

## Short reports

Adherence of *Candida albicans* to oral epithelial cells differentiated by Papanicolaou staining

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**Abstract**

**Objective**—To examine the relative adherence of *Candida albicans* to oral epithelial cells differentiated by Papanicolaou staining.

**Methods**—Oral epithelial cells were collected from 10 healthy adults (five male, five female) and counted. Equal volumes of oral epithelial cells and candida were mixed and incubated. The epithelial cells from this mix were collected by filtration through 10 µm polycarbonate membrane filters. Cells retained on the membrane filters were stained with crystal violet followed by Papanicolaou stain. The number of yeast attached to each of 100 red, orange, and green staining oral epithelial cells was determined by direct microscopic examination.

**Results**—*C albicans* had a higher level of adherence ( $p < 0.001$ ) to red staining oral epithelial cells (mean (SD) number of candida attached to 100 oral epithelial cells 562 (159)) than to cells staining either orange (105 (47)) or green (161 (66)).

**Conclusions**—Oral epithelial cell variability for candidal adherence is confirmed. The technique provides an opportunity to examine the relation between oral epithelial cell type and oral candidosis in specific groups, such as tobacco smokers, where increased epithelial cell keratinisation and candidal colonisation has been reported.

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Keywords: *Candida albicans*; adherence; oral epithelial cells; Papanicolaou staining

The ability of candida to adhere to oral surfaces exposed to the flushing action of saliva is considered a prerequisite for successful colonisation of the mouth. Consequently, the in vitro adherence of candida to oral epithelial cells has been extensively studied<sup>1, 2</sup> and correlation between adherence and virulence reported.<sup>3</sup> Several candidal adherence mechanisms have been proposed, including electrostatic attraction,<sup>4</sup> cell hydrophobic interaction,<sup>5</sup> and specific adhesins.<sup>6</sup> In addition, it has been reported that oral epithelial cells exist as subpopulations with either a high or low affinity for attachment of *Candida albicans*.<sup>7</sup> Therefore, the relative proportion of these “suscepti-

ble cells” within the oral mucosa of an individual may be a factor involved in the establishment of candidal colonisation and subsequent infection. It is also possible that the site of oral candidosis relates to the presence of oral epithelial cells favouring candidal adherence. Indeed, in the case of chronic hyperplastic candidosis, lesions are found in decreasing order of prevalence in the labial commissure regions of the mouth, buccal mucosa, palate, and tongue.<sup>8</sup>

The Papanicolaou staining technique<sup>9</sup> differentiates epithelial cells according to the degree of keratinisation within the cells. Cells of the basal layer stain green, fully keratinised cells stain red, and intermediate cells show shades of orange with this technique. The Papanicolaou staining method, used extensively in cervical cytology, depends on the affinity of acid dyes (light green, eosin, orange G) for basic cytoplasmic constituents, for example keratin. Increased maturation (of epithelial cells) is accompanied by increased cytokeratin, which in turn is responsible for increasing cytoplasmic density. Larger acid dyes (for example light green) penetrate the loose cytoplasm of immature cells more easily. Only the smaller acid dyes—successively orange G and eosin—are able to penetrate the denser cytoplasm of mature keratinised cells.<sup>10</sup>

The technique has been used as the basis for the histological determination of keratin levels in fixed tissue specimens.<sup>11</sup> Following Papanicolaou staining, red cells are presumed to contain more keratin than cells staining orange or green. As stated previously, epithelial cells are known to differ with respect to levels of yeast adherence, although the reason for this remains unclear. Our aim in the present investigation was to examine the relative adherence of *C albicans* to oral epithelial cells differentiated by Papanicolaou staining.

**Methods**

The experimental conditions used for assessing candidal adhesion were based on the recommended procedure of Kennedy,<sup>12</sup> which is aimed at standardising oral epithelial cell adhesion assays. Briefly, oral epithelial cells were collected from the mouths of 10 healthy adult volunteers (five male, five female) by gently rubbing sterile swabs over the surface of

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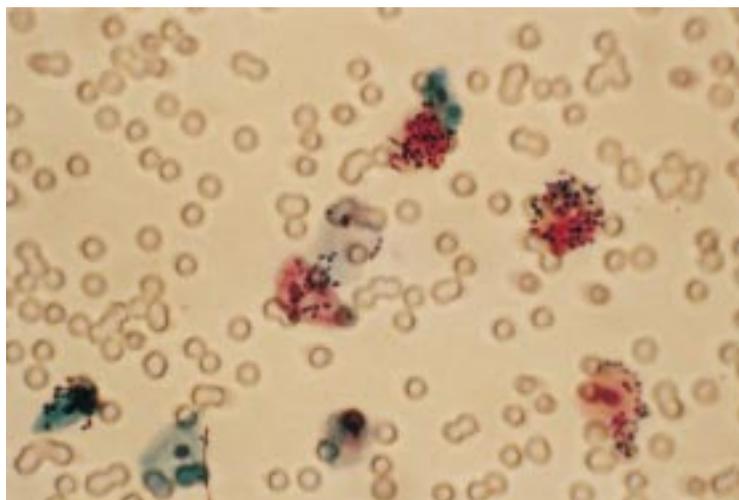


Figure 1 Adherence of *Candida albicans* to oral epithelial cells differentiated by Papanicolaou staining. (Magnification  $\times 86$ ; original magnification  $\times 125$ .)

the mucosa. The swabs were subsequently agitated in 2 ml of phosphate buffered saline (PBS; 0.01 M, pH 7.2) to recover the epithelial cells. The cell suspension was centrifuged (1000 g for 15 minutes) and the cell pellet washed three times in PBS. All 10 samples were pooled and the epithelial cells examined by light microscopy to ensure there was no colonisation by yeast. The oral epithelial cells were counted using an improved Neubauer haemocytometer chamber (Hawksley) and resuspended in PBS to  $10^5$  cells/ml. *Candida albicans* GDH 2346 (isolated from chronic atrophic candidosis; Glasgow Dental Hospital isolate) was cultured in yeast nitrogen base medium (Difco) supplemented with 50 mM galactose at 37°C for 24 hours, and washed three times in PBS. Yeast cells were enumerated as described above and resuspended in PBS at a concentration of  $10^7$  cells/ml.

Equal volumes (0.5 ml) of oral epithelial cells ( $10^5$  cells/ml) and candida ( $10^7$  cells/ml) were mixed and incubated on a rotator (30 rpm) at 37°C for one hour. The epithelial cells from this mix were collected by filtration through 10  $\mu$ m polycarbonate membrane filters (Millipore) and washed six times using 5

ml of PBS. Cells retained on the membrane filters were initially stained with crystal violet and then by Papanicolaou stain. The filters were dehydrated, cleared, and mounted under coverslips using Canada balsam. The number of yeast attached to each of 100 red, orange, and green staining oral epithelial cells was determined by direct microscopic examination ( $\times 125$  magnification). The procedure was repeated in triplicate on four separate occasions by a single observer.

Counts of adherent candida were separated into three groups based on oral epithelial cell colour following Papanicolaou staining. A one way analysis of variance was then performed using the Scheffe multiple comparison method and the computer software program SPSS Version 6 (SPSS Inc, Chicago, Illinois, USA).

## Results

Enumeration of candida adhering to oral epithelial cells differentiated by Papanicolaou staining (fig 1; table 1) showed larger numbers of candida ( $p < 0.001$ ) attached to epithelial cells staining red (mean (SD), 562 (159)) than to those staining orange (105 (47)) or green (161 (66)). There was no apparent difference in the candidal adherence to either green or orange cells ( $p = 0.41$ ).

## Discussion

Adherence of candida to mucosal surfaces in the mouth is regarded as an important virulence factor in oral candidosis. However, despite extensive research in the area of adherence our knowledge of the mechanisms involved is still poor. It is, however, generally accepted that candidal adherence is a multifactorial phenomenon and that oral epithelial cells vary within and between individuals with respect to candida attachment.<sup>7</sup> In the present study we aimed to further examine the role of oral epithelial cell types on *C. albicans* adherence. The differential basis for these oral epithelial cell types was cell coloration after Papanicolaou staining.

It was evident from the data in this study that red staining oral epithelial cells had a greater affinity for candidal adhesion than green and orange staining cells. This may be related to the differential expression of specific candidal receptors associated with oral epithelial cell keratinisation, although other factors may also be significant. It is important to recognise that oral epithelial cells differentiated by Papanicolaou staining may also vary in other aspects, apart from the degree of keratinisation, such as cell viability or their site of origin in the oral cavity. As a result, further investigation is needed to elucidate the exact mechanisms responsible for the differences in candidal adhesion observed for the oral epithelial cell types. However, the results of our study confirm those of previous studies where oral epithelial cell variability for candidal adherence has been reported.<sup>13</sup> Furthermore, for reliable correlations to be made between adherence studies, the initial characterisation of the oral epithelial cell population by Papanicolaou staining could be beneficial. From a clinical

Table 1 Relative adherence of *Candida albicans* to oral epithelial cells (OEC) differentiated by Papanicolaou staining (numbers of yeast cells attached to 100 OEC)

Exp	Sample No	OEC colour		
		Green (non-keratinised)	Orange (moderately keratinised)	Red (highly keratinised)
1	1	81	57	427
	2	130	100	379
	3	126	68	590
2	4	95	26	437
	5	215	127	510
	6	109	88	525
3	7	242	175	437
	8	270	157	600
	9	173	169	494
4	10	227	103	779
	11	188	127	925
	12	86	65	641
Mean (SD)		161 (66)	105 (47)	562 (159)

Exp, experimental occasion.

viewpoint, the technique offers the opportunity to examine the relation between oral epithelial cell type and oral candidosis in specific groups (for example tobacco smokers) where increased epithelial cell keratinisation and candidal colonisation have been reported.<sup>14</sup>

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