

Technical report

A novel technique for assessment of adherence of *Candida albicans* to solid surfaces

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Abstract

A novel approach for the assessment of adherence of *Candida albicans* to translucent acrylic material is described. The method uses the inverted microscope to visualise yeast adhering to acrylic surfaces while the test material remains immersed in buffer. Adherent cells were not subjected to surface tension forces that can occur during drying processes, so that an even distribution of yeast with no aggregation occurred. The process of counting attached yeast was subsequently performed without difficulty. From the 11 *C albicans* isolates examined, two groups were evident with respect to acrylic adherence: one group of four isolates with an adherence level of 400 yeast/mm² acrylic, and one group of seven isolates with adherence levels of 1000 yeast/mm² acrylic.

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Keywords: *Candida albicans*; adherence; acrylic

Adherence of *Candida* spp to oral surfaces is regarded as a prerequisite for the successful colonisation of the mouth, and therefore an important determinant in the development of candidosis. In vitro models to evaluate the adherence of candida to exfoliated buccal epithelial cells have been developed.¹ The in vitro adherence of candida to acrylic surfaces has also been studied, as candidal colonisation of the fitting surface of upper dentures has been implicated in oral candidosis in the palate.² Assessment of adherence to acrylic surfaces has often involved incubation of candida with the solid material followed by washing to remove non-adherent cells, and subsequent staining of the attached candida using either crystal violet or a fluorescent marker. While this approach permits the visualisation of candida, aggregation of yeast cells occurs following staining, which can lead to errors in counting. Cell aggregation can be observed during the drying of the acrylic and is believed to be caused by surface tension forces exerted across the surface of the acrylic. This study assessed the adherence of isolates of *C albicans* to acrylic using the inverted microscope, which minimised the problem of yeast aggregation.

Materials and methods

PREPARATION OF ACRYLIC

The translucent acrylic material used in the present study has been described previously³ and was prepared at the department of basic dental science, Dental School, Cardiff. All samples were made in a highly polished stainless steel mould to minimise surface irregularities. Briefly, denture acrylic was cured in accordance with the manufacturer's instructions (Trevalon; Dentsply Ltd, Weybridge, UK) and cut into strips (10 mm and 3 mm thick). This material was autoclaved and saturated with sterile water for 24 hours.

PREPARATION OF CANDIDA

Eleven strains of *C albicans* were examined: nine clinical isolates (four chronic hyperplastic candidosis, four non-hyperplastic oral candidosis, and one isolate from an asymptomatic carrier) and two laboratory cultures (*C albicans* GDH 2346 and *C albicans* GRI 681). Identification of test isolates was established by germ tube formation and use of the API-20C system (bioMérieux, Basingstoke, UK). Candida were cultured in Sabouraud's broth (Lab M, Bury, UK) supplemented with 500 mM sucrose at 37°C for 24 hours, harvested by centrifugation, and washed three times in phosphate buffered saline (PBS) (0.01 M, pH 7.2). Yeast cells were counted with a haemocytometer and resuspended in PBS at 10⁷ cells/ml.

ADHERENCE ASSAY

A 20 ml volume of test suspension was added to sterile petri dishes containing acrylic strips and incubated for one hour at room temperature without agitation. The acrylic strips were

Table 1 Relative adherence of 11 isolates of *Candida albicans* to acrylic

<i>C albicans</i> isolate	Yeast/mm ² acrylic
GDH 2346	1560.3 (294.7)
705/93	1415.2 (266.5)
632	1469.4 (279.7)
674/93	1359.2 (532.1)
DW1	1889.8 (290.7)
643/93L	1359.6 (458.7)
576/93	1429.7 (458.3)
600/93	398.5 (166.9)
659/93	119.6 (64.2)
GRI 681	152.5 (127.6)
PB1	177.2 (191.8)

Values are mean (SD).

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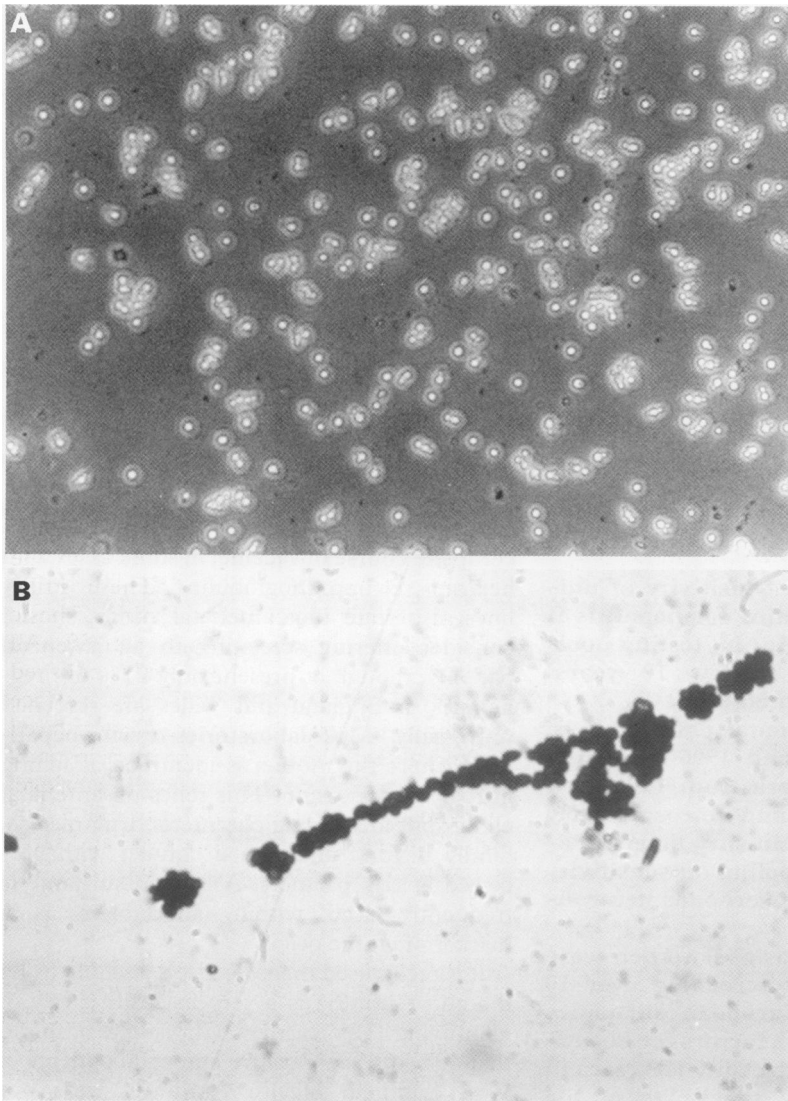


Figure 1 (A) Adherence of *Candida albicans* to acrylic visualised by inverted microscopy (original magnification $\times 200$). (B) Standard light microscopy (original magnification $\times 400$).

washed twice by gentle agitation in fresh PBS for one minute. Adherence of candida was measured while the strips were immersed in PBS using a phase contrast inverted microscope (Olympus CK2; Olympus Optical Co Ltd, London, UK). Adherent yeast cells were counted in 20 randomly selected fields of view (one field was 0.0625 mm^2) avoiding the edges of the acrylic strips. Twenty fields of view had previously been shown to provide a representative sample size using the method described by Weibel.⁴ Although blastospores were the predominant morphological form in all the isolates tested, occasional hyphae were evident. To standardise the measurement of adherent cells, filamentous forms were not counted, and budding daughter cells were counted as individual yeast. Each experiment was repeated in triplicate on two separate occasions.

Results

Examination of the acrylic strips revealed that the adherent candida were evenly distributed across the surface of the acrylic squares (fig 1A). Adherence of *C. albicans* yeast cells to acrylic material was variable, ranging between

a mean (SD) of 119.6 (64.2) and 1889.8 (290.7) yeast/ mm^2 (table 1). The 11 *C. albicans* strains examined could be divided into two groups depending on the degree of adherence to acrylic. Seven isolates gave adherence values in excess of 1000 yeast/ mm^2 , while the adherence of the remaining four isolates was less than 400 yeast/ mm^2 .

Discussion

It is generally accepted that adherence of *C. albicans* to acrylic is strain dependent.⁵ Previous experiments assessing the adherence of candida to acrylic using staining procedures to visualise adherent yeast have been complicated by aggregation of yeast on the surface of the material (fig 1B). Such aggregation can prevent accurate measurement of the number of adherent cells. Microscopic examination of candida on acrylic shows that aggregation occurs during drying of the acrylic material, presumably as a result of surface tension forces. The use of inverted microscopy described in this report enabled us to count uniformly distributed yeast adhering to translucent acrylic strips. Using this methodology, yeast aggregation was minimised as the acrylic remained immersed in buffer throughout the experiment, thus preventing drying. The technique therefore offers an improved method of measuring adherence of candida to acrylic surfaces that could also be used in the examination of adherence of other microorganisms to translucent materials. Of the isolates tested in the present study only two (*C. albicans* GDH 2346 and *C. albicans* GRI 681) have been examined previously using an identical growth medium and standard methodology allowing comparisons to be made between methods.⁵ In the previous investigation, strain GDH 2346 had a fourfold higher level of adherence compared with strain GRI 681 (400 v 100 yeast/ mm^2 of acrylic). The increased levels of adherence for the strains in the present investigation might relate to the different methods, although other variables such as acrylic composition could also have an effect.

Further investigation is required to establish whether differences in hydrophobicity between the 11 candida isolates examined relates to their relative adherence to acrylic, as previously noted with adherence to epithelial cells.⁶

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