of the polymerising mixture and firmly secured. The resultant acrylic strips were cleaned and cut into 5 × 5 mm squares and used in the experiments.

The adherence of yeasts to acrylic surfaces was quantified as described previously.²⁷ Briefly, 0.4 ml of the prepared yeast suspension (1×10^7 cells/ml) was dispensed to a well of a sterile serology plate and an acrylic strip was placed vertically in the well. After incubation at 37°C for 1 h, the strips were removed, washed twice in sterile distilled water, air-dried and stained with crystal violet and Gram's iodine. The strips were then mounted on glass slides and adherent yeasts were quantified as described below. All values quoted represent mean figures derived from three independent assays with duplicate determinations on each occasion.

Microscopy

The number of adherent yeasts on the HeLa cell monolayers and the acrylic strips was estimated by the principle of stratified random sampling.^{25, 27} Thirty fields were counted for each sample. As the samples were set up in duplicate for each experiment, the mean number of yeasts/60 fields was finally expressed as yeasts/0.561 mm² of surface area in the case of HeLa cells and acrylic surfaces. For the BEC assay, the number of yeasts which were adherent to 100 BEC was determined by light microscopy at ×400 magnification. Counts on two separate filters were performed for each sample.

Animal experiments

The murine model of Jones and Russell²⁸ was used. Ten young male Sprague Dawley rats, 200–300 g in weight, were divided equally into two groups for inoculation with either *C. krusei* (CK9) or *C. albicans* (CA2). All animals received tetracycline hydrochloride 1% in drinking water and a carbohydrate-rich diet (carbohydrate 62%, proteins 33%, vitamins and minerals 5%) and were inoculated orally three times per week with 0.1 ml of the appropriate yeast suspension containing *c.* $(1-2) \times 10^8$ yeasts/ml. Once a week, 3 days after the last inoculation, oral swabs were obtained from each rat, the contents of which were thoroughly dispersed in 5 ml of sterile distilled water. The number of colony forming units of the *Candida* isolates in this suspension were assessed by spiral plating (Spiral Systems, OH, USA) 50 μ l on to a Sabouraud's dextrose agar plate which was incubated at 37°C for 48 h and the resultant colonies were quantified.

Statistical analysis

The data from the adhesion assays were analysed by analysis of variance (ANOVA). In particular, the ANOVA test was used to determine the significant differences, if any, between species, isolates and surfaces. The Student-Newman-Keuls test was performed to study pairwise comparisons between isolates within each species-surface combination. The correlation between yeast adherence to all surfaces studied was determined by the StatWorks statistics computer package.

Results

Adhesion

***C. krusei* to epithelial surfaces.** The adhesion of 20 *C. krusei* isolates grown in YNB to HeLa epithelial surfaces is shown in fig. 1. All *C. krusei* isolates adhered well to HeLa cells although wide variations in values were seen; the highest and lowest mean values were 141.7 and 26.1. On further pairwise comparisons, to evaluate intra-species differences in adhesion by the

Fig. 1. The adhesion of 20 *C. krusei* isolates to HeLa epithelial cells ($\blacktriangle$) and acrylic surfaces ($\bullet$). Each point represents a mean of three experiments conducted on separate occasions; bar, SEM.

Table I. The adhesion of *C. krusei* and *C. albicans* to buccal epithelial cells of healthy individuals

<i>C. krusei</i> isolate no.	Mean (SD) number of yeast/100 BEC	<i>C. albicans</i> isolate no.	Mean (SD) number of yeast/100 BEC
CK6	10.33 (3.55)	CA1	113.33 (8.73)
CK9	12.83 (8.77)	CA2	153.66 (31.10)
CK13	18.33 (10.33)	CA3	161.33 (29.82)
CK18	14.16 (1.47)	CA4	206.66 (44.78)
CK19	12.50 (3.14)	CA5	142.50 (47.06)
Mean $\pm$ SD	13.63 $\pm$ 5.45	Mean $\pm$ SD	155.49 $\pm$ 32.29

The results are expressed as means of duplicate counts for each experiment, conducted on three separate occasions.

Fig. 2. The relationship between the adhesion of *C. krusei* isolates to acrylic and HeLa epithelial cell surfaces. Correlation coefficient $r = 0.66$, $p < 0.001$.

Student-Newman-Keuls test, significant differences ($p < 0.05$) in adhesion between 18 (9.5%) of 190 pairs of *C. krusei* were noted.

The foregoing data and those from acrylic studies (see below) were used to select five isolates of *C. krusei* with high, low and moderate degrees of adherence and

Fig. 3. The adhesion of *C. albicans* isolates to HeLa epithelial cells (▲) and acrylic surfaces (●). Each point represents a mean of three experiments conducted on separate occasions; bar, SEM.

Table II. The relative adhesion of *C. albicans* (CA2) and *C. krusei* (CK9) to pooled BEC from healthy and bone marrow transplant patients (BMT)

Experiment no.	Mean number of yeast cells/100 BEC from					
	healthy individuals (n = 16)			BMT patients (n = 5 or 6)		
	<i>C. albicans</i> (CA2) (a)	<i>C. krusei</i> (CK9) (k)	Ratio (a:k)	<i>C. albicans</i> (CA2) (a)	<i>C. krusei</i> (CK9) (k)	Ratio (a:k)
1	149.50	15.50	9.64	30.20	6.80	4.44
2	182.00	5.00	36.40	84.80	18.30	4.63
3	129.50	23.00	5.63	54.30	14.50	3.74
Mean	153.66*	14.50	10.59	56.43*	13.20	4.27

Results are expressed as means of duplicate counts of 100 BEC, for each experiment.

*Significantly different at $p < 0.05$.

these isolates were used in BEC studies. The latter five isolates of *C. krusei* demonstrated a lesser degree of adhesion to BEC from healthy donors than to HeLa cells, although strict comparisons could not be made because of the difficulty in quantifying the total area of BEC. The mean adhesion of *C. krusei* isolates was in the range 10–18 yeasts/100 BEC, indicating little variation between the isolates ($p > 0.05$) (table I).

C. krusei to acrylic surfaces. The remarkably high adherence of the 20 *C. krusei* isolates to acrylic surfaces is shown in fig. 1. As in the case of epithelial surfaces, *C. krusei* isolates demonstrated substantial variations in their adhesion to the acrylic strips. For example, the highest and the lowest values were 354.3 and 96.3 yeasts/unit area. On statistical analysis, significant intra-species differences ($p < 0.05$) in adhesion between 74 (39%) of 190 pair comparisons were noted in contrast to 9.5% observed with the HeLa surfaces.

Relative adhesion of C. krusei to HeLa cells and acrylic surfaces. When the relative adhesion of *C. krusei* isolates to unit areas of HeLa and acrylic surfaces were compared the affinity of yeasts to the

latter was two-fold greater than to the HeLa cell surface ($p < 0.001$). Furthermore, there was a significant positive correlation between the adhesion of *C. krusei* isolates to the two surfaces ($p < 0.001$, $r = 0.663$; fig. 2).

C. albicans to epithelial surfaces. The adhesion of *C. albicans* isolates to HeLa epithelial surfaces is shown in fig. 3. Intra-species variations in adherence values were minimal; the highest and lowest mean adherence values were 14.3 and 20.4. No significant differences in adhesion between *C. albicans* isolates were noted. Furthermore, the mean adherence values of these isolates to HeLa epithelial cells was remarkably lower (17 for *C. albicans* and 86 for *C. krusei*) than for the *C. krusei* isolates.

The results of studies with *C. albicans* and BEC indicated varying mean adherence values with different isolates. Significant differences in adhesion were noted between two pairs of *C. albicans* isolates as opposed to no significant difference between *C. krusei* isolates. In addition, *C. albicans* isolates demonstrated almost 12-fold greater affinity for BEC than *C. krusei* isolates

Table III. Post-inoculation oral carriage of *C. krusei* and *C. albicans* in Sprague-Dawley rats

<i>Candida</i> sp.	Rat no.	Oral carriage (cfu $\times 10^1$ /ml)					
		Week 1	Week 2	Week 3	Week 4	Mean	SEM
<i>C. krusei</i>	1	2.03	4.06	2.03	0.00	2.03	0.83
	2	2.03	4.06	4.06	6.09	4.06	0.83
	3	4.06	6.09	0.00	2.03	3.05	1.31
	4	2.03	4.06	2.03	4.06	3.05	0.59
	5	0.00	0.00	2.03	0.00	0.51	0.51
<i>C. albicans</i>	1	24.39	26.42	34.55	30.48	28.96	2.25
	2	26.42	28.45	30.48	32.52	29.47	1.31
	3	30.48	24.39	36.58	34.55	31.50	2.69
	4	24.39	32.52	28.45	30.48	28.96	1.74
	5	28.45	28.45	26.42	30.48	28.45	0.83

Fig. 4. Oral carriage of *C. albicans* CA2 (□) and *C. krusei* CK9 (▲) in Sprague-Dawley rats over a period of 4 weeks. Each point represents the mean carriage rate (and SEM) in five rats. The rats were inoculated orally three times a week with 0.1 ml of a suspension containing 1.0×10^8 yeasts/ml and swabs were taken 3 days after the last inoculation.

(table I), in contrast to observations with HeLa cells (see above).

The adhesion of a *C. albicans* isolate (CA2) and a *C. krusei* isolate (CK9) to BEC obtained both from healthy individuals and bone marrow transplant patients is shown in table II. When the relative adhesion of the two *Candida* spp. to BEC from the donor groups was compared, *C. albicans* exhibited significantly lower adherence to cells from the patients ($p < 0.05$) although there was no significant difference in the adhesion of *C. krusei* to these cell types. Thus, in relative terms, the avidity of *C. krusei* to BEC of bone marrow transplant patients appeared to be c. three-fold greater than to those from healthy individuals.

***C. albicans* isolates to acrylic and HeLa cell surfaces.** All *C. albicans* isolates demonstrated remarkably similar adherence to the acrylic surfaces (range 17–20 yeasts/unit area) with no significant intra-species difference in adhesion. When the relative adhesion of *C. albicans* isolates to acrylic and HeLa cell surfaces were compared, the affinity of yeasts for both surfaces

did not vary widely and there was no significant correlation between the adhesion of the isolates to the two surfaces.

Relative adhesion of *C. krusei* and *C. albicans* to HeLa cell and acrylic surfaces. *C. krusei* isolates adhered in significantly greater numbers to both HeLa cell and acrylic surfaces than did *C. albicans* isolates ($p < 0.001$). Thus, the mean adhesion of *C. krusei* to HeLa cell and acrylic surfaces was 202 and 86 yeasts/unit area, respectively, compared with 17 and 18 yeasts/unit area in the case of *C. albicans*.

Oral carriage of *C. albicans* and *C. krusei* in rats

The oral carriage rate of both *Candida* spp. is shown in table III and fig. 4. The recovery of *C. krusei* was consistently and significantly less than that of *C. albicans* throughout the experimental period ($p < 0.05$); *C. albicans* demonstrating almost 11-fold greater colonisation potential than *C. krusei*.

Gross oral lesions were not detected in either group

of animals at the termination of the experimental period. Histopathological examination was not performed.

Discussion

Candida spp. are common opportunist yeasts in the oral cavity, gastrointestinal tract and the vagina of many normal individuals.²⁹ They are isolated frequently from the palatal mucosa and the inert, acrylic denture fitting surfaces of patients with denture stomatitis,^{30,31} and are increasingly associated with nosocomial septicaemia originating from intravascular catheters.³² Although the transition of *C. albicans* from saprophytism to pathogenic parasitism remains largely unexplained, it is widely recognised that the ability of these organisms to adhere to susceptible host tissues and inert prostheses, such as dentures or medical implants and catheters, is an essential prerequisite for a transient or a sustained association with the host.^{2,3}

In contrast to *C. albicans*, which is considered to be the most pathogenic and hence the most virulent species, *C. krusei* is a less well known species currently gaining clinical interest as an emerging pathogen. For example, *C. krusei* infections of various organs such as the gut,³³ the eye,¹⁶ the urethra⁹ and many others^{4,11-15,17} have been reported.

Although there has been a multitude of studies with *C. albicans*,^{1,34} only a few investigators¹⁹⁻²¹ have examined the adherence of *C. krusei* to BEC and the maximum number of isolates investigated was seven.²⁰ Several isolates belonging to the same *Candida* spp. need to be studied to obtain a broad perspective of their virulence parameters, as intra-species variations in attributes such as adherence are well known.²² As far as we are aware, there have been no reports on the adhesion parameters of a collection of clinical isolates of *C. krusei* either to cultured epithelial surfaces or to epithelial cells from compromised hosts. Similarly, the oral carriage rate of this organism in an animal model has not been studied previously.

HeLa epithelial cells were chosen to screen all the *C. krusei* isolates as they offer an uncontaminated surface with minimal variability,³⁵ thus rendering the individual results broadly comparable. In contrast, BEC from healthy and diseased hosts were used to elicit baseline data in an attempt to seek relative differences, if any, in candidal adhesion to these cell types. Since a number of parameters are known to affect the adhesion of organisms to BEC,^{1,36} the possible variations in the quality of cells were minimised by collecting and pooling cells from the same group of donors at a specific time of the day and using them within 2 h of collection.

The adhesion of *C. krusei* to BEC was significantly less than that of *C. albicans*, the species most often implicated in human infection. These results agree with those of Tobgi²⁰ and King *et al.*,²¹ in which the

hierarchy of adhesion of *Candida* spp. to BEC was similar. Furthermore, in the study by Tobgi,²⁰ there was an 11-fold increase in the adherence of *C. albicans* to BEC compared with *C. krusei*, a result identical to that of the current study, testifying to the concordance of the experimental method. Generally, all studies revealed minimal adhesion of *C. krusei* to human BEC, tending to confirm the low virulence potential of this yeast.

These in-vitro findings are borne out by the murine oral carriage rate of the two *Candida* spp. over a period of 4 weeks; despite repeated inoculation of equal doses of the yeasts, *C. krusei* carriage was almost 12-fold less than that of *C. albicans*. While this remarkable similarity between the in-vivo carriage rate and the in-vitro adhesion potential of the two *Candida* spp. should not be construed solely as a function of the latter variable, due to the complexity of the oral environment, our results tend to strengthen the belief that adhesion phenomena play a decisive role in oral candidal colonisation.

From the current data it is evident that there are small (4.2 %) but significant intra-species differences in the adherence of *C. krusei* to HeLa epithelial cells, although this figure rose to 72 % with acrylic surfaces. It is difficult to offer a reason for this discrepancy but one possibility may be the hydrophobic forces which operate to a very great extent in the adhesion of *Candida* to inert surfaces (see below).

The adherence of 20 clinical isolates of *C. krusei* to acrylic strips was remarkably high (364 cells/mm²), a result similar to that obtained by Tobgi²⁰ (395 cells/mm²) with seven strains of *C. krusei*. The higher adhesion value obtained by the latter worker may be due to the culture medium, which contained 550 mM sucrose instead of the 27 mM glucose used in the present study. It is known that adhesion of *C. albicans* grown in sucrose media is enhanced considerably when compared with yeasts grown in media with either glucose or low concentrations of other sugars.^{25,37} This is considered to be due to the changes in the yeast cell surface components with resultant formation of extracellular fibrillar material which promotes candidal adherence.³⁸

Compared with *C. albicans*, which adhered in almost equal numbers to both HeLa cells and acrylic surfaces, *C. krusei* demonstrated 2-4-fold greater affinity for acrylic than for HeLa cell surfaces. This may be explicable in terms of the significantly higher (five-fold) cell surface hydrophobicity of *C. krusei* compared with *C. albicans* (Y. H. Samaranayake, unpublished data). Cell surface hydrophobicity and other non-specific forces may promote the attachment of the yeast to plastic medical devices, such as indwelling catheters and prostheses.³⁹⁻⁴¹ Minagi *et al.*⁴⁰ compared the surface hydrophobicity of six *Candida* spp. by contact angle measurement and adherence to hydrocarbons and observed higher adherence of *C. krusei* than *C. albicans*. Others have observed a significant positive correlation between the adherence of *Candida*

spp. to acrylic surfaces and their affinity for hexadecane—an inert organic surface.⁴¹ Furthermore, Nikawa *et al.*⁴² conducted comparative studies on the adherence of *Candida* spp. to surface-modified glass and found *C. krusei* to be the most hydrophobic with the least zeta potential and the most adherent to glass compared to a panel of *Candida* spp., including *C. albicans*. Hence it would seem that the significantly higher adherence of *C. krusei* to acrylic, observed in this study, is to a great extent a reflection of its hydrophobicity.

Although, in the current study, the adherence of *Candida* spp. to acrylic (polymethylmethacrylate) surfaces was studied, these results could arguably be extrapolated to include catheter surfaces, as it is known that the adherence of yeasts to polymers such as teflon and other material of which catheters are made, is directly proportional to their contact angle and hydrophobicity.³⁹ If this were the case, then because of its superior hydrophobicity, *C. krusei* should more preferentially attach to and colonise catheter surfaces than *C. albicans*. Indeed, in a recent in-vitro study, Hawser and Douglas⁴³ demonstrated that *C. krusei* has a significantly greater potential to form biofilms than *C. albicans* on polyvinylchloride catheter material, which may be due to its high affinity for inert surfaces.

In clinical terms, the most interesting finding in this study was the significant reduction in the adherence of *C. albicans* to BEC from bone marrow transplant patients in comparison to cells from healthy individuals (table II). This observation could be explicable in terms of the topical and systemic drug regimens of the patient group, for it is known that oral rinses of

chlorhexidine gluconate 0.2 % and fluconazole therapy result in 16–54 %⁴⁴ and 48–52 %⁴⁵ reduction of *C. albicans* adherence to BEC, respectively. However, we cannot offer an explanation for the almost equal mean adhesion values of *C. krusei* to buccal cells from both healthy and diseased hosts, as no studies are available on the effect of antifungal agents on its adhesion. As *C. krusei* exhibits surface properties^{4,46} and genomic characteristics^{47,48} which are remarkably dissimilar to *C. albicans*, this is an area worthy of further study. However, the results of this study indicate that the drug regimens commonly used in bone marrow transplant patients may select out uncommon organisms such as *C. krusei*, in preference to *C. albicans*. For example, Wingard *et al.*¹⁷ recently found *C. krusei* to be the chief candidal pathogen in 2.6 % of 419 bone marrow transplant patients, compared to 2.4 % infected by *C. albicans*. Hence it is tempting to speculate that the modulation of candidal adhesion dynamics by drug therapy,^{44,45} as well as the intrinsic resistance of *C. krusei* to the newer triazole fluconazole,^{49–50} may result in the increasing emergence of *C. krusei* infections.⁴

In conclusion, the current results taken together indicate that adhesion mechanisms play a significant role in the colonisation of *C. krusei* in both diseased and healthy hosts. However, further research is warranted, particularly to study the intriguing interactions of this and other *Candida* spp. in bone marrow transplant and other compromised patient groups.

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References

- Kennedy MJ. Adhesion and association mechanisms of *Candida albicans*. In: McGinnis MR (ed) Current topics in medical mycology. New York, Springer Verlag. 1987: 73–169.
- Odds FC. *Candida* and candidosis, 2nd edn. London, Bailliere Tindall. 1988.
- Bodey GP. Candidiasis: pathogenesis, diagnosis and treatment, 2nd edn. New York, Raven Press. 1993: 6.
- Samaranayake YH, Samaranayake LP. *Candida krusei*: biology, epidemiology, pathogenicity and clinical manifestations of an emerging pathogen. *J Med Microbiol* 1994; **41**: 295–310.
- Hendrick W, Schonborn C, Spencker FB, Huckel-D. Infection caused by *Candida krusei* in newborn infants. *Paediatr Pathol* 1989; **24**: 289–295.
- Gordon RA, Simmons BP, Appelbaum PC, Aber RC. Intra-abdominal abscess and fungemia caused by *Candida krusei*. *Arch Intern Med* 1980; **140**: 1239–1240.
- Rubinstein E, Noriega ER, Simberkoff MS, Holzman R, Rahal JJ. Fungal endocarditis: analysis of 24 cases and review of the literature. *Medicine* 1975; **54**: 331–344.
- Nguyen VQ, Penn RL. *Candida krusei* infectious arthritis. A rare complication of neutropenia. *Am J Med* 1987; **83**: 963–965.
- Thomalla JV, Steidle CP, Leapman SB, Filo RS. Ureteral obstruction of a renal allograft secondary to *Candida krusei*. *Transplant Proc* 1988; **20**: 551–554.
- Mathieson R, Dutta SK. *Candida* esophagitis. *Dig Dis Sci* 1983; **28**: 365–370.
- Meunier-Carpentier F, Kiehn TE, Armstrong D. Fungemia in the immunocompromised host. Changing patterns, antigenemia, high mortality. *Am J Med* 1981; **71**: 363–370.
- Myerowitz RL, Pazin GJ, Allen CM. Disseminated candidiasis. Changes in incidence, underlying diseases, and pathology. *Am J Clin Pathol* 1977; **68**: 29–38.
- Young RC, Bennet JE, Geelhoed GW, Levine AS. Fungemia with compromised host resistance. A study of 70 cases. *Ann Int Med* 1974; **80**: 605–612.
- Jacobs MI, Magid MS, Jarowski CI. Disseminated candidiasis: newer approaches to early recognition and treatment. *Arch Dermatol* 1980; **116**: 1277–1279.
- Rose HD, Varkey B. Deep mycotic infection in the hospitalized adult: a study of 123 patients. *Medicine* 1975; **54**: 499–507.
- McQuillen DP, Zingman BS, Meunier F, Levitz SM. Invasive infections due to *Candida krusei*: Report of ten cases of fungemia that include three cases of endophthalmitis. *Clin Infect Dis* 1992; **14**: 472–478.
- Wingard JR, Merz WG, Rinaldi MG, Johnson TR, Karp JE, Saral R. Increase in *Candida krusei* infection among patients with bone marrow transplantation and neutropenia treated prophylactically with fluconazole. *N Engl J Med* 1991; **325**: 1274–1277.
- Persons DA, Laughlin M, Tanner D, Perfect J, Gockerman JP, Hawthorn JW. Fluconazole and *Candida krusei* fungemia. *N Engl J Med* 1991; **325**: 1315.
- Critchley IA, Douglas LJ. Differential adhesion of pathogenic *Candida* species to epithelial and inert surfaces. *FEMS Microbiol Lett* 1985; **28**: 199–203.
- Tobgi RS. Differences in the pathogenic potential of *Candida* species, especially *C. albicans*. PhD Thesis. Faculty of Medicine, University of Glasgow. 1989.
- King RD, Lee JC, Morris AL. Adherence of *Candida albicans* and other *Candida* species to mucosal epithelial cells. *Infect Immun* 1980; **27**: 667–674.

22. Samaranayake LP, MacFarlane TW (eds). Oral candidosis. London, Wright. 1990.
23. Lodder J. The yeasts: a taxonomic study, 2nd edn. Amsterdam, North Holland. 1970.
24. Silverman S, Migliorati CA, Epstein JB, Samaranayake LP. Laboratory diagnosis of oral candidosis. In: Samaranayake LP, MacFarlane TW (eds) Oral candidosis. London, Wright. 1990: 213–237.
25. Samaranayake LP, MacFarlane TW. The effect of dietary carbohydrates on the in-vitro adhesion of *Candida albicans* to epithelial cells *J Med Microbiol* 1982; **15**: 511–517.
26. Kimura LH, Pearsall NN. Adherence of *Candida albicans* to human buccal epithelial cells. *Infect Immun* 1978; **21**: 64–68.
27. Samaranayake LP, MacFarlane TW. An in-vitro study of the adherence of *Candida albicans* to acrylic surfaces. *Arch Oral Biol* 1980; **25**: 603–609.
28. Jones JH, Russell C. The histology of chronic candidal infection of the rat's tongue. *J Pathol* 1974; **113**: 97–100.
29. Hurley R, de Louvois J, de Mulhall A. Yeasts as human and animal pathogens. In: Rose AH, Harrison JS (eds) The yeasts, vol 1, 2nd edn. London, Academic Press. 1987: 207–281.
30. Olsen I. Denture stomatitis. Occurrence and distribution of fungi. *Acta Odontol Scand* 1974; **32**: 329–333.
31. Budtz-Jorgensen E. The significance of *Candida albicans* in denture stomatitis. *Scand J Dent Res* 1974; **82**: 151–190.
32. Goldmann DA, Pier GB. Pathogenesis of infections related to intravascular catheterization. *Clin Microbiol Rev* 1993; **6**: 176–192.
33. Merz WG, Karp JE, Schron D, Saral R. Increased incidence of fungemia caused by *Candida krusei*. *J Clin Microbiol* 1986; **24**: 581–584.
34. Segal E, Lehrman O, Dayan D. Adherence in vitro of various *Candida* species to acrylic surfaces. *Oral Surg Oral Med Oral Pathol* 1988; **66**: 670–673.
35. Samaranayake LP, MacFarlane TW. The adhesion of the yeast *Candida albicans* to epithelial cells of human origin *in vitro*. *Arch Oral Biol* 1981; **26**: 815–820.
36. Samaranayake LP, Hamilton D, MacFarlane TW. The effect of indigenous bacterial populations on buccal epithelial cells on subsequent microbial adhesion *in vitro*. *Oral Microbiol Immunol* 1994; (in press).
37. McCourtie J, Douglas LJ. Relationship between cell surface composition, adherence, and virulence of *Candida albicans*. *Infect Immun* 1984; **45**: 6–12.
38. McCourtie J, Douglas LJ. Extracellular polymer of *Candida albicans*. isolation, analysis and role in adhesion. *J Gen Microbiol* 1985; **131**: 495–503.
39. Klotz SA, Drutz DJ, Zajic JE. Factors governing adherence of *Candida* species to plastic surfaces. *Infect Immun* 1985; **50**: 97–101.
40. Minagi S, Miyake Y, Inagaki K, Tsuru H, Suganaka H. Hydrophobic interaction in *Candida albicans* and *Candida tropicalis* adherence to various denture base resin materials. *Infect Immun* 1985; **47**: 11–14.
41. Miyake Y, Fujita Y, Minagi S, Suganaka H. Surface hydrophobicity and adherence of *Candida* to acrylic surfaces. *Microbios* 1986; **46**: 7–14.
42. Nikawa H, Sadamori S, Hamada T, Satou N, Okuda K. Non-specific adherence of *Candida* species to surface-modified glass. *J Med Vet Mycol* 1989; **27**: 269–271.
43. Hawser SP, Douglas LJ. Biofilm formation by *Candida* species on the surface of catheter materials *in vitro*. *Infect Immun* 1994; **62**: 915–921.
44. Tobgi RS, Samaranayake LP, MacFarlane TW. Adhesion of *Candida albicans* to epithelial cells exposed to chlorhexidine gluconate. *J Med Vet Mycol* 1987; **25**: 335–338.
45. Darwazeh AMG, Lamey PJ, Lewis MAO, Samaranayake LP. Systemic fluconazole therapy and in vitro adhesion of *Candida albicans* to human buccal epithelial cells. *J Oral Pathol Med* 1991; **20**: 17–19.
46. Kogan G, Pavliak V, Sandula J, Masler L. Novel structure of the cellular mannan of the pathogenic yeast *Candida krusei*. *Carbohydr Res* 1988; **184**: 171–182.
47. Doi M, Homma M, Chindamporn A, Tanaka K. Estimation of chromosome number and size by pulsed-field gel electrophoresis (PFGE) in medically important *Candida* species. *J Gen Microbiol* 1992; **138**: 2243–2251.
48. Barns SM, Lane DJ, Sogin ML, Bibeau C, Weisburg WG. Evolutionary relationships among pathogenic *Candida* species and relatives. *J Bacteriol* 1991; **173**: 2250–2255.
49. Roder BL, Sonnenschein C, Hartzen SH. Failure of fluconazole therapy in *Candida krusei* fungemia. *Euro J Clin Microbiol Infect Dis* 1991; **10**: 173.
50. Casasnovas RO, Caillot D, Solary E et al. Prophylactic fluconazole and *Candida krusei* infections. *N Engl J Med* 1992; **326**: 891–892.