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Influence of surface characteristics on the adhesion of *Candida albicans* to various denture lining materials

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Abstract

Objective. This study evaluated the influence of surface characteristics of various denture lining materials on the adherence of *Candida albicans.* ***Materials and methods.*** Four different types of materials (tissue conditioners, acrylic and silicone soft liners and hard reline materials) were selected. Disk-shaped material specimens were prepared and their surface roughness values (R_a) measured using a profilometer. The contact angles of four reference liquids were measured on the material surfaces and surface energy parameters (total surface energy, acid and base components, degree of hydrophobicity/hydrophilicity) of the materials were calculated in accordance with acid-base theory. Specimens were incubated with *C. albicans* and adhering fungi quantified using the colony counting method. Data were statistically analyzed using a one-way ANOVA with Games–Howell post-hoc test ($\alpha = 0.05$). Pearson correlation analysis was applied to detect correlations between surface characteristics and *Candida* adhesion. ***Results.*** Significant differences in the surface roughness of the materials were found ($p < 0.001$). The acrylic soft liners were more hydrophilic than the other materials. Overall, the acrylic soft liners and tissue conditioners showed significantly greater *Candida* adhesion than silicone soft liners and hard reline materials ($p < 0.05$). The Pearson correlation analysis indicated that the base component and degree of hydrophobicity/hydrophilicity of the materials ($p = 0.005/0.008$), rather than the total surface energy and the surface roughness ($p = 0.093/0.057$), affected *C. albicans* adherence in a statistically significant way. ***Conclusions.*** The adhesion of *C. albicans* to denture lining materials can be accounted for in terms of interfacial acid-base interactions.

Key Words: *adhesion, Candida albicans*, *denture lining materia*l, *roughness*, *surface energy parameter*

Introduction

Denture stomatitis is a common pathologic change found in the oral mucosa of denture-bearing tissues under complete or partial dentures [1,2]. Although *Candida albicans* is not a determining factor for the disease, it has been reported to play a major role in the disease in most studied patients [3]. Since yeast cells are washed out by saliva and swallowed unless they adhere and replicate [2], an essential pre-requisite for the successful colonization of host tissues by the micro-organism is the ability to adhere to superficial epithelial cells or to a closely associated material surface [2,4]. Thus, denture stomatitis is found more frequently in the maxilla [1], probably indicating that the upper denture acts as a reservoir for infection [2].

The fitting surface of an acrylic denture requires replacement to improve the fit of the denture [5]. According to McCabe and Walls [5], denture lining materials can be classified into three groups: tissue conditioners, soft lining materials and hard reline materials. Although they are usually constructed from either acrylic compounds or silicone elastomers [5,6], commercially available denture lining materials vary greatly in their compositions [1]. Therefore, degree of *Candida* adhesion for the onset of denture stomatitis may depend on the surface characteristics of denture base resins or denture lining materials. Some studies have suggested that the initial attachment of *C. albicans* is greater on soft lining materials than on acrylic and enhanced on rougher surfaces [1,4,7]. However, studies investigating the influence

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Table I. Denture lining materials used.

Code	Material	Manufacturer	Main composition (manufacturer supplied)	Batch number
TC	Tissue Conditioner II	Shofu Inc., Kyoto, Japan	Powder: poly(ethyl methacrylate) Liquid: di-*n*-butyl sebacate, ethanol	Powder: 051095; Liquid: 041066
CC	Coe-Comfort	GC America Inc., Alsip, IL, USA	Powder: zinc undecylenate Liquid: benzoyl benzoate, ethanol	Powder: 1003171; Liquid: 1005261
CS	Coe-Soft	GC America Inc., Alsip, IL, USA	Powder: zinc undecylenate Liquid: benzyl salicylate, ethanol	Powder: 1010251; Liquid: 1011011
DB	DuraBase Soft	Reliance Dental Mfg. Co., Worth, IL, USA	Powder: poly(ethyl methacrylate), benzoyl peroxide Liquid: methyl methacrylate	Powder: 021210; Liquid: 031010
MS	MucoSoft	Parkell Inc., Edgewood, NY, USA	Proprietary compound, methyl methacrylate, methylene chloride	MU-10335/10335
RL	GC Reline Soft	GC Corp., Tokyo, Japan	Base: vinyl dimethyl polysiloxane, silicone dioxide, hydrogen polysiloxane Accelerator: vinyl dimethyl polysiloxane, silicone dioxide	1102031
KL	Kooliner	GC America Inc., Alsip, IL, USA	Powder: poly(ethyl methacrylate), benzoyl peroxide, silicon dioxide Liquid: isobutyl methacrylate, 2,4-dihydroxy benzophenone	Powder: 1010271; Liquid: 1008101
DL	Dura-Liner II	Reliance Dental Mfg. Co., Worth, IL, USA	Powder: poly(ethyl methacrylate), benzoyl peroxide Liquid: methyl methacrylate	Powder: 062596; Liquid: 100496

of surface energy of denture base resins or denture lining materials on *Candida* adherence appear inconsistent. Waters et al. [8] obtained no conclusive relation between the surface energy of denture soft lining materials and the degree of *C. albicans* adherence. Serrano-Granger et al. [3] and Pereira-Cenci et al. [9] reported little or no influence of acrylic resin or denture liner surface energy on *Candida* adhesion. In contrast, Henriques et al. [2] suggested that Lewis acid-base interactions played a major role in the process of *Candida* adhesion to acrylic. Thus, relatively limited attention has been paid to the influence of surface energy on adherence of *C. albicans* to denture base resins or denture lining materials, so that no definite conclusion has been reached about this issue.

For the present *in vitro* study of *C. albicans* adhesion, we selected eight materials including tissue conditioners, soft liners and hard reline materials, all thought to have different surface characteristics from each other. We employed the multi-component acid-base approach to determine which surface energy parameters of the materials might individually affect *Candida* adherence [2].

Materials and methods

Denture lining materials used

Two tissue conditioners (Tissue Conditioner II, TC; Coe-Comfort, CC), two acrylic soft liners (Coe-Soft, CS; DuraBase Soft, DB), two silicone soft liners (MucoSoft, MS; GC Reline Soft, RL) and two hard liners (Kooliner, KL; Dura-Liner II, DL) were tested in this study. Their codes, manufacturers, batch numbers and main compositions are summarized in Table I.

Surface roughness measurements

The surface roughness (R_a) of the eight denture lining materials was determined as follows. Cylindrical Teflon molds (10 mm in diameter, 1 mm in height) were placed on a polyester strip (KerrHawe SA, Bioggio, Switzerland) over a glass slide. Each material was prepared according to the manufacturer's instructions, filled into the mold, covered with another polyester strip and glass slide, gently pressed to expel the excess material, and self-cured. After 6 h, the strips were removed from the specimens and the R_a of each specimen was measured using a previously calibrated profilometer (Surftest SV-400, Mitutoyo Corp., Kawasaki, Japan) at a stylus speed of 0.1 mm/s, a cut-off of 0.8 mm and a range of 600 μm. Ten specimens per material were prepared and the R_a of each specimen was recorded as the average of the five readings.

Contact angle measurements and the calculation of surface energy parameters

Surface energy parameters of the denture lining materials were calculated based on the acid-base theory proposed by van Oss et al. [10,11]. Briefly, the Young

Table II. R_a surface roughness (μm) (SD) of denture lining materials tested.

TC	CC	CS	DB	MS	RL	KL	DL
0.98 (0.17)^{ab}⋆	1.34 (0.30)^{a}	1.13 (0.25)^{ab}	0.87 (0.15)^{b}	0.23 (0.03)^{c}	0.36 (0.05)^{d}	0.11 (0.02)^{e}	0.12 (0.01)^{e}

TC, Tissue Conditioner II; CC, Coe-Comfort; CS, Coe-Soft; DB, DuraBase Soft; MS, MucoSoft; RL, GC Reline Soft; KL, Kooliner; DL, Dura-Liner II.
⋆The same superscripted letter indicates statistically equivalent values among the materials ($p > 0.05$).

equation states [12]: $\gamma_s = \gamma_{sl} + \gamma_l \cos\Theta$, where Θ is the contact angle and γ_s, γ_{sl} and γ_l are the surface tensions of the solid, solid-liquid and liquid surfaces, respectively. The Young-Dupré equation is expressed as $W^{adh} = \gamma_l(1 + \cos\Theta)$ [12], where W^{adh} is the work of adhesion of the liquid to the solid. Combining the equation with the Lifshitz-van der Waals/Lewis acid-base theory yields the following equation [10,11]: $\gamma_l(1 + \cos\Theta) = 2[(\gamma_s^{LW}\gamma_l^{LW})^{1/2} + (\gamma_s^{A}\gamma_l^{B})^{1/2} + (\gamma_s^{B}\gamma_l^{A})^{1/2}]$, where the superscripts LW, A and B refer to the Lifshitz-van der Waals, acid and base components, respectively.

For contact angle measurement, 10 specimens per material were prepared in the same way as for surface roughness measurement. The contact angles were then determined by the sessile drop method at room temperature using a contact angle measurement apparatus (OCA 15 plus, DataPhysics Instrument GmbH, Filderstadt, Germany) on the surfaces of the materials for each of four different test liquids with known surface energy parameters: water, glycerol, ethylene glycol and 1-bromonaphthalene [13,14]. By determination of Θ with the four reference liquids, γ_s^{LW}, γ_s^{A} and γ_s^{B} were calculated with software (SCA20, DataPhysics Instrument GmbH), using the least square method [13,14]. The total surface energy γ_s of the materials was also calculated using the software, based on the equation [15]: $\gamma_s = \gamma_s^{LW} + 2(\gamma_s^{A}\gamma_s^{B})^{1/2}$.

The boundary between hydrophobicity and hydrophilicity of a solid material (s) in the presence of water (w) is equal to the cohesive polar attraction between the water molecules [16]. The work of cohesion, W^{coh}, can be expressed in terms of the free energy, G, using thermodynamic notation, so that $\Delta G^{coh} = -2\gamma = -W^{coh}$ [15]. Thus, the degree of hydrophobicity and hydrophilicity of the denture lining materials can be linked to the magnitude of $\Delta G_{sws} = -2\gamma_{sw}$, where $\gamma_{sw} = \gamma_{sw}^{LW} + \gamma_{sw}^{AB}$ [16]. The LW interfacial tension and the AB interfacial tension are calculated using the following equations, respectively [16]: $\gamma_{sw}^{LW} = [(\gamma_s^{LW})^{1/2} - (\gamma_w^{LW})^{1/2}]^2$ and $\gamma_{sw}^{AB} = 2[(\gamma_s^{A}\gamma_s^{B})^{1/2} + (\gamma_w^{A}\gamma_w^{B})^{1/2} + (\gamma_s^{A}\gamma_w^{B})^{1/2} - (\gamma_w^{A}\gamma_s^{B})^{1/2}]$.

C. albicans adhesion

Specimens for *C. albicans* adhesion test were prepared as described above, except for the size of the diameter of Teflon mold (8 mm). After removing the polyester strips, all specimens were sterilized under ultraviolet light for 16 h. *C. albicans* (ATCC 10231) were streaked on Sabouraud dextrose agar (BD Difco, Sparks, MD) and incubated at 37°C for 24 h, then further incubated in a 3% sucrose biofilm medium (BM) whose composition has been detailed elsewhere [17,18], overnight without shaking. Thereafter, 2% of the culture broth was inoculated to fresh BM-sucrose and incubated for 2–4 h to adjust the optical density of the yeast suspension to 0.2 at 600 nm. The optical density was monitored using a microplate reader (Sunrise, Tecan Austria GmbH, Grödig, Austria). Each specimen was placed into a tube containing 5 mL of the prepared *C. albicans* broth and incubated for 2.5 h at 37°C [19]. After washing 3-times with PBS, each specimen was transferred to a microtube containing 1 mL PBS. The adherent yeast cells were then detached by sonication using four 30-s pluses with three 30-s intermittent coolings [20]. The yeast suspensions were serially diluted up to 10^{-1}–10^{-3} and 100 μL of each dilution was plated on Sabouraud dextrose agar. After 24–48 h incubation at 37°C, the colony forming units per milliliter (CFU mL^{-1}) were calculated. Colony counts were expressed as a CFU per unit area of the specimen (mm^2). These experiments were run in triplicate or quadruplicate and repeated three separate times (total 10-times).

Scanning electron microscopy for C. albicans-attached specimens

C. albicans was adhered to the denture lining materials as described above and the adhesion was observed using a field emission-scanning electron microscope (FE-SEM; JSM-6700F, Jeol, Tokyo, Japan). The specimens with the adhering fungi were fixed, dried and sputter coated with platinum for investigation with FE-SEM.

Statistical analysis

The surface roughness and CFU data were statistically analyzed using a one-way ANOVA with Games-Howell post-hoc multiple comparison test. Pearson correlation analysis was applied to detect possible correlations between each calculated surface energy parameter and the *Candida* adhesion. All statistical

Table III. Values of contact angles (°) (SD) measured with water (Θ_w), glycerol (Θ_g), ethylene glycol (Θ_e) and 1-bromonaphthalene (Θ_b) on denture lining materials.

Material	Θ_w	Θ_g	Θ_e	Θ_b
TC	91.7 (4.7)	89.6 (2.7)	69.5 (3.8)	37.8 (3.6)
CC	84.5 (1.3)	82.8 (3.0)	69.0 (3.6)	41.4 (1.4)
CS	72.3 (3.4)	97.7 (1.8)	67.4 (1.8)	46.5 (2.7)
DB	63.7 (2.7)	91.0 (3.7)	56.5 (1.2)	40.1 (3.5)
MS	105.9 (5.4)	94.3 (5.8)	87.0 (2.2)	49.9 (4.8)
RL	103.3 (3.2)	103.4 (1.5)	90.9 (3.0)	44.8 (2.7)
KL	88.1 (2.7)	91.6 (3.3)	88.0 (4.9)	32.5 (2.0)
DL	82.3 (2.1)	82.4 (4.3)	58.4 (2.3)	36.6 (1.8)

TC, Tissue Conditioner II; CC, Coe-Comfort; CS,Coe-Soft; DB, DuraBase Soft; MS, MucoSoft; RL, GC Reline Soft; KL, Kooliner; DL, Dura-Liner II.

analyses were performed using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL) at a level of significance of 0.05.

Results

The surface roughness values (R_a) of the denture lining materials are summarized in Table II, their means ranging from 0.11–1.34 μm (one-way ANOVA, $p < 0.001$). Overall, the tissue conditioners (TC and CC) and the acrylic soft liners (CS and DB) showed rougher surfaces than silicone soft liners (MS and RL) and the hard reline materials (KL and DL).

Tables III and IV show the contact angles of the four reference liquids measured on the denture lining materials and the surface energy parameters derived from the contact angle measurements, respectively. The total surface energy (γ_s) values ranged from 29.87–40.99 mJ/m^2. All materials tested in the present study showed large base components and very small acid components, except for the MS and RL liners, whose base components were similar to their acid components. ΔG_{sws} values indicating the degree of hydrophobicity/hydrophilicity demonstrated that the acrylic soft liners (CS and DB) were more hydrophilic than the other materials.

Representative *Candida* adhesion patterns examined by FE-SEM are shown in Figure 1. The majority of cells observed on the surfaces of the denture lining materials were hyphal forms and occasionally in the blastospore phase. The *C. albicans* adhesion expressed in CFU mm^{-2} is depicted in Figure 2. DB showed the highest yeast cell adhesion, followed by CS, without any statistically significant difference between them ($p = 0.266$). TC and CC exhibited moderate *Candida* adhesion, the former significantly higher than the latter ($p = 0.028$). The silicone soft liners and hard liners (MS, RL, KL and DL), all statistically equivalent ($p > 0.05$), yielded significantly lower *Candida* adhesion than the other four materials ($p < 0.05$).

Table V summarizes the results of the Pearson correlation analyses between individual surface characteristics and *C. albicans* adhesion. A significant positive linear correlation was found between the base component, γ_s^B, and *Candida* adherence ($r = 0.869$, $p = 0.005$). In addition, γ_{sw}^{AB} and ΔG_{sws} showed significant negative and positive linear correlations with adherence, respectively ($r = -0.844/0.846$, both $p = 0.008$). On the other hand, no statistically significant correlation was found between surface roughness and the adhesion ($p = 0.057$).

Discussion

Adherence of a micro-organism to a surface is believed to be a two-stage process: non-specific initial interactions and specific adhesion-receptor interactions [8]. The initial adhesion of micro-organisms to surfaces may be related to the surface characteristics (e.g. surface free energy, roughness) of the surfaces and the micro-organisms [8,21]. In the present study, a simple *in vitro* model was employed to compare the adherence of *C. albicans* to various commercial

Table IV. Total surface energy (γ_s) and surface energy parameters calculated from the contact angle measurements.

Material	γ_s	γ_s^{LW}	γ_s^A	γ_s^B	$\gamma_s^A\gamma_s^B$	γ_{sw}^{LW}	γ_{sw}^{AB}	ΔG_{sws}
TC	37.77	35.69	0.25	4.37	1.09	1.70	26.93	−57.26
CC	35.41	33.97	0.07	7.61	0.53	1.34	21.93	−46.54
CS	40.99	32.18	0.68	28.39	19.31	1.01	−2.35	2.69
DB	40.63	35.57	0.20	31.34	6.27	1.68	−5.05	6.74
MS	29.87	29.33	0.33	0.22	0.07	0.56	41.00	−83.12
RL	33.75	32.08	0.76	0.92	0.70	0.99	34.18	−70.34
KL	40.21	36.39	0.60	6.13	3.68	1.86	22.01	−47.73
DL	36.68	36.19	0.01	7.81	0.08	1.81	22.32	−48.28

TC, Tissue Conditioner II; CC, Coe-Comfort; CS, Coe-Soft; DB, DuraBase Soft; MS, MucoSoft; RL, GC Reline Soft; KL, Kooliner; DL, Dura-Liner II; γ_s^{LW}, Lifshitz-van der Waals component; γ_s^A, acid component; γ_s^B:, base component; γ_{sw}^{LW}, LW interfacial tension; γ_{sw}^{AB}, AB interfacial tension; ΔG_{sws}, degree of hydrophobicity/hydrophilicity. All values are in mJ/m^2.

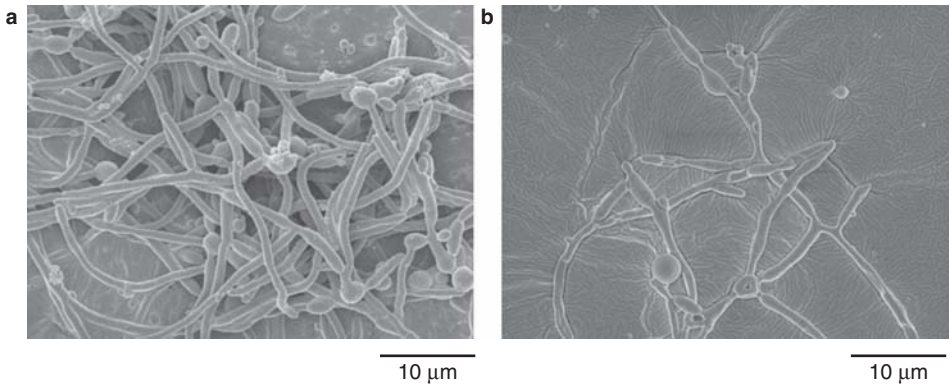

Figure 1. Scanning electron micrographic images of *C. albicans* attachment: DuraBase Soft (A) and Dura-Liner II (B) (original magnification × 1500, bar represents 10 μm).

denture lining materials in terms of thermodynamics. The findings suggest that certain surface energy parameters of the materials account for the degree of *Candida* adhesion.

This study of *C. albicans* adhesion to the eight materials found higher yeast counts in the tissue conditioners and acrylic soft liners than in the silicone soft liners and hard reline materials (Figures 1 and 2), suggesting that acrylic soft liners are less favorable than silicone soft liners in terms of initial adhesion of *C. albicans*. Although tissue conditioners are susceptible to colonization by micro-organisms, clinicians are aware that tissue conditioner should be replaced regularly (e.g. every 2–3 days) for adequate conditioning and to prevent it from serving as a reservoir for micro-organisms [5,6]. On the other hand, soft lining materials are maintained longer (e.g. a month or two, or permanently) in the oral cavity [5]. For use as a relatively long-term soft lining material, therefore, silicone elastomers may be a better choice than acrylic in terms of initial adherence of *C. albicans*. It should be noted, however, that relatively smooth-surfaced silicone soft liners were used for the *C. albicans* adhesion test (Table II). In practice, silicone soft liners may be difficult to finish and thus aggravate yeast infection [7,22]. Although the hard reline materials showed smoother surfaces than the other types of denture lining materials (Table II), their surface may also be susceptible to colonization by micro-organisms such as *C. albicans* due to porosity resulting from air inclusions during mixing [5].

The current study suggests that surface energy parameters derived from contact angle measurements account for the adherence of *C. albicans*. We used four reference liquids, including water, to calculate various components related to the surface-free energy of the denture lining materials. Although the water contact angle on a material surface alone may provide primary information about surface hydrophobicity/hydrophilicity [19,23], it cannot quantify the surface in such terms. In the present study, we used ΔG_{sws} to quantitatively compare denture lining material hydrophobicity and hydrophilicity. For all materials tested, the γ_{sw}^{LW} values were relatively constant and very small (varying between 0.56–1.86 mJ/m^2) (Table IV). As far as polar cohesion is concerned, thus, ΔG_{sws} indicates the degree to which the polar attraction of solid to water is greater (hydrophilic) or smaller (hydrophobic) than the polar attraction of water molecules to each other [16]. The order in the ΔG_{sws} was similar to that in the water contact angle, but not necessarily consistent (Tables III and IV). As regards the degree of hydrophobicity/hydrophilocity of a material surface, ΔG_{sws} obtained using water and other reference liquids constitutes a more appropriate measure than water contact angle [16]. The ΔG_{sws} calculated from the contact angle measurements in this study showed the hydrophilic characteristics of the two acrylic soft lining materials to be 2.69 and 6.74 mJ/m^2 for CS and DB, respectively. Among the other materials, the tissue conditioners exhibited similar ΔG_{sws} values to the hard relining materials. The silicone soft lining materials had the most negative ΔG_{sws} values (i.e. hydrophobic) among all materials

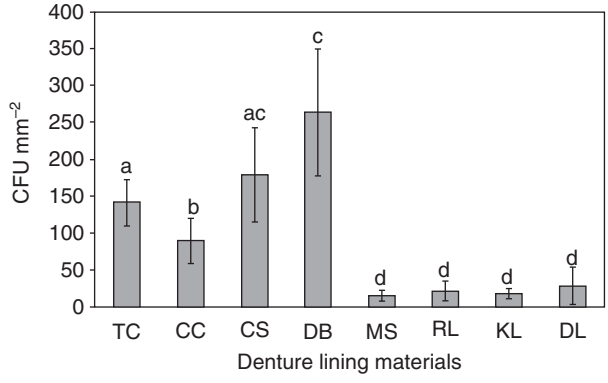

Figure 2. Bar chart of colony-forming unit (CFU) mm^{-2} for each denture lining material (vertical bar = ± 1 SD). TC, Tissue Conditioner II; CC, Coe-Comfort; CS, Coe-Soft; DB, DuraBase Soft; MS, MucoSoft; RL, GC Reline Soft; KL, Kooliner; DL, Dura-Liner II. The same lower case letters indicate statistically equivalent values among the materials ($p > 0.05$).

Table V. Pearson correlation coefficients (r) between surface characteristics (surface energy parameters and surface roughness) and *C. albicans* adhesion.

	γ_s	γ_s^{LW}	γ_s^{A}	γ_s^{B}	$\gamma_s^{A}\gamma_s^{B}$	γ_{sw}^{LW}	γ_{sw}^{AB}	ΔG_{sws}	R_a
r	0.632	0.230	−0.142	0.869	0.564	0.206	−0.844	0.846	0.693
p*	0.093	0.584	0.738	0.005	0.145	0.624	0.008	0.008	0.057

γ_s, total surface energy; γ_s^{LW}, Lifshitz-van der Waals component; γ_s^{A}, acid component; γ_s^{B}, base component, γ_{sw}^{LW}, LW interfacial tension; γ_{sw}^{AB}, AB interfacial tension; ΔG_{sws}, degree of hydrophobicity/hydrophilicity; R_a, surface roughness.
*In this study, a correlation coefficient is considered significant if the p-value is less than 0.05.

tested. In fact, the large differences in hydrophobicity between the tissue conditioners and acrylic soft lining materials were unexpected, given their similar composition [5]. Although the manufacturers do not fully disclose the composition of the materials (Table I), incorporated additives may considerably change their surface hydrophobicity/hydrophilicity.

Despite the remaining controversy regarding the influence of hydrophobicity and hydrophilicity on fungal adhesion, preferential adherence of hydrophobic and hydrophilic micro-organisms is apparent [2,19]. Therefore, the adherence of relatively hydrophilic species *C. albicans* was expected to increase in materials with more hydrophilic surfaces [24]. In the current study, the Pearson correlation coefficient between the ΔG_{sws} and the *C. albicans* adhesion showed a significant positive linear correlation (Table V). This implies that the hydrophobicity/hydrophilicity of the denture lining materials plays a role in the process of *Candida* adhesion.

As stated earlier, some studies on the effect of total surface energy of acrylic resins and denture lining materials have shown inconsistent results [2,3,8,9]. It should be noted that different methods for calculating surface-free energy of solids after contact angle measurements on the surfaces may yield different results [25]. Applying the equation-of-state (EOS) approach, Minagi et al. [24] demonstrated that the adherence of *C. albicans* increased with greater surface-free energy of various denture base resin materials, whereas Serrano-Granger et al. [3] and Pereira-Cenci et al. [9] suggested no correlation between the two variables. It is possible that specific ingredients of the materials directly reduce the adhesion of *C. albicans* as well as alter surface characteristics [1,3], although such an inhibitory effect of soft lining materials on yeast growth was not apparent in the present study (Figure 2). Otherwise, the EOS approach, still believed useful [26], may sometimes fall short in interpreting the contact angles of liquids with similar surface tensions and differing fractions of non-dispersive interactions because it considers solid–liquid interfacial tension a function of the total solid and liquid surface tensions alone [25]. In the present study, therefore, we employed one of the surface tension component approaches, acid-base theory, to obtain several surface energy components together with total surface energy of the denture lining materials used. Despite the many controversies over results obtained by this approach, it helps better account for the examined phenomena in terms of interfacial acid-base interactions. According to the Pearson correlation analysis, *C. albicans* adhesion was significantly affected by the base component of the materials, but not by the total surface energy (Table V). As shown in Table IV, the non-dispersive components also appeared to govern the hydrophobicity/hydrophilicity of the materials.

It seems that surface roughness is related to deposit formation and micro-organism colonization of surfaces. Greater surface roughness means a greater specific surface area, and thus more available surface active sites for thermodynamic reaction [27]. In the present study, surface roughness of the denture lining materials was also measured, as it is a factor in the entrapment of micro-organisms on surfaces and in their protection from shear forces [7]. While all the material surfaces were prepared against a smooth polyester strip, significant differences in the surface roughness values (R_a) were detected (Table II), though no statistically significant correlation between the R_a values and *C. albicans* adhesion were revealed by the Pearson correlation analysis (Table V). This finding implies that surface energy parameters of the materials play a greater role in *C. albicans* adhesion rather than surface roughness when the latter is controlled within a range (e.g. $R_a < \sim 1.5$ μm).

Moreover, changes in surface roughness can influence the contact angle, thereby changing surface energy characteristics [28–30]. Most theories of solid surface energy have their basis in the Young equation, which assumes an ideal (i.e. chemically homogeneous, rigid and flat at an atomic scale) surface [15]. Moreover, the solid should not interact in any fashion with the probe liquid [31]. However, real surfaces in most experimental conditions differ from ideal ones, firstly in terms of surface roughness [31]. According to Busscher et al. [28], changes in solid surface R_a below 0.1 μm have no effect on contact angle. In the present study, surface energy parameters were calculated based on initial contact angle measurements of the denture lining materials because the initial angle is a measure of adhesion [9,32]. As the R_a values of the denture lining

materials ranged from 0.11–1.34 μm in this study (Table II), the actual contact angles could have deviated from the estimated contact angles obtained from Young's equation [15,31]. Therefore, more accurate experimental approaches to measuring contact angles should be developed for the surface characterization of denture lining and other dental materials.

The current study focused on the influence of surface energy parameters, including hydrophobicity/hydrophilicity, of eight selected denture lining materials on the initial adhesion of *C. albicans*. The results of this study suggest that the adhesion of *C. albicans* to denture lining materials can be accounted for in terms of interfacial acid-base interactions. Although this micro-organism is the major pathogen in both oral and systemic candidosis [19], other related *Candida* species should also be considered to draw a general conclusion, particularly because non-*albicans* species such as *C. tropicalis*, *C. glabrata* and *C. dubliniensis* are hydrophobic and thus may have different tendencies to adhere to denture lining materials [24]. Moreover, dental materials including denture lining ones are coated by saliva when exposed in the oral cavity. It is possible that coating of denture lining materials by a liquid medium such as saliva influences the results of *Candida* adhesion [2]. According to Millsap et al. [33] and Busscher et al. [34], saliva enhances *Candida* adhesion to acrylic surfaces, whereas it discourages adhesion to silicone elastomer. However, there remains conflicting evidence whether such salivary coating reduces or enhances the adhesion of *C. albicans* [19]. One reason for this discrepancy may be the great difference among saliva formulations used in the *in vitro* studies [1,2,8,9]. In the present study, the influence of saliva on fungal adhesion to the materials was not observed, our focus being only on direct interactions between the materials and *C. albicans*. Therefore, further study on the influence of adhesion medium on *Candida* adhesion using both artificial and natural saliva is needed to obtain more clinically relevant results. Also, further investigation using accurate, appropriate methods for contact angle measurement and calculation of surface-fee energy is required to clarify the relationship between the surface characteristics of denture lining materials and their resultant susceptibility to *Candida* adherence.

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