Hiroshima Journal of Medical Sciences
Vol.35, No.2, 93~97, June, 1986
**HIJM** 35-15



# Effect of Saliva on Candidal Adherence to Polymethyl Methacrylate Resin

Shogo MINAGI[1)], Yoichiro MIYAKE[2)], Yasumasa AKAGAWA[1)], Hiromichi TSURU[1)] and Hidekazu SUGINAKA[2)]

*1) Department of Removable Prosthodontics,*
*2) Department of Microbiology and Oral Bacteriology, Hiroshima University School of Dentistry, 2-3, Kasumi 1-chome, Minami-ku, Hiroshima 734, Japan*

(Received February 6, 1986)

*Key words: Candida, Adherence, Saliva, Free energy*

## ABSTRACT

The adherence of *Candida* species to saliva-coated plates of polymethyl methacrylate resin was studied physico-chemically. Saliva-coating of the resin plate caused increasing adherence of *C. albicans*, whereas it caused decreasing adherence of *C. tropicalis*. The hydrophobicities of the surfaces of the resin plate and the candidal cells were evaluated by a contact angle method, and surface free energies of these surfaces were calculated. The candidal cell adherence to the saliva-coated plates was highly correlated with the free energy change in hydrophobic interaction.

*Candida* species, especially *Candida albicans*, is frequently recovered from denture surface[1,3,11]. Candidal adherence to the denture surface or oral mucosa is thought to be an important step of an infectious process in denture stomatitis (chronic atrophic candidosis)[12,15]. Several investigators have analyzed the adherence of *C. albicans* to acrylic surfaces. Samaranayake, McCourtie and MacFarlane[14] reported that incubation of *C. albicans* with sucrose or glucose enhanced its adherence to the acrylic surface. McCourtie and Douglas[8] suggested that an additional surface layer produced after incubation of *C. albicans* with sugars takes part in the adherence of *C. albicans* to the acrylic surface. Hydrophobic forces have been suggested to play an important role in bacterial adherence to solid surfaces or human epithelial cells[2,4-6,13].

We previously reported that adherences of *C. albicans* and *C. tropicalis* to the acrylic surfaces are highly correlated with the changes of free energy which corresponds to the process of adherence[9]. Our theory have well agreed with data of following studies by Klotz et al[7] and van Pelt et al [16].

In the work reported here we attempted to investigate the effect of salivary pellicle on the adherence of *Candida* species to the acrylic surface.

## MATERIALS AND METHODS

*Organisms and culture condition*

The organisms used were *C. albicans* IFO 1385 and *C. tropicalis* IFO 1400. The fungi maintained on Sabouraud glucose (1.0% peptone, 0.5% yeast extract, 2.0% glucose) slopes were precultured in Sabouraud glucose broth overnight. Ten milliliters of the preculture was inoculated into 40 ml of Sabouraud glucose broth and incubated at 37°C with shaking to a turbidity of 2.0 at 660 nm (18-mm light path). Cells were harvested by centrifugation and washed three times with 0.01 M phosphate-buffered saline (PBS, pH 7.3). *C. albicans* and *C. tropicalis* were resuspended in PBS at concentrations of $10^7$ $ml^{-1}$ and $10^5$ $ml^{-1}$, respectively.

*Preparation of resin plates*

Heat-curing polymethyl methacrylate (PMMA) resin for denture base ("Bio" resin, Shofu dental MFG. Co., Kyoto, Japan) was used in this experiment. The material was prepared to make

plates (10 by 10 mm) with a smooth surface. The plates were washed with running water for 3 days to remove remaining monomer and five times with distilled water, and then dried.

*Saliva-coating of resin plates*

Sample of whole unstimulated saliva, which has no detectable *Candida* agglutinating activity, was collected in a container chilled in ice, and was clarified by centrifugation at 10,000 *g* for 10 min.

The resin plates were placed in a petri dish (60 by 15 mm). Seven milliliters of the clarified saliva was poured into the dish and incubated at 37°C for 1, 2, 5, 7, 10 and 15 min. The saliva-coated plates were vigorously rinsed with distilled water after incubation.

*Adherence assay*

The methods used were the same as description in the previous study[9] with the following exceptions. Four samples of each saliva-coated plate were placed in a petri dish, and 7.0 ml of the candidal suspension was poured into the dish. After incubation at 37°C for 1 hr, plates were washed three times in PBS, fixed in 35% formaldehyde and methanol, and then stained with 1% crystal violet. Adherent cells in 10 high power fields (14 $mm^2$) were counted by a microscope and totaled. The mean number of adherent cells was calculated.

*Contact angles and thermodynamical analysis*

The Young contact angles of distilled water on non-coated plate and on candidal cells were measured by a drop-on method which was previously described by Minagi et al[9].

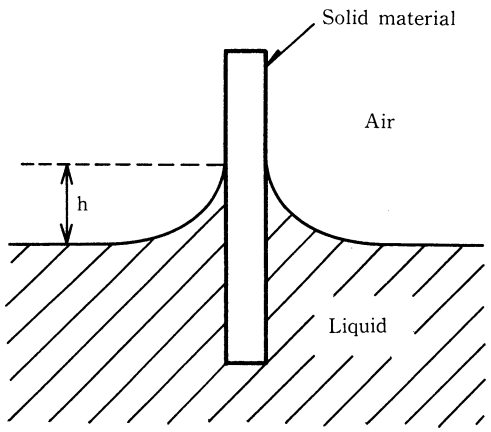


**Fig. 1.** Schema of a vertical plate method. h : height of modified capillary rise of liquid medium.

The contact angles on the saliva-coated plates were measured in a wet condition by a vertical plate method (Fig. 1), which is appropriate to evaluate a exact value of contact angle ($\theta$) on hydrophilic surface. The contact angles of distilled water is calculated from equation (1):

$$\sin \theta = 1 - (\rho g h^2/2\gamma) \quad (1)$$

where

$\rho$ ; density of liquid medium.

g ; gravity.

h ; height of modified capillary rise of liquid medium (Fig. 1).

$\gamma$ ; surface tension of liquid medium.

The change in interfacial free energy which corresponds to the process of adherence ($\Delta Ga$) was calculated from equation (2)[6,9].

$$\Delta Ga = \gamma^{SF} - \gamma^{SV} - \gamma^{FV} \quad (2)$$

where

$\gamma^{SF}$ ; interfacial free energy between solid and fungal cell.

$\gamma^{SV}$ ; surface free energy of solid surface.

$\gamma^{FV}$ ; surface free energy of fungal cell surface.

**Table 1.** Heights of modified capillary rise and contact angles of the saliva-coated resin plates

| Incubation time (min) | height[a] (h) (mm) | Contact angle[b] ($\theta$) (degrees) |
|---|---|---|
| 1 | 2.676 ± 0.035 | 31.15 |
| 2 | 2.677 ± 0.092 | 31.14 |
| 5 | 2.496 ± 0.028 | 35.47 |
| 7 | 2.505 ± 0.234 | 35.25 |
| 10 | 2.670 ± 0.120 | 31.31 |
| 15 | 2.477 ± 0.066 | 35.92 |

[a]mean heights of the modified capillary rise ± the standard deviation.

[b]contact angles calculated from the mean values of the height.

## RESULTS

Table 1 shows the contact angles and the heights of the modified capillary rise of distilled water on the saliva-coated plates. The contact angle on the non-coated plate, which was measured by the drop-on method, was 81.3 ± 2.8 degrees. Fig. 2 shows the calculated surface free energies of the plates as a function of the saliva-coating time. The surface free energy of the non-coated plate was 34.53 ergs $cm^{-2}$, and

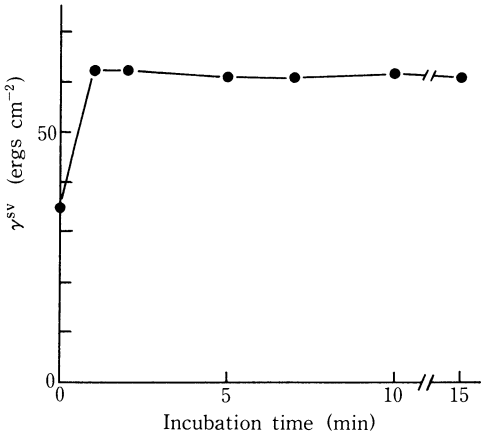


**Fig. 2.** The surface free energy of the resin plate covered with pellicle as a function of saliva-coating time.

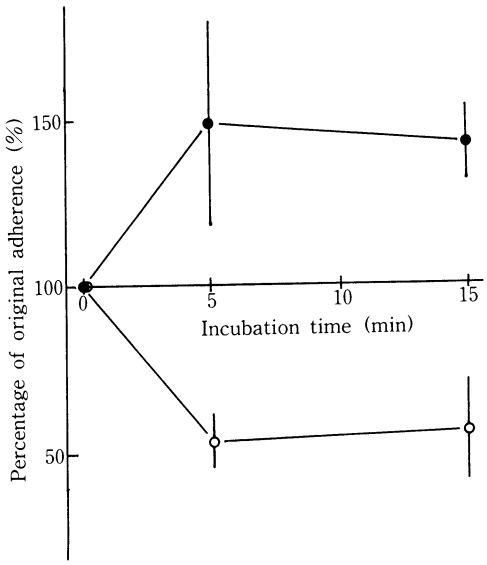


**Fig. 3.** Effect of saliva-coating on the adherence of *C. albicans* and *C. tropicalis*. The results are presented as the percent adherence of candidal cells to surfaces relative to those to the non-coated plates ± the standard error of the mean. (○); *C. albicans*, (●); *C. tropicalis*

it changed to more than 60 ergs cm$^{-2}$ after 1 min incubation with saliva. Candidal adherences to non-coated and saliva-coated plates are shown in Fig. 3. Saliva-coating of the plate caused higher adherence of *C. albicans* to the plates than that to non-coated plates, whereas it caused lower adherence of *C. tropicalis* to the saliva-coated plates than that to the non-coated plates. The relationships between the surface free energy of the test plates and adherence of *C. albicans* or *C. tropicalis* are shown in Fig. 4. The logarithm of adherent-cell number of *C. albicans* was proportional to the surface free energy of the test plates, whereas that of *C. tropicalis* was inversely proportional to the surface free energy of the test plates. Fig. 5 shows the relationship between the logarithm of adherent-cell number and the change in interfacial free energy which corresponds to the process of adherence. A decrease of the change in interfacial

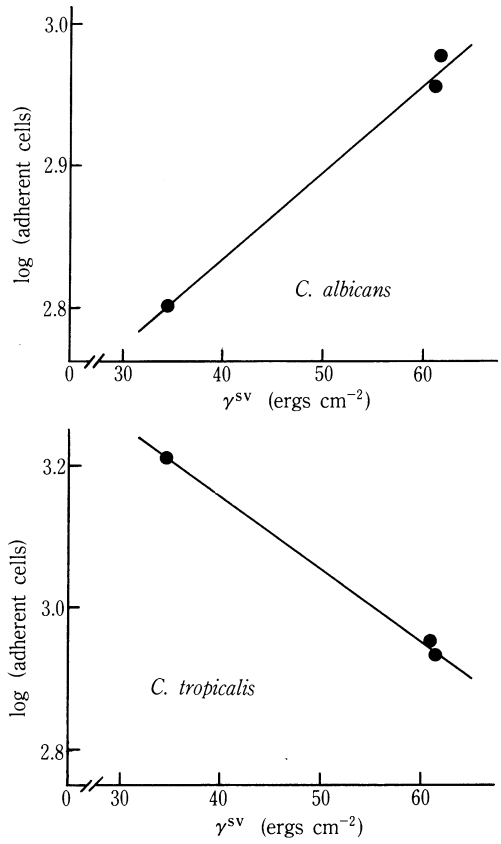


**Fig. 4.** Relationship between the surface free energy ($\gamma^{sv}$) of the resin plates and the adherence of *C. albicans* and *C. tropicalis* to the saliva-coated and non-coated plates.

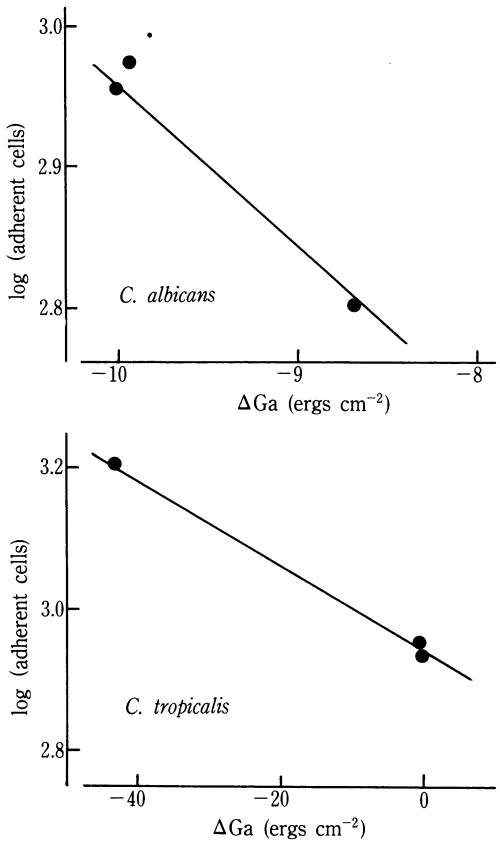


**Fig. 5.** Relationship between the change of the free energy accompanying adherence (ΔGa) and the adherence of *C. albicans* and *C. tropicalis* to the saliva-coated and non-coated plates. $r$=-0.967 for *C. albicans* and $r$=-0.999 for *C. tropicalis*.

free energy (ΔGa) resulted in increasing adherences of both *C. albicans* (correlation coefficient [$r$]=-0.967, $p<0.05$) and *C. tropicalis* ($r$=-0.999, $p<0.001$).

## DISCUSSION

The candidal adherence to the saliva-coated plate of PMMA resin was examined by using *C. tropicalis* IFO 1400 (a hydrophobic strain) and *C. albicans* IFO 1385 (a hydrophilic strain)[9]. Saliva-coating of the resin plate caused a change of surface free energy (Fig. 2) and a change in candidal adherence (Fig. 3). As the drying of salivary pellicle may cause a change in its conformation, the surface free energy of the pellicle was evaluated in a wet condition with the vertical plate method in this study. With increasing surface free energy of the plate by saliva-coating, the adherence of *C. albicans* increased, whereas those of *C. tropicalis* decreased (Fig. 4). Minagi et al[9] demonstrated that the adherences of *C. albicans* and *C. tropicalis* to the various acrylic surfaces are highly correlated with the change of free energy which corresponds to the process of adherence. In other words, with regard to hydrophobic interaction, a higher adherence of microorganisms to the material, whose surface hydrophobicity was closer to that of the microorganisms, had been observed. The change of free energy accompanying the candidal adherence to the saliva-coated plates was calculated in this study, and linear relationships were also observed between the change of free energy which corresponds to the process of adherence and the adherent-cell numbers of both *C. albicans* and *C. tropicalis* to the saliva-coated plates (Fig. 5). Although the change of free energy in the adherence of *C. tropicalis* to the saliva-coated plate was approximately equal to zero, candidal adherence was still observed. The result suggests that other factors, such as specific interaction, electrostatic interaction, mechanical attachment, and/or other unknown factor (s), may participate in the candidal adherence to the plate. As the concentration of *Candida* are different from that under *in-vivo* condition, the number of adherent cells could not be compared between the two strains. However, it is revealed that saliva-coating of PMMA plates caused increasing adherence of the hydrophilic strain (*C. albicans*) and decreasing adherence of the hydrophobic strain (*C. tropicalis*). In the present study, the hydrophobic interaction is demonstrated to be an important factor which affects to the probability of candidal adherence to PMMA surface. These results well agreed with the data of van Pelt et al[16] and Klotz et al[7], and were consistent with the theory of hydrophobic interaction[9].

Olsen[11], in investigating the frequency and distribution of fungi from denture plaque in denture stomatitis, showed that *C. albicans* is most frequently isolated from the patients, followed

by *C. glabrata* and *C. tropicalis*. As a result of measuring the surface free energies of six species of *Candida*, we demonstrated that *C. tropicalis* was most hydrophobic, followed by *C. glabrata*, *C. albicans*[10]. These results well agreed with the frequency of fungi, and suggested that hydrophobic interaction, not all but in part, may affect the probability of candidal adherence to denture pellicle.

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