In Vitro Study of the Adherence of *Candida Albicans* to Acrylic Resins: Relationship to Surface Energy

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Purpose: This study aimed to determine whether small variations in the composition of the polymethylmethacrylate (PMMA) of widely used dentures produce differences in the degree of *Candida albicans* adherence and to relate any differences found to the surface energy of the resins, which appears to play a major role in the initial phases of microorganism adhesion. ***Materials and Methods:*** A reference strain of *C albicans* (18.804 ATCC) and 11 different PMMAs (Vacalon, Inkotherm 85, Veracril, Probase Cold, Inkotherm Press, Inkotherm 85 T, Ruthinium, Vertex, SR Ivocap, Idoacryl, Lucitone) were used. Fifty specimens (15 × 10 × 1 mm) of each type were prepared. *C albicans* adhesion was determined by microorganism count under fluorescent optical microscope, and the surface energy of the resins was calculated by the contact angle method. $P < .05$ was regarded as significant. ***Results:*** *C albicans* adhesion on the resins ranged from 7.12 cells/mm^2 to 330.8 cells/mm^2, with statistically significant ($P > .05$) differences in some cases. Despite small variations in the composition of the resins, their surface energy values were very similar (38.78 to 41.2 mJ/m^2), and no relationship was found between *C albicans* adhesion and surface energy. ***Conclusion:*** The adhesion of *C albicans* to different resins varied in vitro, possibly as a result of the action of residual postpolymerization products. According to these results, variations in surface energy that result from differences in the composition of the different PMMA resins appear to have no influence on the adhesion of *C albicans* or, therefore, on the onset of denture stomatitis. *Int J Prosthodont 2005;18:392–398.*

Yeast and bacteria generally colonize dental materials in saprophytic form without producing disease. However, they can sometimes act as a coadjuvant factor, triggering or perpetuating a disease, as in the situation of denture stomatitis,[1–5] which is widely prevalent (11% to 67%). Although *Candida albicans* is not a determining factor for denture stomatitis, it has been reported to play a major role in this disease in most studied patients.[6–9] Analyses of the microorganisms present in denture stomatitis–associated plaque showed a greater presence of *C albicans* in more severe situations of the disease.[3,8]

Numerous factors are involved in the adhesion of *C albicans* to the polymethylmethacrylate (PMMA) of the acrylic resin base, and in vitro studies have sometimes found contradictory results. Thus, although there is agreement that saliva plays a protective role against denture stomatitis,[2,10,11] some authors found greater adhesion of *C albicans* to saliva-treated PMMA specimens.[12,13] The adhesion of *C albicans* initially depends

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Table 1 Acrylic Resins Used and Their Specifications

PMMA specimens	Specifications
Vacalon (Vacalon System)	Special vacuum, dry heat furnace at high temperature
Inkotherm 85 (Hedent)	Self-polymerized
Veracril (New Stetic)	Heat-polymerized
Probase Cold (Ivoclar)	Self-polymerized
Inkotherm Press (Hedent)	Heat-polymerized by injection
Inkotherm 85 T (Hedent)	Self-polymerized
Ruthinium (Dental Manufacturing)	Heat-polymerized
Vertex (Supra, Benelux)	Heat-polymerized
SR Ivocap (Ivoclar)	Heat-polymerized by injection
Idoacryl (Odilux, Villarejo de Salvanés)	Heat-polymerized
Lucitone (De Trey/Dentsply)	Heat-polymerized

on the roughness of the denture surface (denture foundation area), the surface energy of the microorganisms, and the surface energy of the material of the acrylic resin base; some products that form part of the composition of soft rebase materials reduce the adhesion and inhibit the growth of *C albicans*.[14–18] After a few hours, the thin saliva-dependent protein film coating the denture also plays a role in the adhesion.[12,13,19–24]

Although numerous studies have addressed the adhesion of *C albicans* to acrylic resin,[14–18,25–29] few have investigated the relationship between *C albicans* adhesion and the surface energy of the PMMA.[20,21,30,31] There have been no recent studies on the adhesion of *C albicans* to the acrylic resins currently used in dentures (tinted denture bases) or on the possible effect of variations in composition among PMMAs on their surface energy and on the degree of adhesion of *C albicans*. There is no standardized method for studying microorganism adhesion to plastic polymers. The most widely applied techniques are by optical count of the microorganisms per field,[10,20,22,30,32,33] calculating the mean value and the equivalent value in yeast/mm^2, or via labeling with radioactive elements such as methionine [^{35}S] or uridine [^{3}H][12,34,35] for subsequent count by scintillation camera. Other less widely used systems are counts under electronic microscope,[13,23,36] determination of the pH variation,[14,15,25] or assessment of the growth capacity of colonies after serial dilutions.[26] Standardized procedures are available for calculating the surface energy of solids.

The objectives of the present study were to determine, in vitro, the greater or lesser adhesion of *C albicans* to different types of PMMA currently used for tinted denture bases to establish whether small differences in the composition of different PMMAs are translated into variations in their surface energy, and to relate the adhesion of *C albicans* to different types of PMMA to their surface energies. If a relationship were found, manufacturers could make small modifications in the composition of resins to reduce the adhesion of *C albicans* for use in patients susceptible to developing prosthetic stomatitis.

Materials and Methods

In this study, the adhesion of *C albicans* to the different PMMA specimens was determined by using an optical fluorescent microscope, and their surface energy was measured.

Preparation of C Albicans Suspension

C albicans strain 1,002 of the (Spanish) National Type Culture Collection, corresponding to strain 18,804 of the American Type Culture Collection, was cultured for 24 h at 37°C in Sabouraud-dextrose broth (Difco, Becton, Dickinson).[20,22,23,30,37] The presence of *C albicans* in yeast form (blastospore) was then tested by acridine orange staining (0.003%) in acetic acid (2.5%), followed by visualization under a fluorescent microscope (Laborlux-D, Leitz). The yeast was separated from the culture broth by centrifugation (Selecta-P centrifuge; Abrera) at 1,200 rpm for 10 min and resuspension (vortex mixer Mixo-Tub 30, Abrera) in phosphate-buffered saline (PBS). This procedure was repeated twice. The time periods and centrifuge speed, which are not standardized for this procedure, were those most widely applied in the literature.[25,30,37] PBS dilutions were used to obtain a yeast suspension with optical density of 1 and wavelength of 540 nm (Spectronic 20 spectrophotometer; Bausch & Lomb), corresponding to a yeast concentration of $1.29 \pm 0.6 \times 10^7$ cells/mL^{-1}.

Preparation of PMMA Specimens

PMMA specimens were prepared using a brass study model, from which wax preforms of the required dimensions ($15 \times 10 \times 1$ mm) were obtained for transformation into the different acrylic resin polymers. Each acrylic resin polymer was processed according to the manufacturer's specifications. For those requiring flasking in their polymerization, the acrylic resin polymer was achieved by using the same type of gypsum materials (Hebör Española).

The different types of resin used to prepare the samples are listed in Table 1. Fifty specimens were prepared from each type. These were examined by magnifying glass (50×), and defective specimens or those with surface pores were discarded. They were used in a randomized fashion, and a small mark was made to identify the side for staining (unmarked side).

Incubation and Staining of PMMA Specimens with *C Albicans* Suspension. PMMA specimens were kept in distilled water for 17 days; the water was changed daily to achieve saturation. The polymer specimen was then collected in a tube (Soria Genlab), 2 mL of yeast suspension were added, and the tube was incubated in an oven (Model 207 Selecta-P; Abrera) at 37°C for 2 hours. The specimens were removed from the oven and rinsed twice with 25 mL sterile PBS solution for 1 minute and gently shaken manually to remove yeast not adhered to the polymer specimens. This procedure was performed 5 times for each PMMA type and in duplicate.[22,23,30] Once the specimens were dry, they were placed on petri dishes (Soria Genlab) with 8 drops of acridine orange solution added to the surface, rinsed after 1 minute with 25 mL sterile distilled water, and left to dry.[22,30]

Count of *C Albicans* Adhered to the Surface of PMMA Specimens. A count of 10 fields was done on each specimen, selecting areas uniformly distributed on the specimen surface. The area of each field, studied at 40 × 10 magnification, was 0.152 mm^2.

Surface Energy Determination of PMMA Specimens. The surface energy is the force exerted on the surface of the liquid per unit of length and is produced by cohesion forces (Van der Waals, ionic); the units for its measurement are $J.m^{-2}$ and $N.m^{-1}$, since $J.m^{-2} \times m/m^2 = N \times m^{-1}$. The contact angle θ of a liquid with a solid surface derives from the balance of a set of forces (γsg, γsl, γlg) that result from the interaction of the liquid with the solid and the surrounding gas. The Young equation can be calculated from these variables:

$$\cos \theta = \gamma_{sg} - \gamma_{sl}/\gamma_{lg}$$

According to this formula, a drop of liquid placed on an ideal solid surface will spread until the angle θ reaches the value given by the Young equation. The surface energy of a solid is calculated using the state equations of Antonov and Berthelot: γsl is related to γsg and γlg as $\gamma sl = [\gamma lg - \gamma sg]$ in the former and as $\gamma sl = \gamma lg + \gamma sg - 2\sqrt{\gamma lg - \gamma sg}$ in the latter. Combining these equations with that of Young, the following expression is obtained:

$$\cos \theta = -1 + 2\sqrt{\frac{\gamma sg}{\gamma lg}}\, e^{\beta(\gamma lg - \gamma sg)^2}$$

according to which the surface energy of a solid can be determined from the contact angles when the surface energy of the liquid and β are known. Therefore, γlg and β can be determined by least-squared fit of the experimental data to the above equation.[38,39] In the present case, the following liquids were used of known surface energy (in parentheses): Water (72.70 mJ/m^2), glycerol (63.13 mJ/m^2), formamide (59.08 mJ/m^2), diethylene glycol (45.16 mJ/m^2), and dimethyl sulfoxide (42.68 mJ/m^2).

The cleanest and most ideal surfaces possible were used to determine the contact angle, and a new surface was used for each determination. To obtain the best possible surface, the specimens underwent water polishing treatment. Although it is desirable to polymerize the material between 2 glass or mica slides, it was not feasible to perform the polishing until after the polymerization. The water polishing was performed manually, first with a silicone carbide water sander (P1200 [3/0]; El Tren) using circular movements for 30 s, and then with a water sander (P1600 [4/0]; El Tren), again using circular movements for 30 s. A contact angle goniometer (model 100-00, Ramé-Hart) was used to determine the contact angles between the different liquids and the PMMA specimens. Thirty-three measurements of each acrylic resin specimen were made with each liquid.

Data Treatment

Excel 2000 spreadsheets (Microsoft) were used to record data on the adhesion of yeast to the different acrylic resin types and data obtained from measurements of the contact angles of diverse liquids with different acrylic polymers. Statistic analyses of the data were done by means of the SAS 8.1 statistical program (SAS Institute). A variance analysis and Duncan's multiple range test were used to determine the differences in *C albicans* adhesion among the different resin types. Surface energy values of the different PMMA types were calculated from the state equation by a least-squares curve fit procedure using the MATHCAD 3.1 program (Mathsoft). The relationship between the surface energy of the acrylic resins and the degree of yeast adhesion was determined by fitting the regression lines.

Results

The adhesion values are summarized in Table 2 as means ± standard deviations and depicted graphically in Fig 1, showing the differences in adhesion among the different resin types. The value assigned to each PMMA type corresponds to a count of 10 fields in 10 specimens of each type. Hence, the total mean value for each PMMA is obtained from 100 counts. The variance analysis showed a value of $P = .0001$, indicating statistically significant differences in the adhesion of *C albicans* among the different resin types. Figure 2, obtained from the results of Duncan's multiple range test, shows the statistically significant differences among resin types.

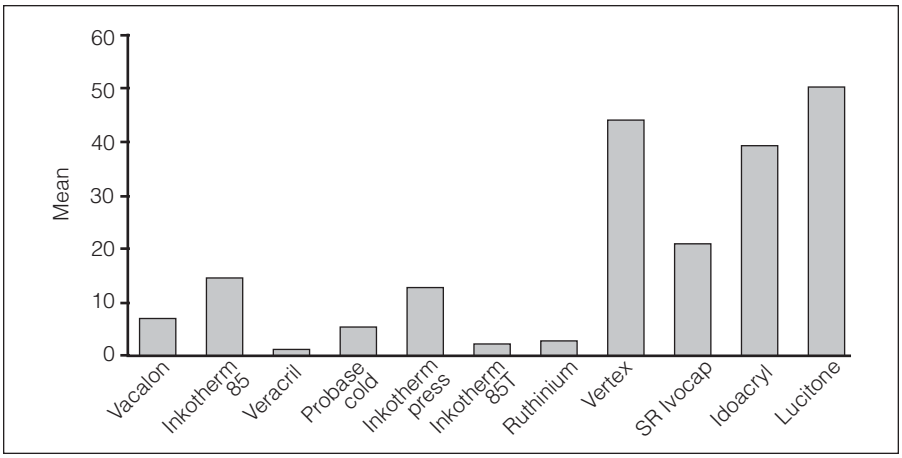

Fig 1 Bar chart of final mean yeast count/field for each resin type, using fluorescence microscope.

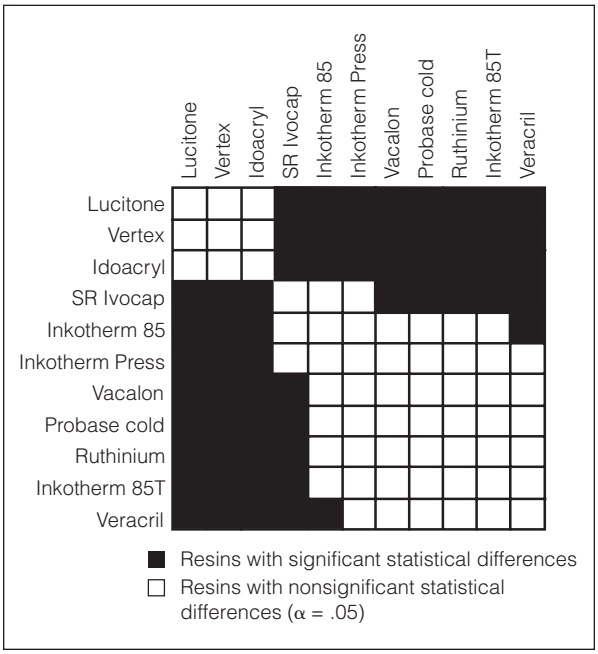

Fig 2 Presence and absence of statistically significant differences in adhesion of *C albicans* among resin types (α = .05).

Table 2 Mean Yeast Count Per Field and mm² for Each Resin Type

Resin	Mean yeast/ field	SD	Mean yeast/ mm²
Vacalon	6.89	2.4	46.00
Inkotherm 85	14.57	2.8	95.85
Veracril	1.09	0.2	7.12
Probase Cold	5.30	1.1	34.90
Inkotherm Press	12.91	2.6	84.93
Inkotherm 85 T	1.96	1.3	12.80
Ruthinium	2.65	1.7	17.43
Vertex	44.16	23.1	297.10
SR Ivocap	21.01	8.3	138.20
Idoacryl	39.49	16.2	264.40
Lucitone	50.28	25.3	330.80

Table 3 shows the mean value (in degrees) of the tangent of the angle formed between the surface of each resin type and a drop of each liquid used. The value expressed for each liquid is the mean of 33 measurements. This table also displays the resulting surface energy value for each PMMA type, calculated from values of the tangents between the different liquids and specimens. The *C albicans* adhesion values (Table 2) were compared with the surface energy values (Table 3) of the different PMMA specimens by simple regression analysis, but no significant relationship was observed (Fig 3).

Discussion

The diversity of methods used to determine the adhesion of *C albicans* to PMMA resins hampers the comparison of results. Even if the same system were used, the absence of an established protocol means that research groups use different *C albicans* strains, yeast concentrations, time and temperature conditions, procedures for removal of unattached yeast, and staining methods. In the present study, the adhesion of *C albicans* to different types of PMMA was measured by optical count, and statistically significant differences were observed after application of a parametric (analysis of variance) analysis (Fig 2). Differences in *C albicans* adhesion among different resin specimens were also reported by other authors, such as Minagi et al,[20] Klotz and Drutz,[19] Waters et al,[30] and Radford et al.[5] These variations derive from mechanisms operating during the first phase of the adhesion and are determined by the surface energies of the PMMA and the *C albicans*.[21]

A wide variation in adhesion was found among fields on the same PMMA specimen. Clusters of yeast were observed in the acrylic resin base with greatest adhesion to *C albicans*, making an accurate count very difficult. Microscopic observation of the same PMMA specimen showed some fields with very little yeast and others with numerous clusters of yeast, as also de-

Table 3 Mean Values (degrees) of the Tangent of the Angle Formed Between the Surface of Each Resin Type and a Drop of Each Liquid Used, and Surface Energy Values of the Different PMMA Specimens

Resin	Water	Glycerol	Fomamide	Dethylenglycol	Dimethylsulfoxide	Surface energy
Vacalon	70.52	65.39	50.94	33.82	16.70	40.86
Inkotherm 85	72.52	66.76	51.33	33.30	15.88	40.93
Veracril	71.97	67.12	48.82	32.52	14.97	41.20
Probase Cold	70.76	67.48	49.61	32.85	14.64	40.60
Inkotherm Press	67.48	64.91	49.79	34.06	15.79	40.10
Inkotherm 85 T	71.42	65.06	49.82	34.55	14.94	40.30
Ruthinium	62.50	66.12	48.30	34.48	15.36	40.00
Vertex	69.76	67.00	49.18	32.18	15.67	40.45
SR Ivocap	70.48	69.09	53.55	36.00	21.97	38.78
Idoacryl	71.67	66.61	49.36	33.36	14.12	40.62
Lucitone	68.64	68.70	52.18	35.85	22.33	

scribed by Verran and Maryan[22] and Radford et al.[5] The count also varied widely among different specimens of the same type of PMMA, as found by Minagi et al[20] and Waters et al,[30] who reported standard deviations of 550 and 102 for mean counts of 1,749 and 373 yeast/mm^2, respectively. These differences are generally more marked when the mean yeast adhesion is greater. Thus, differences in the mean count of adhered *C albicans* were much wider among Veracril than among Lucitone tinted denture base specimens. Without the coloring, coupling, or stabilizing agents added for its dental use, PMMA has a surface energy of 38.5 $\pm$ 0.5 mJ/m^2.[39] Surface energy values obtained for the present specimens ranged from 38.78 mJ/m^2 to 40.93 mJ/m^2. As shown in Table 2, variations in the composition of the specimens were not translated into large differences in surface energy. Minagi et al[20] reported a much wider variation in surface energy values, ranging from $<$ 20 mJ/m^2 to $>$ 40 mJ/m^2. This discrepancy may be related to differences in the method used to calculate the surface energy. Minagi et al[20] used only distilled water and made the calculation from the tangents of the angle formed between the water and the acrylic resin surface. Values obtained with a single liquid are subject to greater error, and the more different liquids that are used, the more accurate the result. A surface energy value for the Lucitone resin could not be established, because it was not possible to fit a least-squares curve to the data obtained. This error may be a result of alterations of the angles by an interaction of some of the liquids used with 1 or more PMMA components. For an accurate calculation of the surface energy, there must be no interaction between the study material and the liquids used to determine the tangent of the contact angle.

No association was found between the *C albicans* adhesion results and the surface energy values of the different PMMA specimens studied (Fig 3). In contrast, Minagi et al[20] found a positive linear relationship

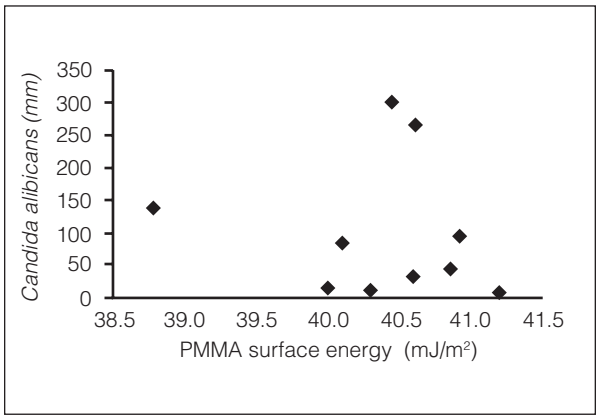

Fig 3 Surface energy values in relation to the adherence values obtained by optical count.

between the surface energy and *C albicans* adherence. This association was also reported by Klotz and Drutz,[19] who studied very different plastic polymers, such as polystyrene and Teflon, as well as PMMA. Other authors, such as Waters et al,[30] found no clear relationship between the degree of adhesion and the surface energy, and indicated that the surface energies of the microorganism and culture medium used also play a role.[31] These differences in adhesion may also be influenced by the action of some components of PMMA specimens, as occurs with some soft denture lining materials, which reduce the adhesion and inhibit the growth of *C albicans*.[14–18] Dibutyltin dilaurate, vinyl silane, and zinc dimethyldithiocarbamate, among other substances, are associated with an inhibitory effect.[16] In the case of PMMA, no specific substance has been identified, although the residual monomer produces differences in the resin surface charge that can affect the adhesion.[22]

Therefore, the role played by the surface area of the material to which *C albicans* adheres is not clear because of the influence of other variables, such as the

surface energies of the microorganism and culture medium. The components of the material can modify the degree of adhesion, which can also vary when the polymer is subjected to a large number of thermal cycles, possibly because residual substances are modified.[27–29] However, the above factors are of less importance than the roughness of the surface and the saliva-dependent acquired pellicle.

Conclusions

For a surface of the same roughness, specimens of different PMMA types showed statistically significant differences in *C albicans* adhesion per square millimeter. Variations in the composition of different PMMA types had no major effect on their surface energy; hence variations in *C albicans* adhesion could be attributed to this factor. No relationship was found between *C albicans* adhesion and the surface energy of the PMMA specimens. According to these results, variations in the adhesion of *C albicans* to different types of PMMA are not related to the differences in surface energies that different resin types should theoretically have because of the variability of their composition. Knowledge of the causes of these differences in the adhesion of *C albicans* would allow manufacturers to develop more suitable resins for patients who are susceptible to prosthetic stomatitis.

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Literature Abstract

Inability to relate tooth forms to face shape and gender

Gender and face shape have been considered for selecting the maxillary anterior teeth in removable prosthodontics. This selection has been issued with increasing esthetic demand. The purpose of this study was to examine gender-dependent correlations between inverted face shape and maxillary central incisor shape. Two hundred four (102 males, 102 females) dental students participated in the study. Photographs for portrait and of the anterior teeth were taken with standardization. The inverted face and the right maxillary central incisor shapes were evaluated by a quotient of 3 categories: tapered, ovoid, and square. The quotient was calculated with ratio of length between 2 lines on the photographs. Five professional clinicians and 5 postgraduate students participated. They were asked to determine the gender of the subject on the basis of the tooth form. A second survey was performed with the same participants and with the same subjects, in different order. All of the statistical analysis was conducted at a 95% level of confidence. Tooth shape was classified in 25% of cases as tapered, in 39% of cases as ovoid, and in 36% of cases as square-shaped. No significant correlation was found between tooth form and gender ($P > .05$). There was no significant correlation ($P > .05$) between tooth shape and face shape. Only one-third of all cases showed the same classification in face and tooth shapes. Participating clinicians made decisions on gender by anterior teeth with an accuracy of 55 ± 4% in the first survey, and 57 ± 8% in the second. The correspondence between the 2 studies was 66 ± 8%. The authors suggested that the traditional rules for selecting anterior teeth should be reevaluated. They concluded that the patient's involvement should be ensured for optimal selection in each individual.

Wolfart S, Menzel H, Kern M. *Eur J Oral Sci* 2004;112:471–476. **References:** 27. **Reprints:** Dr Stefan Wolfart, Department of Prosthodontics, Propaedeutics and Dental Materials, School of Dentistry, Christian-Albrechts Universtiy, Arnold-Heller-Str 16, D-24105 Kiel, Germany. E-mail: swolfart@proth.uni-kiel.de—*Eunghwan Kim, Lincoln, NE*