Oral adhesion of yeasts

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Oral adhesion of yeasts probably occurs by interaction between yeast cell adhesins and oral epithelial cell receptors. In *Candida albicans* mannoprotein, glucan, chitin, cell wall proteins, and lipids are possible adhesins. Mannoprotein appears as a fibrillar or floccular outermost layer in stationary-phase cells grown in sugar-rich medium. Preincubation of buccal epithelial cells (BECs) with concanavalin A inhibits adhesion, as does suppression of mannoprotein production by tunicamycin. Germ tubes adhere more easily to BECs and plastic than do blastospores. Methyl-α-D-mannoside may be analogous to the yeast adhesin or epithelial cell receptor because it inhibits adhesion of *C. albicans* to BECs. L-Fucose, *N*-acetyl-D-glucosamine, or D-mannose, having the same effect, may also function as epithelial cell receptors. Other factors affecting yeast adhesion may be fibronectin, hydrophobicity, s-IgA, and indigenous bacteria. Growth of yeasts to stationary phase in sugar-rich media promotes adhesion to acrylic, as do divalent cations and serum. Saliva, chlorhexidine, and *Streptococcus salivarius* inhibit adhesion of yeasts. □ *Adherence; fungi; mouth*

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Adhesion of yeasts to mucosa and denture is probably an important initial step in the pathogenesis of oral yeast infections. A direct correlation between the adherence of *Candida albicans* strains and pathogenicity for mice has been demonstrated (1, 2). Most studies of adhesion so far have been performed in vitro and are based on assessment of adhesion of *C. albicans* and related species to buccal epithelial cells (BECs). Excellent reviews on experiments with adhesion of yeasts have been published (for example, 3–5), and the reader should consult these reviews for more extensive information. In the present paper emphasis will be placed on adhesion factors discussed most recently in the literature. Reviewing the factors possibly affecting yeast adhesion, it may be reasonable to organize these into factors pertaining to the yeast cell, factors related to mucosal cells and denture, and environmental factors affecting the yeast–host cell/denture interaction (Table 1).

Factors related to yeast cells

Medium/cultivation

Adhesin (cell surface structure promoting adhesion) production is highly dependent on the cultivation conditions of the yeast. By culturing *C. albicans* in 13 media (10 complex

Table 1. Factors affecting adhesion of yeasts

Factors related to yeast cells
Medium/cultivation
Phenotype
Germ tubes/hyphae
Extracellular polymeric material (EP)
Floccular/fibrillar surface layers
Mannan
Chitin
Hydrophobicity
Proteinase/phospholipase
Cellular lipids
Factors related to host cells
Cell source
Mucosal cell size and viability
Fibronectin
Fibrin
Sex hormones
Yeast carriers versus patients with overt candidosis
Environmental factors affecting adhesion
Cations
pH
Sugars
Saliva
Humoral antibody and serum
Antibacterial drugs
Bacteria
Lectins

and 3 synthetic) and by altering the growth conditions, adhesion of this organism to BECs was significantly modified (6). Optimal adhesive activity of *C. albicans* occurred when the cells were grown in defined media (depending on the carbohydrate used), and/or at 25°C. There were also significant differences in the adhesion of BECs when *C. albicans* was grown in the same complex medium from different manufacturers and even in different batches of medium from the same manufacturer. Cultivation in different media also resulted in differences in surface topography and cell wall ultrastructure of *C. albicans*. It was noteworthy that none of these differences, including presence or absence of an outer floccular layer, frequently held to contain major yeast adhesins (3–5), correlated with the adhesive changes observed.

Phenotype

Under identical growth conditions adhesion of *C. albicans* is significantly altered, depending on the phenotype state of the organism. Pathogenic isolates of *C. albicans* are able to switch heritably, reversibly, and at high frequency between two phenotypes ('white' or 'opaque') readily distinguishable by size, shape, and color of the colonies formed on agar at 25°C. 'White' cells were significantly more adhesive to BECs than 'opaque' cells, and a strong correlation existed between phenotype adhesiveness and the percentage of BECs to which *C. albicans* attached (7). The difference in adhesion to plastic shown by the two phenotypes was not statistically significant.

Germ tubes and hyphae

Germ tubes of *C. albicans* produced an additional surface layer compared with blastoconidia; this was responsible for their enhanced adherence to plastic (8). This layer, which is composed of fibrils, was retained on the plastic. The fibrils contained four different mannoproteins of various molecular weights.

The adherence of the hyphal phase of *C. albicans* to HeLa cells was significantly greater than that of the blastospore phase (9). In scrapings from tongue and buccal mucosa of patients with oral candidosis, blastospores adhered to hyphae (10). The difference in the ability of blastospores and hyphae to adhere to glass is demonstrated in Fig. 1.

Extracellular polymeric material

In adhesion much importance has been placed on the role of extracellular polymeric material (EP) originating from the yeast cell surface (3, 4). Galactose-grown yeasts, which were most adherent, produced more EP in culture supernatants than did sucrose-grown organisms, particularly after incubation for 5 days (11). Glucose-grown yeasts, being least adherent, gave the lowest yield. EP produced from all three carbon sources (50 mM glucose, 500 mM sucrose or galactose) was of similar composition and contained carbohydrate (65–82%), mannose with some glucose, protein (7%), phosphorous (0.5%), and glucosamine (1.5%). Pretreatment of acrylic strips with EP to form a polymeric coating promoted yeast adhesion to the acrylic surface. Similar pretreatment of BECs with EP inhibited subsequent yeast adhesion. It was suggested that EP originating from the cell surface of *C. albicans* contains the surface component(s), probably mannoprotein in nature, responsible for yeast adhesion. The protein portion of the mannoprotein adhesin is probably more important than the carbohydrate moiety in mediating yeast attachment to BECs (12) and may provide the predominant interaction between yeast and epithelial cell. The purified adhesin inhibited adhesion to BECs 30 times more efficiently (on a weight basis) than unfractionated EP. It was possible to inhibit (30–50%) adhesion of four strains of *C. albicans* to BECs with EP isolated from another strain (13). EP from all five strains contained lectin-like proteins with affinities for L-fucose, *N*-acetyl-D-glucosamine and D-mannose in different amounts. It is thought that the mannoprotein of culture supernatants originates, at least in part, from the fibrillar layer on the yeast surface (3, 4).

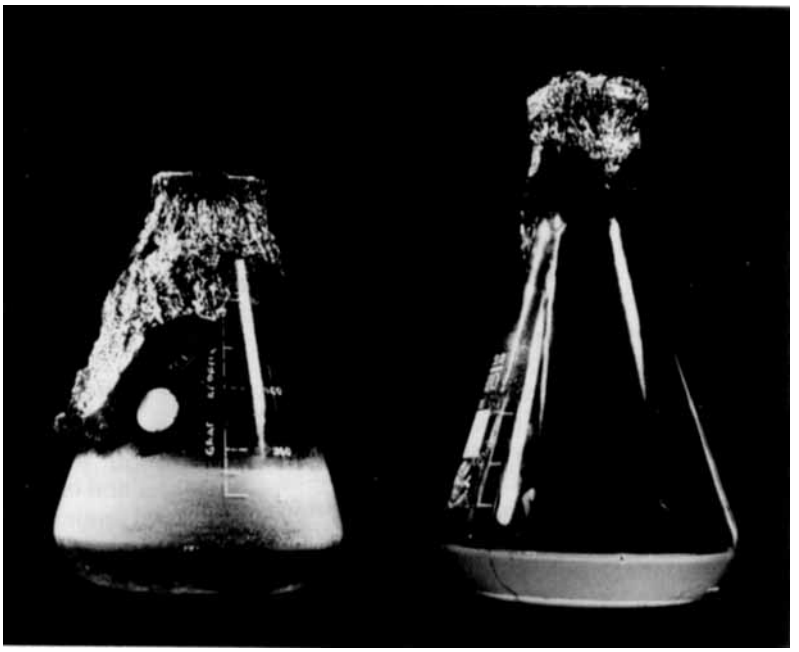

Fig. 1. *Candida albicans* cultured aerobically for 27 h at 37°C under shaking has been brought to multiply in a yeast phase (right) and a mycelial phase (left). The left flask shows abundant adhesion of hyphae to glass, whereas the right one shows that blastospores have not adhered.

There is also evidence to suggest that protein alone may serve as a yeast adhesin (5).

Floccular and fibrillar surface layers

There are at least two surface types of *Candida* adhesins: floccular and fibrillar (5). The floccular surface layer mediates adhesion of *C. albicans* to oral mucosal cells (14). *C. albicans* strains able to synthesize a fibrillar surface layer when grown in medium with a high galactose or sucrose content showed increased adhesion to BECs and increased virulence for mice (1). Less pathogenic *Candida* species and *Saccharomyces cerevisiae* do not show increased adhesion under these conditions (15). Development of the fibrillar layer is prevented by the antibiotic tunicamycin (16). Fibrils are synthesized in the presence of high sugar concentrations during the exponential growth phase of yeast cells (1); 500 mM galactose-grown yeasts have more antigenic determinants and probably a more extensive fibrillar layer than 500 mM glucose-grown yeasts. *C. albicans* growing in vivo has been shown to possess a similar layer (3, 4). It should be noted that even *Candida* cells devoid of floccular or fibrillar structures can be highly adhesive to BECs (5).

Mannan

There is a possibility that candida adhesion is mediated by an alkali-soluble, mannan-containing moiety(ies) of the yeast cell wall (17). *C. albicans* cells were treated with alkali and acid to extract the α-mannan. Cells were recovered at three stages as extraction proceeded from mild to more extensive: Alk-1, Alk-2, and Alk + Acid. Yeasts from all three stages of extraction adhered in significantly lower numbers to buccal mucosal cells than did unextracted cells. Adhesion was as low for Alk-1 cells as for those submitted to more complete extraction. Treatment of cells from all stages with concanavalin A, a lectin probe with strong affinity for yeast α-mannan, showed no significant change in adhesion. Abundant agglutination exerted by concanavalin A occurred only with untreated cells. Blockage of alkali-soluble, mannan-containing moiety(ies) inhibited adhesion of untreated

cells. Extracted cells lacked this moiety but still possessed enough mannan for concanavalin A recognition and agglutination.

Chitin

Another possible adhesin-like substance of the yeast cell wall may be chitin. A chitin-soluble extract from cell walls of *C. albicans* inhibited adhesion of this organism to human vaginal epithelial cells (VECs) and blocked in vivo attachment to the murine vaginal mucosa, thereby preventing candidal infection (18). The chitin-soluble extract gave two fractions, of which only one had adhesion-inhibitory activity. Chemical analyses showed that over 70% of the chitin-soluble extract contained proteins, most of which were found in the non-active fraction. Doubt has been expressed as to the role of chitin in adhesion, since it is mainly located in the inner layer of the yeast cell wall (3, 4).

Hydrophobicity

Conflicting views have also been expressed as to the significance of cell hydrophobicity in yeast adhesion. Peptide moieties of mannans from *C. albicans* formed hydrophobic bonds with the plastic molecule of polystyrene microtiter plates (19). On the other hand, Kennedy et al. (7), examining 'white' and 'opaque' phenotypes of *C. albicans*, suggested that cell surface hydrophobicity is of minor importance in direct adhesion to epithelial cells but that it may contribute to yeast co-adhesion. Even if 'opaque' cells were more hydrophobic than 'white' cells, the latter were significantly more adhesive to BECs. Hazen (20), measuring adhesion of *C. albicans* to HeLa cells, suggested that cell surface hydrophobicity of the yeast is involved in adherence but is not the predominant mechanism and that the effect of cell surface hydrophobicity on adherence is isolate-dependent.

The different degrees of cell surface hydrophobicity of *Candida* species (21) could be correlated with the ability of the yeasts to adhere to denture base materials and plastics (22–24). However, the surface-free energy of the denture material may also influence *Candida* adhesion (6).

Proteinase/phospholipase

Extracellular proteinase of *C. albicans* has been claimed to facilitate fungal adherence and invasion of the mucosa (25–27). A similar correlation between *C. albicans* isolates and their phospholipase production has been reported (28).

Cellular lipids

Surface lipids of yeasts may also be involved in adherence. Ceramide monohexosides and ceramide dihexosides isolated from total lipids of yeast forms of *C. albicans* and steryl glycosides isolated from the mycelial forms inhibited the adherence of yeast cells to BECs by 48%, 49%, and 54%, respectively (29). Total lipids extracted from a membrane-free preparation of yeast cell walls and from whole epithelial cells blocked adherence by 57% and 53%.

Factors related to host cells

Cell source

In vitro adherence of *C. albicans* may be influenced by the mucosal cell donor, time of collection of mucosal cells, and body site of their origin. In vitro assay of adherence of *C. albicans* to human epithelial cells from 24 donors showed statistically significant differences in the number of attached yeast cells between individuals (30). Sex did not have any significant influence on adhesion. Yeast attachment to mucosal cells varied significantly within subjects with time (5 days). Cells from some donors showed greater date-to-date variation in yeast adhesion than others. Yeast adherence was highest to BECs and lowest to urinary tract cells, whereas VECs were intermediate.

Mucosal cell size and viability

The number of *C. albicans* cells attaching to individual buccal cells varies greatly. In one study most of the BECs (88%) had none

or very few yeasts attached, whereas a few of the cells (12%) bound more than half of all the attached yeasts (31). Cells of an intermediate size (36–70 μm) had a greater affinity for yeasts than had cells of other sizes. Buccal cell viability appeared not to be necessary for adhesion of yeasts. The number of yeast cells attached to single buccal cells did not differ from that attached to cells within sheets.

Fibronectin

Some experimental studies have demonstrated fibronectin binding by *Candida* species, whereas other studies have not. Organisms that are frequently involved in endocarditis, such as *C. tropicalis* and *C. albicans*, bound significantly better ($p < 0.01$) to fibronectin in vitro than did *C. krusei*, which is rarely implicated (32). Fibronectin also seemed to be involved in the adhesion of *C. albicans* to the genital mucosal surface (33). Epithelial cell receptors for fibronectin have, however, not been characterized. Putative receptor analogues of fibronectin, such as *N*-acetyl-D-glucosamine and D-galactose, and several non-specific saccharides, such as α-D-methylglucopyranoside, D-ribose, and D-xylose, failed to decrease adhesion of *C. albicans* to human BECs (34).

Fibrin

C. albicans and *C. tropicalis* showed marked adherence to fibrin clots, whereas *C. krusei, C. guilliermondii*, and *C. glabrata* adhered less readily (35). *C. parapsilosis* was intermediate. *C. albicans* strains with little ability to adhere in vitro to a fibrin platelet matrix were relatively avirulent in a rabbit endocarditis model (36). The fibrinogen binding factor of *Candida* is thought to be a glycoprotein, probably a mannoprotein present at the cell wall surface (37).

Sex hormones

There seems to be a correlation between the levels of adherence in vitro of *C. albicans* to epithelial cells and the hormonal status of their donors. Thus, estradiol, progesterone, and testosterone affected to various extents the adhesion of yeasts to HeLa cells or VECs (38). Progesterone had the most marked effect, leading to a significant increase in the number of yeasts adherent to these cells. There was an increased binding to intermediate-positioned cells as opposed to superficial cells. Increased numbers of intermediate cells, appearing during high levels of progesterone, were found in patients predisposed to vaginal candidosis.

Yeast carriers versus patients with overt candidosis

There seem to be remarkable differences in the adhesiveness of yeasts isolated from patients with candidosis and those from asymptomatic carriers. Five *C. albicans* strains isolated from candidosis patients possessed on the average higher adhesiveness to epithelial cells than did four strains isolated from persons without symptoms of candidosis (39). *C. albicans*, the most virulent oral yeast species, also tends to be more adhesive than other *Candida* species (40). *C. albicans* strain-related differences in adhesiveness may be minor or substantial.

Environmental factors affecting adhesion of yeasts

Cations

Using epithelial cells from the oral cavity, Karaev et al. (41) found that introduction of cations such as Ca^{++} (1 and 10 mM) and Mg^{++} (10 mM) into their experimental system led to increase in adhesion by 80%, 100%, and 24%, respectively. Divalent cations were also shown to promote the adhesion of *C. albicans* to acrylic, and at high concentrations these cations caused extensive co-adhesion and aggregation of *C. albicans* (42). Ion-binding mechanisms and electrostatic forces may thus be important in the adhesion of *C. albicans* to BECs and acrylic.

pH

Conflicting evidence has been presented as to the role of pH in the adhesion of yeasts. Adherence of two strains of *C. albicans* to HeLa cells varied with the pH of the test medium (9). Maximal adherence occurred at pH 3, while less adhesion occurred under neutral pH conditions. On the other hand, Karaev et al. (41) found that adhesion of fungi belonging to the genus *Candida* to oral epithelial cells reached its maximum at pH 6.2–7.0.

Sugars

Exogenous and endogenous carbon sources may affect the oral carriage of *C. albicans* cells by modifying their adhesive properties. A denture stomatitis isolate of *C. albicans* showed increased adherence after cultivation in media containing various sugars, the most effective of which was galactose (43, 44). Samaranayake & MacFarlane (45) also found that preincubation of *Candida* in medium with sugars produced significant enhancement of adhesion to HeLa cells and BECs. Maltose was the most effective sugar. A clinical isolate of *C. albicans* demonstrated greater overall enhancement in adhesion after preincubation with sugar than did a laboratory reference strain. Adhesion of *C. albicans* strains to epithelial cells has been found to be inhibited by L-fucose, *N*-acetyl-D-glucosamine, methyl-α-D-mannoside, D-mannose, or D-mannosamine, possibly by blocking glycosides serving as epithelial cell receptors in adhesion (3, 4). Such experiments suggest that several possible surface components may serve as receptors for *C. albicans*. Sandin (34), however, found that receptor analogues such as *N*-acetyl-D-glucosamine and D-galactose and several non-specific saccharides such as α-D-methylglucopyranoside, D-ribose, and D-xylose failed to decrease significantly *C. albicans* adhesion to BECs.

Saliva

The role of saliva in adhesion of yeasts is unclear. Secretory immunoglobulin A tended to inhibit binding of *C. albicans* to BECs (46, 47). A mixed salivary pellicle on HeLa cells significantly enhanced candidal adhesion (9). Yeasts preincubated in whole saliva for 3 h showed significantly greater adhesion to HeLa and human embryonic kidney epithelial cells than yeasts in phosphate-buffered saline. On the other hand, pretreatment of acrylic with unstimulated mixed saliva for 30 min led to a reduced adherence for all *Candida* strains tested (48). This finding is in accord with the observation in monkeys that colonization of prostheses by *Candida* increases with decreased salivary flow (49). Binding of *C. albicans* to acquired denture pellicle is mediated by specific salivary or serum components (50).

Humoral antibody and serum

Humoral antibody against *C. albicans* may protect against *C. albicans* endocarditis through inhibition of adhesion, which is a crucial step in the pathogenesis of this disease (51). Pretreatment of acrylic with serum, however, slightly increased adhesion of *Candida* strains (48).

Antibacterial drugs

Antibacterial drugs, particularly broad-spectrum ones, promote candidal infection. Administration of 0.1% tetracycline hydrochloride solution to mice for 4 days increased the adhesive number (AN) and adhesion index (AI) of *C. albicans* to epitheliocytes up to 175% and 250%, respectively, as compared with the control (52). After 3 weeks of antibiotic use the AN and AI amounted to 220% and 350%, respectively.

There was a significant reduction in the adherence of yeasts to BECs collected immediately after an oral rinse with chlorhexidine gluconate (53). A significant positive correlation was also noted between the time elapsed after a chlorhexidine rinse and yeast adhesion to BECs. In vitro exposure of BECs from children and adults to 0.002–0.2% chlorhexidine also reduced candidal adhesion. Adherence can be inhibited by the antibiotic tunicamycin,

which decreases the synthesis of mannoprotein but not of chitin or glucan in yeasts (16), and by a sublethal concentration of ketoconazole, which decreases germ tube formation (54). Nystatin, amphotericin B, 5-fluorocytosine, clotrimazole, and ketoconazole significantly inhibited the adherence of *C. albicans* to BECs (55). Pretreatment of denture acrylic with chlorhexidine (48, 56) and exposure of *Candida* to sublethal concentrations of chlorhexidine (56) resulted in reduced yeast adhesion to denture material.

Bacteria

The indigenous bacterial flora may interfere with adhesion of yeasts (57). Preexposure of HeLa cells to *Streptococcus salivarius* and *S. mitior* reduced candidal adhesion to these cells, whereas *S. mutans* had no significant effect (9). Adhesion of *C. albicans* to denture acrylic surfaces in vitro was significantly inhibited by *S. salivarius* (58). In the gut the indigenous microflora suppressed *C. albicans* colonization and dissemination from the gut by inhibiting *Candida*–mucosal association (59).

Lectins

Adhesion of *C. albicans* strain GDH 2346 to BECs was inhibited by winged-pea lectin, and that of GDH 2023 by wheat germ agglutinin (13). Lectin-like protein with affinities for L-fucose, *N*-acetyl-D-glucosamine, and D-mannose was detected in EP from all strains in different amounts. Glycosides containing L-fucose or *N*-acetyl-D-glucosamine may function as epithelial cell receptors for *C. albicans*. Addition of concanavalin A to human BECs at 0, 10, or 20 min of incubation decreased adhesion of *C. albicans* to 38%, 45%, and 63%, respectively, of control values (34). Concanavalin A, which is a lectin binding to α-mannosyl residues, could not inhibit adhesion after 20 min. It was suggested that in vitro attachment to human mucosal cells by *C. albicans* is inhibited up to a defined point of time by a lectin with affinity for mannose-containing surface moieties but becomes non-reversible thereafter.

Conclusion

There are several factors that may influence adhesion of *C. albicans* to epithelial cells. Some of these factors may stimulate adhesion; others may prevent it. In the mouth a series of such factors probably act at the same time. Unfortunately, no in vitro system developed for adhesion experiments so far is likely to imitate in full extension the processes of mucosa–yeast cell associations occurring in the oral cavity, since existing in vitro systems are limited by several pitfalls (5). This makes comparison of results, obtained by different authors under various experimental conditions, difficult. One way to make interpretation of adhesion experiments easier is to set standards for in vitro assays. In vitro standard test assays should be adopted for studying adhesion of a reference organism—for example, *C. albicans* to reference cells at the molecular level—using a defined basal medium. Nevertheless, in vivo experiments should be applied whenever possible, using as experimental models the oral cavity of monkeys, rats, or mice (49, 60) or BECs or dentures of the human oral cavity. There is no doubt that knowledge of fungal adherence mechanisms is in a comparatively early stage.

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