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Effect of surface roughness on the hydrophobicity of a denture-base acrylic resin and *Candida albicans* colonization

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Abstract

Aim: The aim of the present study was to evaluate the effect of surface roughness (roughness average [Ra] μm) on the hydrophobicity of a denture-base acrylic resin and the initial adherence and biofilm formation of *Candida albicans* (*C. albicans*).

Methods: Disk-shaped specimens were divided into six groups: Ra 0.05, Ra 0.2, Ra 0.4, Ra 0.8, Ra 1.5, and Ra 3.0. Water contact angles (WCA) were measured, and the specimens incubated with *C. albicans* for 90 min (initial adherence, $n = 108$) or 48 h (biofilm formation, $n = 108$). Adhered and biofilm cells were evaluated by c.f.u./mL and 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT), and the correlation between the two methods was evaluated. The surface of the specimens and cells (adhered and biofilm) were also analyzed by scanning electron microscopy (SEM).

Results: Groups Ra 0.05 and 3.0 exhibited the lowest (~75°) and the highest (~100°) WCA mean values, respectively. For both initial adherence and biofilm formation, no statistically-significant differences were observed among all groups, as determined by c.f.u./mL and XTT. A positive correlation between these two methods was found. SEM analysis showed the presence of scratches and valleys on the acrylic specimens and densely-packed yeast cells covering the entire surface.

Conclusions: Roughness significantly increased hydrophobicity (WCA), but had no effect on the number and metabolic activity of adherent and biofilm cells of *C. albicans*.

Introduction

Denture stomatitis is a frequent disease in denture wearers, and has been associated with microorganisms adhering to the inner surfaces of the dentures, which act as a reservoir of pathogens. Therefore, besides the high incidence in denture wearers, denture stomatitis is also of great concern because it increases the risk of serious systemic infections,[1] mainly in immunocompromised patients.[2] Although the etiology of denture stomatitis is multifactorial, *Candida* species play a critical role in the pathogenesis of this disease, with *Candida albicans* (*C. albicans*) the predominant isolate.[3] The pathogenicity of *Candida* species is mediated by virulence factors, such as the ability to adhere to surfaces.[4] The initial attachment is followed by cell division, proliferation, and extracellular matrix production,[4,5] resulting in complex microbial communities known as biofilms, which are much more tolerant to antifungal therapy and host immune defenses.[4,5]

Although surface roughness has been considered an important determinant of *C. albicans* adhesion, as well as proliferation on the most commonly-used denture-base material, polymethylmethacrylate, conflicting results have been reported. While some studies have shown that, during initial adherence, *C. albicans* is retained in greater

amounts on rough surfaces in comparison to smooth acrylic surfaces,[6–11] others have observed no significant influence of surface roughness on the initial attachment of *C. albicans*.[12–15] It is important to note, however, that although in these studies the substrates were prepared to produce different surface morphologies, only a few investigated the adherence of *C. albicans* to denture-base resins over wider range of roughness.[7–9,11,12] Moreover, to date, only a few studies have reported on the effect of surface roughness on *C. albicans* biofilm formation (mature biofilm) on denture-base resins.[9,16] Thus, to test the influence of surface roughness on *Candida* biofilm adequately, an appropriate study design, modifying the surface roughness, has been suggested.[17]

Roughness can have an effect on the surface physicochemical properties, such as hydrophobicity,[18] which is also related to *C. albicans* adherence. However, there are inconsistent data for concerning the effect of surface hydrophobicity on initial *C. albicans* adhesion to denture-base resins. A relationship between *C. albicans* adherence and the contact angle of the substrate, that is, a higher number of cells adhering on more hydrophobic surfaces, was observed by Klotz *et al.*, whereas Minagi *et al.* observed lower adhesion of *C. albicans* to more hydrophobic surfaces.[19,20] Other studies on this have found a weak-positive or no significant correlation between hydrophobicity and initial adherence and the biofilm formation of *C. albicans* on acrylic resins.[13,21–24]

Thus, the aim of the present study was to evaluate the effect of surface roughness on the hydrophobicity of a denture-base acrylic resin, initial adherence, and the biofilm formation of *C. albicans*. Surface topography of the acrylic resin with different roughness and the *C. albicans* cells (adhered and biofilms) were also evaluated using scanning electron microscopy (SEM). The null hypothesis tested was that the surface roughness has no effect on hydrophobicity of the denture-base acrylic resin, initial adherence, and biofilm formation of *C. albicans*.

Materials and methods

Preparation of acrylic resin specimens

Disk-shaped specimens ($n = 216$) were prepared using a colorless denture-base acrylic resin polymerized by microwave energy (Vipi Wave; Vipi Indústria Comércio de Exportação e Importação de Produtos Odontológicos, São Paulo, Brazil). Initially, a metal mold with circular holes (10-mm diameter and 2-mm thickness) was invested between glass slides in dental stone in microwave flaks. After the stone had set, the flasks were separated, and the acrylic resin was proportioned and mixed according to the manufacturer's instructions. Thereafter, the mixture was packed into the circular holes, a trial pack was performed, and excess material was removed. A final pack was performed and held for 15 min. The acrylic resin was then polymerized in a 500-W domestic microwave oven (Brastemp; Brastemp da Amazônia SA, Amazon State, Manaus, Brazil) for 20 min at 20% power, followed by 5 min at 90% power. The flasks were allowed to bench cool at room temperature, the specimens were deflasked, and the excess was removed with a sterilized bur (Maxi-Cut; Lesfils de August Malleifer SA, Ballaigues, Switzerland).

Six experimental groups were evaluated, with different surface roughness values (roughness average [Ra] μm) as follows: 0.05, 0.2, 0.4, 0.8, 1.5, and 3.0. To obtain these values, specimens were processed, as described, between two glass slides with smooth surfaces. Thereafter, the specimens were either left unpolished (group Ra 0.2, $n = 36$) or were polished using polishing paste (group Ra 0.05, $n = 36$) or with 600-, 400-, and 200-grit silicon carbide paper under running water (group Ra 0.4, $n = 36$, group Ra 0.8, $n = 36$, and group Ra 1.5, $n = 36$, respectively). For group Ra 3.0 ($n = 36$), the specimens were invested between glass slides with known roughness, and were not polished.

All specimens were washed in an ultrasonic bath using distilled water for 20 min to remove the residues from polishing.

Surface roughness measurements

Measurements of the surface roughness (Ra μm) of all specimens were performed using a profilometer (Mitutoyo SJ 400; Mitutoyo, Tokyo, Japan), with a resolution of 0.01 μm, an interval (cut-off length) of 0.8 mm, a transverse length of 2.4 mm, a stylus speed of 0.5 mm/s, and a diamond stylus tip radius of 5 μm. For each specimen, readings were performed at four points, and the average reading of surface roughness was designated as the Ra value of that specimen. All measurements were recorded by the same operator.

Water contact angle measurements

The surface hydrophobicity of each specimen was determined by contact angle measurements performed with a goniometer (Ramé-Hart 200 00; Ramé-Hart Instrument, Succasunna, NJ, USA), using deionized water as the test liquid. For each measurement, a droplet of deionized water was placed on the surface, and an image of the sessile drop was obtained. The right and left contact angles of the droplet were then measured by image-processing software, and the mean value between them was calculated. Measurements in two different positions were made

for each specimen, and the average was calculated. The specimens were then stored in sterile, distilled water for 48 h at room temperature to release residual monomers before the microbiological tests.

Microorganism and culture conditions

C. albicans (American Type Culture Collection 90028) was the fungal strain used in the present study. To prepare the inoculum, a loopful of the stock culture was streaked onto Sabouraud dextrose agar (SDA; Himedia, Mumbai, India) and incubated at 37°C for 48 h. One loopful of this young culture were transferred to 20 mL RPMI-1640 culture medium (Sigma, St Louis, MO, USA) and incubated at 37°C for 21 h. Fungal cells of the resultant culture were harvested, washed twice with sterile phosphate-buffered saline (PBS) (pH 6.8) at 4000 × *g* for 5 min, and resuspended in RPMI-1640. Inoculum was standardized to 1×10^7 c.f.u./mL[5], spectrophotometrically.

Adherence and biofilm formation assays

Half of the specimens of each experimental group ($n = 18$) were used for the adherence assay (90 min of incubation), and the other half ($n = 18$) was used for biofilm formation assay (48 h of incubation). Prior to the microbiological tests, the specimens were ultrasonicated in distilled water for 20 min and then exposed to UV light under dry conditions for another 20 min (each side) for sterilization. Two milliliters of the inoculum was added to each well of a 24-well microplate containing the specimens, and then incubated at 37°C in an orbital shaker.[24] The non-adherent cells were removed from the specimens by gently washing twice with 2 mL PBS after 90 min (initial adherence). In the case of biofilm formation assay, after the washing procedure, 2 mL RPMI-1640 culture medium was placed in each well and maintained for 24 h at 37°C in an orbital shaker.[24] At 24 h, 1 mL RPMI-1640 medium was removed, and an equal volume of fresh RPMI-1640 culture medium was added. The 24-well microplates containing the specimens were then incubated again for a further 24 h (until 48-h biofilm formation). The medium was then aspirated, and non-adherent cells were removed by washing the biofilms twice with PBS. For all experiments, negative controls were acrylic specimens to which no cells were added. All experiments were performed in triplicate on three independent occasions.

The initial adherence and 48-h biofilm formation of *C. albicans* were evaluated using c.f.u./mL and 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) reduction assays.

C.f.u. method

Viable microorganisms after the initial adherence ($n = 9$) and 48-h biofilm formation ($n = 9$) of *C. albicans* were quantified by counting the c.f.u. After washing the non-adherent cells with PBS, each specimen was transferred to a Falcon tube containing 10 mL PBS, which was vortexed vigorously for 1 min to resuspend the adhered and biofilm cells. *Candida* suspensions obtained were serially diluted (from 10^{-1} to 10^{-8}) in PBS, and 10 μL of each dilution plated on SDA in duplicate. After 48 h of incubation at 37°C, the number of *C. albicans* colonies was counted and expressed as c.f.u./mL, which were then $\log_{10}$ transformed.

2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide reduction assay

The initial adherence ($n = 9$) and 48-h biofilm formation ($n = 9$) of *C. albicans* were also evaluated using the XTT reduction assay, a semiquantitative technique that measures cell viability based on mitochondrial enzyme activity. The colorimetric change in the XTT assay represents a direct correlation with the metabolic activity of biofilm.[25] An XTT stock solution was prepared by mixing XTT salt (Sigma) in ultrapure water to a final concentration of 1 mg/mL, filtered through a 0.22-μm filter, and stored at −70°C until use. Menadione (Sigma) was prepared in acetone at 0.4 mM immediately before each assay. After washing, the specimens were transferred to new 24-well microplates each containing 1580 μL PBS supplemented with 200 mM glucose, 400 μL XTT, and 20 of menadione. After 3 h of dark incubation at 37°C, the contents of each well were centrifuged at 5000 × *g* for 2 min, and the colorimetric change, measured at 492 nm, was determined using a 96-well microtiter plate reader (TP Reader–ThermoPlate®, Shenzhen, China).

Scanning electron microscopy

An SEM (JEOL JSM 6610LV; JEOL, Peabody, MA, USA) was used to analyze the surface topography of the acrylic resin with different roughness, as well as the morphological characteristics of *Candida*'s initial adhesion and biofilm formation on the acrylic specimens. For this purpose, one additional sample from each group was coated with carbon. In the case of the adhered *Candida* and the 48-h biofilms on the different surfaces, the additional specimens were washed in PBS and the cells were fixed in 2.5% glutaraldehyde for 24 h at room temperature. Thereafter, they were dehydrated through a graded ethanol series (70%, 85%, and 100%) and coated with carbon.

Statistical analysis

For each variable (hydrophobicity–WCA, initial adherence, and biofilm formation of *C. albicans*), the effects of the factor surface roughness were analyzed by one-way ANOVA, followed by Tukey's test. Additionally, Pearson's coefficient was used to evaluate correlations between the results obtained by c.f.u./mL and XTT assays, c.f.u./mL assay and WCA measurements, and XTT assay and WCA measurements. A significance level of 0.05 was used for all statistical tests.

Results

The mean values and standard deviations (SD) of the surface roughness of the specimens prepared for the initial adherence and biofilm formation assays are presented in Table 1. As proposed, groups with pre-established different surface roughness values were obtained.

The means and SD of WCA for all groups evaluated are presented in Table 2. Groups Ra 0.05 and Ra 3.0 exhibited the lowest and the highest WCA mean values, respectively ($P < 0.001$). The mean WCA value of group Ra 0.2 was higher than that obtained for group Ra 0.05, but lower when compared to groups Ra 0.4, Ra 0.8, Ra 1.5, and Ra 3.0 ($P < 0.001$).

The means and SD of log c.f.u./mL from the initial adherence and biofilm formation assays are shown in Table 3. No statistically-significant differences were found among all groups evaluated ($P > 0.05$). For the XTT reduction assay, one-way ANOVA also showed that there were no statistically-significant differences among all groups ($P > 0.05$) for both initial adherence and biofilm formation (Table 4).

Pearson's coefficient correlation revealed that there were significant positive correlations between XTT and c.f.u./mL assays in the initial adherence ($R = 0.2969$ and $P = 0.0293$) and biofilm formation ($R = 0.2865$ and $P = 0.0357$). No significant correlations were found between c.f.u./mL assay and WCA ($R = -0.0751$ and $P = 0.5891$) or between XTT assay and WCA ($R = -0.0956$ and $P = 0.4513$) for initial adherence. Statistically-significant correlations were also not observed between c.f.u./mL assay and WCA ($R = 0.008$ and $P = 0.9542$) or between XTT assay and WCA ($R = -0.2133$ and $P = 0.1215$) for biofilm formation.

Table 1. Means and standard deviations of surface roughness (Ra μm) of the specimens prepared for the initial adherence and biofilm formation assays for all groups evaluated

Groups ($n = 36$)	Initial adherence ($n = 18$)	Biofilm formation ($n = 18$)
Ra 0.05	0.05 ± 0.01	0.05 ± 0.01
Ra 0.2	0.20 ± 0.02	0.21 ± 0.02
Ra 0.4	0.39 ± 0.02	0.39 ± 0.04
Ra 0.8	0.79 ± 0.03	0.77 ± 0.01
Ra 1.5	1.50 ± 0.06	1.45 ± 0.05
Ra 3.0	3.11 ± 0.09	3.09 ± 0.13

Ra, roughness average.

Table 2. Means and standard deviations of water contact angle (°) of the specimens prepared for the initial adherence and biofilm formation assays for all groups evaluated

Groups ($n = 36$)	Initial adherence ($n = 18$)	Biofilm formation ($n = 18$)
Ra 0.05	74.55 ± 1.78^{A}	74.72 ± 1.72^{A}
Ra 0.2	78.82 ± 2.25^{B}	78.75 ± 2.07^{B}
Ra 0.4	90.96 ± 1.55^{C}	90.65 ± 1.61^{C}
Ra 0.8	92.06 ± 2.17^{C}	92.01 ± 1.87^{C}
Ra 1.5	91.54 ± 2.42^{C}	91.16 ± 2.72^{C}
Ra 3.0	98.64 ± 2.40^{D}	99.08 ± 2.45^{D}

Means in each column with different superscript capital letters differ significantly at $P < 0.05$.
RA, roughness average.

Table 3. Means and standard deviations of c.f.u./mL of *Candida albicans* obtained in the initial adherence and biofilm formation assays for all groups evaluated

Groups ($n = 18$)	Initial adherence ($n = 9$)	Biofilm formation ($n = 9$)
Ra 0.05	5.23 ± 0.18	5.90 ± 0.45
Ra 0.2	5.23 ± 0.20	5.96 ± 0.47
Ra 0.4	5.03 ± 0.28	5.98 ± 0.55
Ra 0.8	5.15 ± 0.17	5.90 ± 0.50
Ra 1.5	5.22 ± 0.23	5.95 ± 0.44
Ra 3.0	5.24 ± 0.22	5.90 ± 0.40

In each column, there were no significant differences among all groups ($P > 0.05$).

Table 4. Means and standard deviations of 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide values (absorbance at 492 nm) obtained in the initial adherence and biofilm formation assays for all groups evaluated

Groups ($n = 18$)	Initial adherence ($n = 9$)	Biofilm formation ($n = 9$)
Ra 0.05	0.72 ± 0.24	1.71 ± 0.31
Ra 0.2	0.54 ± 0.21	1.61 ± 0.39
Ra 0.4	0.55 ± 0.17	1.70 ± 0.26
Ra 0.8	0.55 ± 0.21	1.61 ± 0.32
Ra 1.5	0.50 ± 0.23	1.46 ± 0.36
Ra 3.0	0.55 ± 0.22	1.48 ± 0.32

In each column, there were no significant differences among all groups ($P > 0.05$).

SEM analysis of the acrylic specimens showed the presence of scratches and valleys, which increased, particularly in groups Ra 0.8, Ra 1.5, and Ra 3.0. SEM photographs of both *C. albicans* cells initially adhered to and biofilm developed on the specimens with different surface roughness showed densely-packed yeast cells covering the entire surface (Figure 1).

Discussion

On the basis of the data obtained, the null hypothesis was partially accepted, as surface roughness altered the hydrophobicity of a denture-base acrylic resin, but had no significant effect on *C. albicans* colonization (initial adherence and biofilm formation) on the prepared surfaces.

As in previous investigations on the adherence and biofilm formation of *Candida albicans* to denture-base resins, the arithmetic Ra was chosen to represent the surface roughness in the present study.[6–16,22,23]

The contact angle has been used to determine the hydrophobicity of a surface and to qualitatively characterize the complete or partial wetting that might occur when a non-volatile liquid, such as water, is brought into contact with a solid surface. When the liquid spreads out completely (hydrophilic surfaces), the contact angle is zero, while for partial wetting, where the liquid spreads to a limited extent and maintains the drop shape on the surface (more hydrophobic surfaces), the contact angle is greater than zero.[26] The results of the present study demonstrated that the higher the surface roughness, the higher the hydrophobicity of the denture-base resin. Among other factors, the hydrophobicity of a material depends on the surface roughness. It is well known that the surface roughness increases the surface area, and thus the affinity of the surface with the liquid is enhanced. Therefore, if the roughness of a hydrophilic surface has increased, it becomes more hydrophilic. Conversely, the surface roughness increases the hydrophobicity (WCA) of hydrophobic materials.[27,28] Therefore, the increase of the hydrophobicity observed in the present study can be attributed to the greater surface area presented by rough surfaces, which increased the contact between the drop (water) and the functional groups of the acrylic resin, thus intensifying the interaction forces. Similar to this study, Monsénégo *et al.* also found that the WCA for the sand-abraded or polished denture-base acrylic resins were higher compared to the prepared, untreated surfaces.[18] These results can be explained by the increased roughness of the sand-abraded surfaces and by the reorientation of the superficial polymer chains brought about by the polishing procedure. Nishioka *et al.* also observed that the WCA values decreased as the surface roughness of the denture-base acrylic resin specimens decreased.[28]

Two methods based on different principles were used in the present study to evaluate the adherence and biofilm formation of *C. albicans* on the denture-base acrylic resin: the counting of viable microorganisms (c.f.u. on plates) and the XTT reduction assay, in which mitochondrial dehydrogenases of live cells convert the XTT salt (yellow) into an orange product that can be measured spectrophotometrically.[5,14,17,24,25,29] While the XTT reduction assay

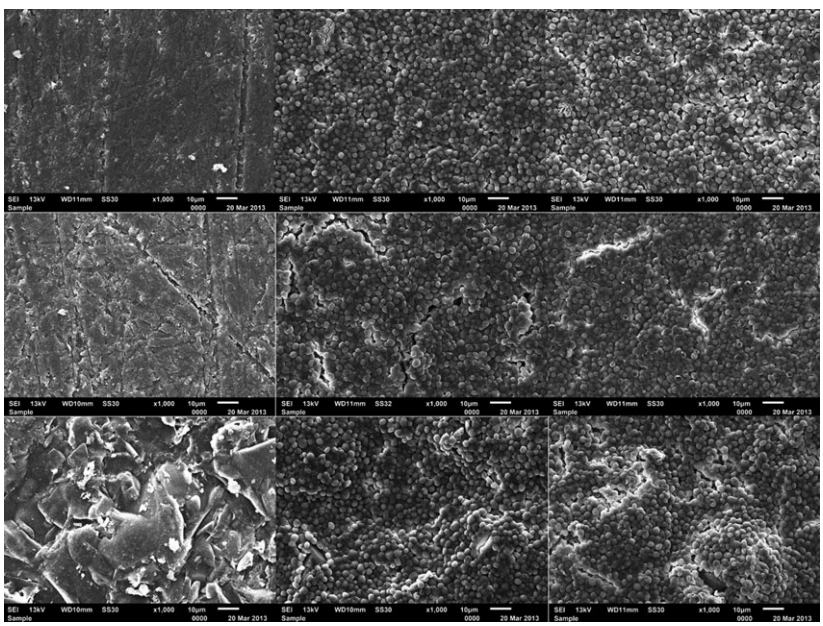

Figure 1. Scanning electron microscopy images (×1000 magnification) showing the denture-base acrylic resin surfaces (left), *C. albicans* adhesion (center), and *C. albicans* biofilm formation (right). From top to bottom: representative images of typical fields of view for the smoothest surface (group roughness average [Ra] 0.05), intermediate roughness (group Ra 0.4), and roughest surface (group Ra 3.0).

evaluates metabolic activity, c.f.u./mL is unlikely to be significantly affected by the metabolic state of the cells.[29] A significant positive correlation between c.f.u./mL and XTT assays was found, which is in accordance with previously-reported results.[29]

The results of the adherence assay demonstrated that, even though a wide range of Ra values was evaluated, there was no significant effect of surface roughness on the number of viable cells (c.f.u./mL), as well as on the metabolic activity of the adhered *C. albicans* (XTT assay). This is in line with recent studies, in which no significant effect of surface roughness on *C. albicans* adhesion to denture-base acrylic resins was observed.[12–15] In contrast, Odagiri *et al.* found that disinfection of an autopolymerized denture-base resin with 5% sodium hypochlorite promoted an increase in roughness (Ra 1.04) and the initial attachment of *C. albicans* when compared to the control (immersion in water, Ra 0.38).[10] It should be noted, however, that the increase in *C. albicans* adherence, although significant, was small (<0.5 $\log_{10}$ c.f.u./mL). In Mayahara *et al.*'s study, resin strips were polished to three surface roughness levels (Ra 3.2, Ra 0.48, and Ra 0.06), immersed in *C. albicans* suspensions of the logarithmic phase (9 h: yeast form) or stationary phase (24 h: mycelial form), and analyzed by SEM.[11] They observed that although mycelial adhesion increased with surface roughness, the adhesion of yeast-form *C. albicans* was indifferent to surface roughness. In the present study, SEM images of the acrylic specimens prepared for the adherence assay showed that the *C. albicans* cells were predominantly in the yeast form.

In the present study, surface roughness did not have a significant effect on the biofilm formation of *C. albicans* in terms of both the number of viable cells and metabolic activity. SEM images of *C. albicans* biofilms formed on the substrates with different surface roughness showed cells covering the entire specimen, with a stratified multilayer structure that followed some surface features, such as undulations (mainly in group Ra 3.0). Nikawa *et al.* evaluated the biofilm formation of *C. albicans* on the surfaces of a denture-base acrylic resin and seven soft lining materials artificially deteriorated by immersion into water or commercially-available denture cleansers.[16] The relationship between biofilm formation on the samples of each material and their surface roughness was analyzed, and no significant correlation was observed. In Lamfon *et al.*'s[9] study, *C. albicans* was grown on a cold-cure acrylic denture with different surface roughness (Ra 2.5, Ra 3.6, and Ra 9.3) in a constant depth film fermenter, and maintained with artificial saliva over 96 h. The results showed that the surface roughness affected the number of yeast cells during the first 6 h. However, after this time period, no differences in the numbers were observed, regardless of the surface roughness. More recently, the development of hyphal and blastospore biofilms on low-, medium-, and high-abraded denture acrylic resin specimens, and the ease of removal of these biofilms, has been studied.[30] It was found that even though the retention of hyphae and yeast cells was enhanced by an increase in surface roughness, the amount of biofilm after maturation at 48 h of growth was not affected.[30] Although no direct comparison can be made because of different study designs, the findings reported by Nikawa *et al.*, Lamfon *et al.*, and Jackson *et al.* are in general agreement with those observed in the present study for the 48-h biofilms.[9,16,30]

Although previous studies have evaluated the correlation between the hydrophobicity (surface energy) of different acrylic resins and the adhesion of *C. albicans*, the resins evaluated had similar surface roughness and the variations on their contact angle values or surface energy were very small.[13,21,22] In the present investigation, the surfaces evaluated had different roughness values, and the WCA ranged from approximately 75° to 100°. This variation is higher compared to the previous studies. It was also found that the rougher surfaces presented higher WCA (hydrophobicity). Nevertheless, similar to previous studies, no significant correlations could be established between the hydrophobicity of the denture-base acrylic resin and both the initial adherence and biofilm formation of *C. albicans*.[13,21,22] Other researchers have observed a weak-positive correlation between WCA on samples and the initial adhesion and biofilm formation of *C. albicans*.[23] A recent study found that differences in the surface free energy of a denture-base resin does not affect the adhesion of *C. albicans*, but leads to robust, mature biofilms.[24] These conflicting results could be due to the different protocols used in each study. Li *et al.* evaluated several uncoated or saliva-coated materials used in implant overdenture, among them only one heat-polymerized denture-base resin.[23] In da Silva *et al.*'s[24] study, one poly(methyl methacrylate) acrylic resin and one poly (ethyl methacrylate) denture liner were pellicle coated with saliva alone, saliva + blood plasma, or blood plasma alone.

One limitation of this study is that only one strain of *C. albicans* was evaluated. Other microorganisms can behave differently due to variations in cell shape and size, which could facilitate their retention on the surfaces when the substrates topographic features have dimensions comparable to cell size. Moreover, mixed biofilms can exhibit different results, because one microorganism (with specific size) and the extracellular polysaccharide matrix might act as anchors for others. These are limitations of the present investigation, and deserve to be addressed in future research.

In conclusion, in the present study, denture-base acrylic resin specimens were prepared in order to obtain a wide range of roughness values, ranging from Ra 0.05 to Ra 3.0. Surface roughness significantly increased the WCA (hydrophobicity), but had no significant effect on the number and the metabolic activity of adherent and biofilm cells of *C. albicans*. Additionally, the results demonstrated that there were no significant correlations between hydrophobicity and *C. albicans* adhesion and biofilm formation on the denture-base acrylic resin.

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References

1 Sánchez-Vargas LO, Estrada-Barraza D, Pozos-Guillen AJ, Rivas-Caceres R. Biofilm formation by oral clinical isolates of *Candida* species. *Arch Oral Biol* 2013; **58**: 1318–26.
2 Epstein JB. Diagnosis and treatment of oropharyngeal candidiasis. *Oral Maxillofac Surg Clin N Am* 2003; **15**: 91–102.
3 Sanitá PV, Pavarina AC, Giampaolo ET *et al. Candida* spp. prevalence in well controlled type 2 diabetic patients with denture stomatitis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2011; **111**: 726–33.
4 Ramage G, Martínez JP, López-Ribot JL. *Candida* biofilms on implanted biomaterials: a clinically significant problem. *FEMS Yeast Res* 2006; **6**: 979–86.
5 Chandra J, Kuhn DM, Mukherjee PK, Hoyer LL, McCormick T, Ghannoum MA. Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance. *J Bacteriol* 2001; **183**: 5385–94.
6 Verran J, Maryan CJ. Retention of *Candida albicans* on acrylic resin and silicone of different surface topography. *J Prosthet Dent* 1997; **77**: 535–39.
7 Radford DR, Sweet SP, Challacombe SH, Walter JD. Adherence of *Candida albicans* to denture-base materials with different surface finishes. *J Dent* 1998; **26**: 577–83.
8 Taylor R, Marian C, Verran J. Retention of oral microorganisms on cobalt–chromium alloy and dental acrylic resin with different surface finishes. *J Prosthet Dent* 1998; **80**: 592–7.
9 Lamfon H, Porter SR, McCullough M, Pratten J. Formation of *Candida albicans* biofilms on non-shedding oral surfaces. *Eur J Oral Sci* 2003; **111**: 465–71.
10 Odagiri K, Sawada T, Hori N *et al.* Evaluation of denture base resin after disinfection method using reactive oxygen species (ROS). *Dent Mater J* 2012; **31**: 443–8.
11 Mayahara M, Kataoaka R, Arimoto T *et al.* Effects of surface roughness and dimorphism on the adhesion of *Candida albicans* to the surface of resins: scanning electron microscope analyses of mode and number of adhesions. *J Investig Clin Dent* 2014; **7**: 307–12.
12 Nevzatoglu EU, Özcan M, Kulak-Ozkan Y, Kadir T. Adherence of *Candida albicans* to denture base acrylics and silicone-based resilient liner materials with different surface finishes. *Clin Oral Investig* 2007; **11**: 231–36.
13 Hahnel S, Rosentritt M, Handel G, Bürgers R. *In vitro* evaluation of artificial ageing on surface properties and early *Candida albicans* adhesion to prosthetic resins. *J Mater Sci: Mater Med* 2009; **20**: 249–55.
14 Zamperini CA, Machado AL, Vergani CE, Pavarina AC, Giampaolo ET, da Cruz NC. Adherence *in vitro* of *Candida albicans* to plasma treated acrylic resin. Effect of plasma parameters, surface roughness and salivary pellicle. *Arch Oral Biol* 2010; **55**: 763–70.
15 Zamperini CA, Machado AL, Vergani CE, Pavarina AC, Rangel EC, Cruz NC. Evaluation of fungal adherence to plasma-modified polymethylmethacrylate. *Mycoses* 2011; **54**: e344–51.
16 Nikawa H, Jin C, Makihira S, Egusa H, Hamada T, Kumagai H. Biofilm formation of *Candida albicans* on the surfaces of deteriorated soft denture lining materials caused by denture cleansers *in vitro*. *J Oral Rehabil* 2003; **30**: 243–50.
17 da Silva WJ, Seneviratne J, Samaranayake LP, Del Bel Cury AA. Bioactivity and architecture of *Candida albicans* biofilms developed on poly(methyl methacrylate) resin surface. *J Biomed Mater Res B Appl Biomater* 2010; **94**: 149–56.
18 Monsénégo P, Baszkin A, Costa ML, Lejoyeux J. Complete denture retention. Part II: wettability studies on various acrylic resin denture base materials. *J Prosthet Dent* 1989; **62**: 308–12.
19 Klotz SA, Drutz DJ, Zajic JE. Factors governing adherence of *Candida* species to plastic surfaces. *Infect Immun* 1985; **50**: 97–101.
20 Minagi S, Miyake Y, Inagaki K, Tsuru H, Suginaka H. Hydrophobic interaction in *Candida albicans* and *Candida tropicalis adherence* to various denture base resin materials. *Infect Immun* 1985; **47**: 11–4.
21 Serrano-Granger C, Cerero-Lapiedra R, Campo-Trapero J, Del Río-Highsmith J. *In vitro* study of the adherence of *Candida albicans* to acrylic resins: relationship to surface energy. *Int J Prosthodont* 2005; **18**: 392–8.
22 Moura JS, Silva WJ, Pereira T, Cury ADB, Garcia RCMR. Influence of acrylic resin polymerization methods and saliva on the adherence of four *Candida* species. *J Prosthet Dent* 2006; **96**: 205–11.
23 Li J, Hirota K, Goto T, Yumoto H, Miyake Y, Ichikawa T. Biofilm formation of *Candida albicans* on implant overdenture materials and its removal. *J Dent* 2012; **40**: 686–92.

24 da Silva WJ, Leal CM, Viu FC, Gonçalves LM, Barbosa CM, Del Bel Cury AA. Influence of surface free energy of denture base and liner materials on *Candida albicans* biofilms. *J Investig Clin Dent* 2015; **6**: 141–6.
25 Ramage G, Jose A, Coco B *et al.* Commercial mouthwashes are more effective than azole antifungals against *Candida albicans* biofilms *in vitro*. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2011; **111**: 456–60.
26 Ramiasa M, Ralston J, Fetzer R, Sedev R. The influence of topography on dynamic wetting. *Adv Colloid Interface Sci* 2014; **206**: 275–293.
27 Elkin B, Mayer J, Schindler B, Vohrer U. Wettability, chemical and morphological data of hydrophobic layers by plasma polymerization on smooth substrates. *Surf Coat Technol* 1999; **116–119**: 836–40.
28 Nishioka M, Yamabe Y, Hisatsune K, Fujii H. Influence of polishing of denture base resin and metal surfaces on wettability with water and saliva. *Dent Mater J* 2006; **25**: 161–5.
29 Jin Y, Samaranayake LP, Samaranayake Y, Yip HK. Biofilm formation of *Candida albicans* is variably affected by saliva and dietary sugars. *Arch Oral Biol* 2004; **49**: 789–98.
30 Jackson S, Coulthwaite L, Loewy Z, Scallan A, Verran J. Biofilm development by blastospores and hyphae of *Candida albicans* on abraded denture acrylic resin surfaces. *J Prosthet Dent* 2014; **112**: 988–93.