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In vitro adhesion of *Candida glabrata* to denture base acrylic resin modified by glow-discharge plasma treatment

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Summary

This study evaluated the potential of plasma treatments to modify the surface chemistry and hydrophobicity of a denture base acrylic resin to reduce the *Candida glabrata* adhesion. Specimens ($n = 54$) with smooth surfaces were made and divided into three groups ($n = 18$): control – non-treated; experimental groups – submitted to plasma treatment (Ar/50 W; AAt/130 W). The effects of these treatments on chemical composition and surface topography of the acrylic resin were evaluated. Surface free energy measurements (SFE) were performed after the treatments and after 48 h of immersion in water. For each group, half ($n = 9$) of the specimens were preconditionated with saliva before the adhesion assay. The number of adhered *C. glabrata* was evaluated by cell counting after crystal violet staining. The Ar/50 W and AAt/130 W treatments altered the chemistry composition, hydrophobicity and topography of acrylic surface. The Ar/50 W group showed significantly lower *C. glabrata* adherence than the control group, in the absence of saliva. After preconditioning with saliva, *C. glabrata* adherence in experimental and control groups did not differ significantly. There were significant changes in the SFE after immersion in water. The results demonstrated that Ar/50 W treated surfaces have potential for reducing *C. glabrata* adhesion to denture base resins and deserve further investigation, especially to tailor the parameters to prolong the increased wettability.

Key words: *Candida*, *Candida glabrata*, saliva, acrylic resins.

Introduction

The ability of *Candida* to grow attached to oral surfaces in communities known as biofilms is an important factor in the development of denture stomatitis. Although *Candida albicans* is still the microorganism most often associated with this infection, non-*albicans* species have been isolated from denture surfaces and oral mucosa.[1] *Candida glabrata* was the second most commonly isolated pathogen in patients with denture-induced stomatitis, followed by *C. pseudotropicalis*, *C. Krusei*, *C. tropicalis*, *C. parapsilosis* and others.[1] In addition, mixed *Candida albicans* and *Candida glabrata* biofilms have been associated with denture stomatitis,[2] indicating that *Candida glabrata* may also play an integral role in this pathogenesis.[2] Moreover, in recent years, the prevalence of *C. glabrata* infections has increased.[3] This fact must receive attention because the mortality rate of *C. glabrata* infections is higher compared with infection with other non-*albicans Candida* and are more often difficult to treat.[3]

Among the virulence attributes of *Candida*, the ability of adherence to acrylic is a prerequisite for colonisation and development of biofilms on denture surfaces. Thus, the inhibition of the adhesion could be effective to treat or prevent the denture stomatitis.[4] Although this attribute has been extensively studied in *C. albicans*, few studies evaluated *C. glabrata* adhesion, mainly to

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modified surfaces.[4] This is particularly important due to increasing prevalence of *C. glabrata* in human infections as a consequence of the emergence of the acquired immunodeficiency syndrome and the wide use of immunosuppressive medications.[3]

Many factors that affect *Candida* adherence have been described, among them the hydrophobic interactions. It has been demonstrated that these interactions are involved in the adherence of *Candida* to acrylic.[4–8] The closer the surface free energy of the substrate and the yeast, the higher the probability of adherence.[6] A significant positive correlation between cell surface hydrophobicity and adhesion to acrylic surfaces of *Candida glabrata*, *Candida krusei* and *Candida albicans* has also been observed.[7,8] Moreover, higher cell surface hydrophobicity and greater avidity to acrylic of *Candida glabrata* as compared with *Candida albicans* has been observed.[8] Thus, these results suggest that hydrophilic surfaces could inhibit the adherence of *Candida* to acrylic surfaces, particularly the adherence of relatively hydrophobic fungal cells,[4] such as *Candida glabrata*.

Efforts have been made to modify acrylic resins to decrease the adherence of *Candida* spp.[4,9–14] Coatings with or without antifungal medications have been used to prevent denture stomatitis.[4,11,13] Other approaches are the chemical modification of the surface charge of acrylic resins [9,11] and the incorporation of phosphate groups in the monomer.[10,12] Although these methods have demonstrated positive results in reducing the adhesion of *Candida* to the acrylic surfaces, there are concerns regarding the physical properties and the biocompatibility of these modified polymers.[9–12] Moreover, few studies evaluate the effectiveness of these approaches against the adhesion of *Candida glabrata*.[4] Among the methods for surface modification, there is the glow-discharge plasma treatment, a type of cold plasma. This treatment is a gaseous mixture comprising high energy electrons, ions, ultraviolet photons and reactive neutral species with energy to break covalent bonds on the material surface and thus, to change its characteristics.[15] Various advantages have been attributed to plasma treatment. As the depth of plasma treatment is limited to a few nanometers of the surface, it is an economical and effective method by which surface modifications can be achieved while the bulk properties and function of the material are retained.[15,16] As gas temperature can remain as low as room temperature,[17] an advantage of this technique is that it allows the treatment of materials that cannot be subjected to high temperatures,[15] such as polymers used for removable denture bases. Moreover, this technique is dry, fast and can alter the surface properties of a wide variety of materials.[15] Despite these advantages, in dentistry, the glow-discharge plasma treatment has received little attention.[18,19] Recently, the effect of different plasma treatments on the adherence of *Candida albicans* to a denture base acrylic resin was investigated.[14,20] However, to date, there is no information about the adhesion of *C. glabrata* to plasma treated denture resins. Therefore, in this *in vitro* study we evaluate the potential of two plasma treatments to modify a denture base acrylic resin to reduce the *C. glabrata* adhesion. To the author's knowledge, this has yet not been investigated.

Some studies also have demonstrated that the salivary pellicle is involved in adherence of *Candida* to acrylic. However, the role of saliva during initial adhesion and biofilm formation of *Candida* is poorly understood. Although some studies have demonstrated that the saliva pellicle increased the colonisation of *Candida*,[21–25] others have showed that preconditioning the materials with saliva either did not affect [26–29] or reduce *Candida* adhesion.[30–34] Furthermore, few studies evaluated the effect of saliva on *C. glabrata* adhesion.[31,33,34]

Therefore, the main purpose of this *in vitro* study was to investigate the potential of two glow-discharge plasma treatments to modify the surface chemistry and hydrophobicity of a denture base acrylic resin to reduce the *C. glabrata* adhesion. Moreover, the effect of saliva coating was also evaluated.

Materials and methods

Acrylic resin specimens

A microwave denture base acrylic resin (Vipi Wave – VIPI Indústria e Comércio Exportação e Importação de Produtos Odontológicos Ltda Pirassununga, SP, Brazil) was used to obtain the specimens ($n = 54$), using a conventional pressure-pack technique. Firstly, disk-shaped silicone patterns (Zetaplus/Indurent – Zhermack, Badia Polesine, Rovigo, Italy) measuring 13.8×2 mm were made using a metal mould. For surface standardisation, these patterns were invested between glass slides in dental stone in microwave flaks. After the stone had set, the silicone patterns were removed. For each specimen, 1 g of powder and 0.47 ml of liquid were processed according to the manufacturer's instructions. The mixture was packed into the moulds, a trial pack was completed and excess material was removed. A final pack was performed for 15 min. The denture base acrylic resin was processed (20 min at 20% power, followed by 5 min at 90% power) in a 500 W domestic microwave oven (Brastemp – Brastemp Amazonia SA, Manaus, AM, Brazil). Afterwards, the specimens were deflasked, and the excess material was removed with a sterile bur (Maxi-Cut; Lesfils de August Malleifer SA, Ballaigues, Switzerland).

Surface roughness measurements

Measurements of the average surface roughness (Ra – μm) of all specimens were obtained using a profilometer (Mitutoyo SJ 400; Mitutoyo Corporation, Tokyo, Japan). Four measurements were made for each specimen. Resolution was 0.01 μm, interval (cut-off length) was 0.8 mm, transverse length was 2.4 mm, the stylus speed was 0.5 mm s^{-1} and the diamond stylus tip radius was 5 μm. All measurements were made by the same operator.

Glow-discharge plasma treatments

After roughness measurements, the specimens were washed in ultrasonic water and detergent bath for 15 min. Following, the specimens were sonicated in distilled water for 15 min and dried in air. Then, the specimens were divided into three groups, each one including 18 specimens. The specimens of the control group were left without treatment. For the two experimental groups, both specimen surfaces were exposed to plasma treatment, using the following conditions: argon atmosphere at 50 W (group Ar/50 W); atmospheric air at 130 W (AAt/130 W). According to the feedstock gas, the plasma treatments can induce different physical and chemical changes on polymer surfaces.[14,20,35,36] In this study, argon (noble gas) was chosen because it is biochemically inert and did not react with the polymer surface. The changes caused by inert gases can occur due to etching, cross-linking of polymer surfaces and/or by creating free radical species able to react with species from the environment.[35,36] The choice of the atmospheric air plasma was due to its ability of producing reactive species that could act as precursors of the hydrophilic groups to be incorporated on the polymer surface.[35,36] The plasma parameters were established based on the degree of surface hydrophobicity. Parameters that produced surfaces with contact angle values close to zero were used for group AAt/130 W. While, for the group Ar/50 W, parameters that provided hydrophobicity values between those of the control group (higher hydrophobic) and those of the group AAt/130 W specimens (lower hydrophobicity) were chosen.

Plasma treatments were performed through the application of radiofrequency power (13.56 MHz) to two parallel plate electrodes fitted inside a homemade stainless steel vacuum chamber. In this technique, gas temperature remains at room temperature, preserving the integrity of the material.[17,37] In addition, during plasma treatment, specific active agents such as, ultraviolet photons and radicals are generated, resulting in sterilisation of the samples.[38]

Surface free energy measurements

The surface free energy of the specimens was calculated from contact angle measurements using the sessile drop method and a goniometer (Ramé-Hart 100-00; Ramé-Hart Instrument Co., Succasunna, NJ, USA). The goniometer comprises a CCD camera to record the image of a droplet placed onto the surface using a microsyringe and an image processing software to determine the contact angle.[39] In the contact angle measurements, deionised water was used as the polar liquid and diiodomethane (Sigma-Aldrich, St. Louis, IL, USA) as the non-polar (dispersive) liquid. Surface free energy components (polar and dispersive) were evaluated by so-called two-liquid or Owens–Wendt method based on the contact angles of two test liquids with different polarities.[40] Specimens were then stored at room temperature in sterile distilled water (48 h) to release residual monomers.[33] Afterwards, the surface free energy measurements for each specimen (with and without saliva) were again measured.

For each experimental group, Ar/50 W and AAt/130 W, nine additional specimens were made and treated as described and their water contact angles were measured immediately after plasma treatments and at periods of 5, 15, 30, 60 and 120 min of immersion in water. These measurements were made to evaluate the durability of plasma treatment.

Chemical composition

The effect of the plasma treatments on the chemical composition of the acrylic resin surfaces were evaluated using X-ray photoelectron spectroscopy analysis (XPS). This technique was carried out at a pressure of less than 10^{-7} Pa using a commercial spectrometer (UNI-SPECS UHV) to verify the changes in surface chemical composition of the treated specimens. The Mg K_α line was used ($h\nu = 1253.6$ eV) and the analyser pass energy was set to 10 eV. The inelastic background of the C 1s and O 1s electron core-level spectra was subtracted using Shirley's method. The composition of the near surface region was determined with an accuracy of ±10% from the ratio of the relative peak areas corrected by Scofield's sensitivity factors of the corresponding elements. The spectra were fitted without placing constraints using multiple Voigt profiles. The width at half maximum (FWHM) varied between 1.2 and 2.1 eV and the accuracy of the peak positions was ±0.1 eV. One specimen of untreated denture base acrylic resin and one specimen of each experimental group (Ar/50 W and AAt/130 W) were analysed.

Surface topography

Surface topography was evaluated using a nanoindenter system (Hysitron Tribo Indenter – 8.1.1; Hysitron Inc., Minneapolis, MN, USA). One specimen of untreated denture base acrylic resin (control) and one specimen of each experimental group (Ar/50 W and AAt/130 W) were analysed and surface images were obtained. In addition, the topography properties also were evaluated using surface profilometer (Veeco Dektak 150 Surface Profilometer; Veeco Instruments Inc., Woodbury, New York, USA). One specimen of control group and one specimen of each experimental group were analysed and mean roughness (Ra) of each specimen were again obtained.

Adhesion assay

Candida glabrata strain ATCC 2001 was used in this study. For preparation of the yeast inoculum, one loopful of the stock culture was streaked onto YEPD medium [1% yeast extract, 2% peptone, 2% dextrose and 2% agar] and incubated at 37 °C for 48 h. One loopful of this young culture was transferred into 20 ml of yeast nitrogen base medium containing 50 mmol L^{-1} glucose and incubated at 37 °C for 24 h. Cells were then harvested, washed twice with phosphate-buffered saline (PBS, pH 7.2) at 5 000 *g* for 5 min and resuspended in yeast nitrogen base medium containing 100 mmol L^{-1} glucose. *Candida* suspensions were standardised to a concentration of 1×10^7 cells ml^{-1}, spectrophotometrically. The acrylic resin specimens were placed into the 12-well microtiter plates and immersed in 3 ml of the standardised *C. glabrata* cell suspension for 90 min at 37 °C – adhesion phase.[25] Non-adherent cells were removed from the specimens by gently washing with 3 ml PBS twice. Acrylic specimens without cells were negative controls. All experiments were performed in triplicate and repeated on three occasions.

Saliva collection and preconditioning

Unstimulated whole saliva was collected from 15 healthy adult volunteers. The saliva was expectorated into sterile 50 ml Falcon tubes on ice, pooled and clarified by centrifugation at 10 000 *g* for 5 min at 4 °C.[33] Then, the saliva was filtered through 0.22 μm membrane filter.[25,28,41] The filtered saliva was stored at −70 °C until use. All subjects volunteered to participate and signed an informed consent form approved by Ethics Committee of Araraquara Dental School.

To investigate the effect of preconditioning with saliva on *Candida glabrata* adhesion to the denture acrylic resin, half of specimens from each group ($n = 9$) were placed into the 12-well microtiter plates and coated with 3 ml of prepared saliva for 30 min at room temperature prior to the adhesion assay.

Crystal violet staining

After the washing of non-adherent cells, all specimens were fixed with 80% ethanol, stained with crystal violet (1 min) and washed twice with PBS.[42] Adherent yeast cells were counted in 10 different fields for each specimen, using a light microscope (Olympus BX51; Olympus, Tokyo, Japan) at 400 × magnification and the mean values were calculated. Adherent yeast cells were counted in a 'blind' manner to avoid subjective bias. The results were expressed as cells mm^{-2}.

Statistical analysis

A random effect linear model was used to determine differences in the contact angle measurements and surface free energy among the experimental conditions.

To determine differences in the water contact angle measurements among the periods of measurements, a repeated-measures analysis of variance was used.

Comparison of the roughness values among the groups was performed by non-parametric Kruskal–Wallis tests.

Differences in the adherent yeast cells among the experimental conditions were evaluated by two-way analysis of variance followed by Tukey's test. Data of yeast counts (cells mm^{-2}) were transformed by log.

A significance level of 0.05 was used for all statistical tests.

Results

Surface free energy

It can be seen from Table 1 that, immediately after plasma treatments, control group exhibited the highest water contact angle (WCA), whereas the lowest mean value was observed in group AAt/130 W ($P < 0.05$). In

Table 1 Means and standard deviations (SD) of water and diiodomethane contact angles (°) obtained immediately after plasma treatments (0 h) and after immersion in water (48 h) in absence and presence of saliva.

Groups	Water contact angle (WCA)				Diiodomethane contact angle (DCA)			
	Absent saliva		Present saliva*		Absent saliva		Present saliva*	
	0 h	48 h	0 h	48 h	0 h	48 h	0 h	48 h
Control	72.87 (5.11) ^{aA}	63.32 (4.06) ^{aB}	72.48 (4.54) ^{aA}	8.57 (3.04) ^{aB}	35.80 (3.13) ^{aA}	32.09 (2.89) ^{aA}	34.62 (3.07)^{aA}	40.27 (2.51)^{aB}
Ar/50 W	36.96 (3.97)^{bA}	54.35 (2.44)^{bC}	39.73 (4.37)^{bA}	10.96 (3.49)^{aB}	25.52 (2.45)^{bA}	38.95 (1.65)^{bB}	26.48 (2.47)^{bA}	37.77 (6.19)^{aB}
AAt/130 W	4.89 (1.22)^{cA}	57.02 (1.65)^{bC}	4.39 (0.96)^{cA}	8.69 (1.60)^{aB}	25.00 (3.54)^{bA}	30.48 (3.59)^{aB}	24.19 (3.43)^{bA}	36.49 (5.25)^{aB}

Horizontally, means with different capital superscript letters are significantly different ($P < 0.05$). Vertically, means with different small superscript letters are significantly different ($P < 0.05$).

*Present saliva only after 48 h of immersion in water.

the absence of saliva and after 48 h of immersion in water, control group demonstrated higher WCA than the experimental groups ($P < 0.05$), which did not differ from each other ($P > 0.05$). For the specimens treated with saliva, there were no significant differences among the groups. The presence of saliva did alter the WCA for all groups.

The diiodomethane contact angles (DCA) measured immediately after plasma treatments showed that the groups Ar/50 W and AAt/130 W did not differ from each other and both were significantly lower than control ($P < 0.05$) (Table 1). After immersion in water for 48 h, for the specimens not treated with saliva, no significant difference was observed between control and AAt/130 W groups, whereas the higher DCA was observed in the Ar/50 W group. In presence of saliva, no significant differences in the DCA were found among the groups. The presence of saliva did alter the DCA for control and AAt/130 W groups.

Figure 1 shows that, immediately after treatments, the lowest surface free energy (SFE) was observed for control and the highest for AAt/130 W group. After 48 h of immersion in water, there were significant changes in the SFE for all groups ($P < 0.05$). In the absence of saliva, control group exhibited the lower SFE mean value ($P < 0.05$), whereas Ar/50 W and AAt/130 W did not differ from each other. In the presence of saliva, there were no significant differences in the SFE values among all groups.

Figure 2 presents the WCA of the additional specimens of Ar/50 W and AAt/130 W groups, measured immediately after treatments and at periods of 5, 15, 30, 60 and 120 min after the plasma treatment. For the AAt/130 W group, there was a significant increase in the contact angles after 5 min of immersion in water. At the 30, 60 and 120-min periods, the mean contact angles were significantly lower than that of the 5-min period. The Ar/50 W specimens showed significant increase in the contact angle after five immersions in water and then levelled off.

Chemical composition

The chemical compositions of the surfaces evaluated by XPS analysis are shown in Table 2. The spectra of the unmodified surface (control) showed mainly peaks for carbon (C-H: 60.06%; C-O: 24.91% and O-C = O: 15.04%) and oxygen (O = C: 47.96%; and O-C = O: 52.04%). After Ar/50 W plasma treatment, there were small changes in the spectra with a decrease in the percentage of C 1s (C-H: 62.11%; C-O: 25.03%; and

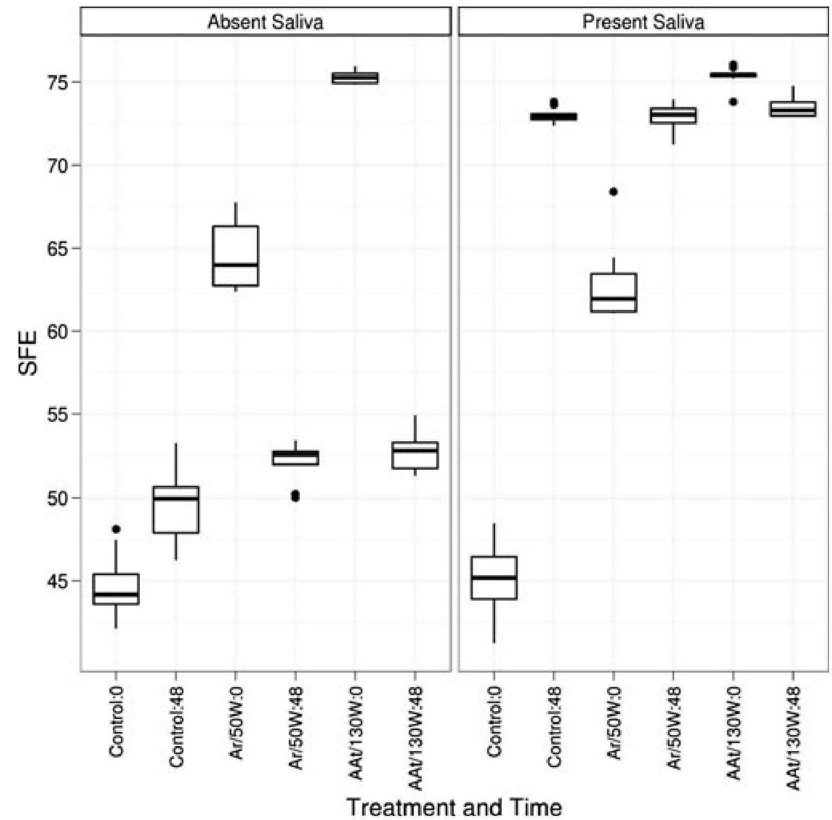

Figure 1 Box plot of surface free energy (SFE) values (Dyn/cm), according to groups, time of measurements (0 or 48 h) and saliva (present or absent).

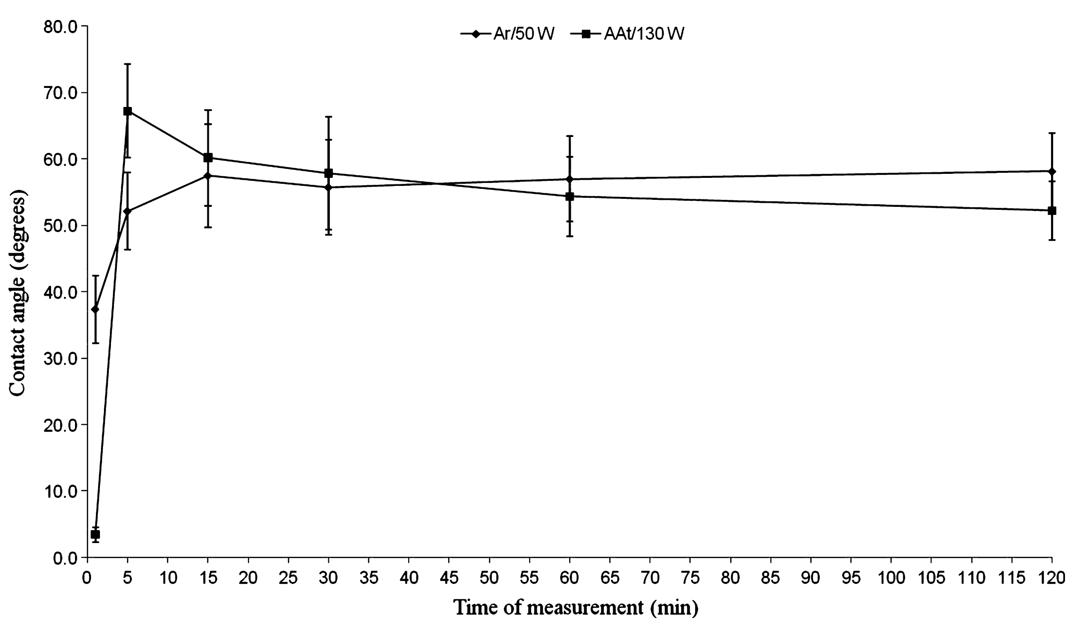

Figure 2 Means and SDs of water contact angles (°) obtained immediately after plasma treatments and after different periods of immersion in water (5, 15, 30, 60 and 120 min).

O-C = O: 12.86%) and an increase in the percentage of O 1s (O = C, O-H: 50.35%; O-C = O: 45.44%). The spectra of the AAt/130 W modified surface showed larger differences compared with the control. There was a decrease in the C 1s peak (C-H: 55.77%; C-O: 25.79%; and O-C = O: 18.45%) and a significant increase in the

Table 2 Elemental surface composition (at %) of the control and experimental groups evaluated by X-ray photoelectron spectroscopy analysis.

	Groups		
Elements (at %)	Control	Ar/50 W	AAt/130 W
C 1s	74.21	70.64	55.85
O 1s	24.58	25.55	34.94
N 1s	0.22	0.84	1.63

Table 3 Mean roughness values (Ra-μm) of all acrylic resin specimens after preparation (before plasma treatments).

Groups	Ra
Control	0.23
Ar/50 W	0.22
AAt/130 W	0.23

No significant differences were found among all groups evaluated ($P > 0.05$).

peak for O 1s (O = C, O-H: 64.79%; O-C = O: 30.81%) and N 1s.

Surface topography

Table 3 shows that, for the roughness mean values obtained after preparation of the acrylic resin specimens (before plasma treatments), there were no significant differences among all groups evaluated ($P > 0.05$).

After plasma treatments, the images obtained by nanoindenter (Fig. 3a–c) demonstrated that there was significant alteration on surface topography, particularly after Ar/50 W plasma treatment. The images of untreated (control) and AAt/130 W-treated specimens (Fig. 3a,c) show topographies very similar to each other, with irregular protrusions or peaks (whiter regions) on the surface. The surface topography of the Ar/50 W treated specimen is shown in Fig. 3b. The surface morphology became much smoother (mean roughness – Ra < 10 nm) with few and lower peaks compared to other specimens.

The results obtained by profilometer also demonstrated that the surface of the Ar/50 W group was smoother (mean Ra = 0.026 μm; $P < 0.05$) compared with AAt/130 W (mean Ra = 0.362 μm) and control (mean Ra = 0.360 μm) groups, which did not differ from each other ($P > 0.05$).

Candida glabrata adhesion

Candida glabrata adherence as determined by crystal violet staining assay is shown in Fig. 4. In the absence of saliva, group Ar/50W showed significantly lower *C. glabrata* adherence than the control group ($P < 0.05$). Experimental and control groups did not differ ($P > 0.05$), in the presence of saliva. Figure 4 also shows that, within each group, there was no significant difference between absence and presence of saliva. All

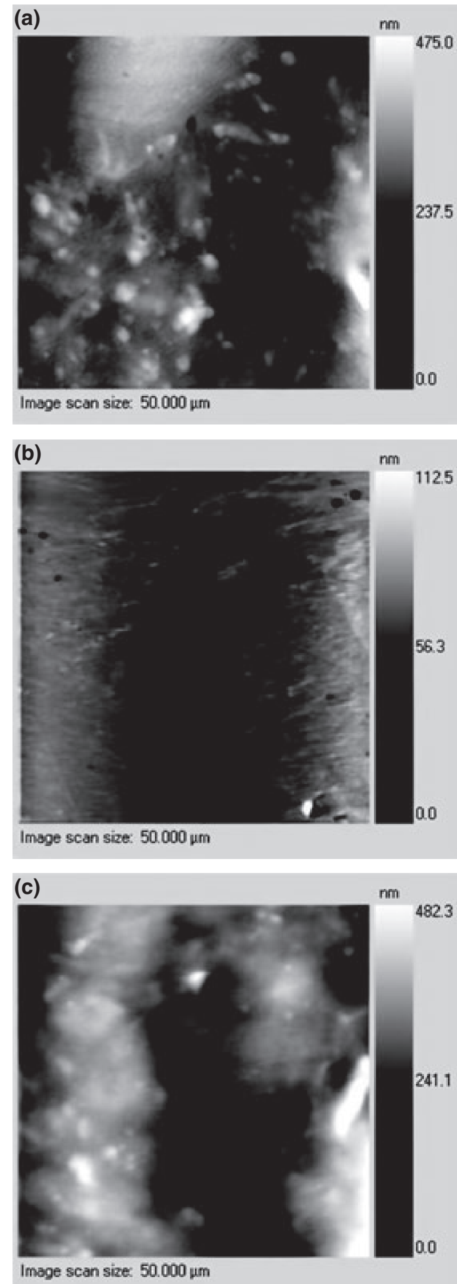

Figure 3 Nanoindenter images of (a) untreated (control) specimen; (b) Ar/50 W treated specimen and (c) AAt/130 W-treated specimen.

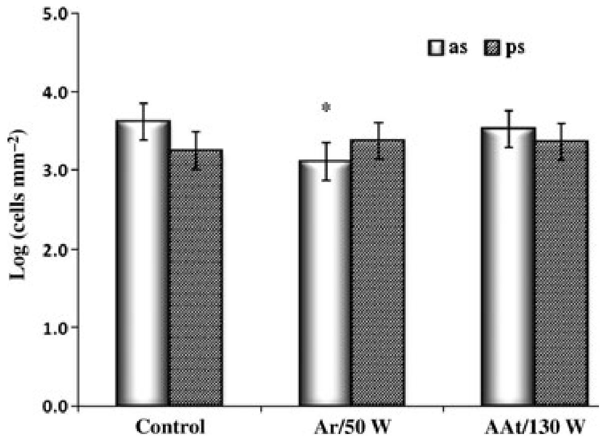

Figure 4 Mean log numbers (cells mm^{-2}) and 95% CIs for all groups as: absence of saliva; ps: presence of saliva. (*) Statistically different mean compared to control.

negative controls exhibited no metabolic activity (data not shown).

Discussion

In this study, the plasma treatments intended to modify the surface chemistry and hydrophobicity of a denture base acrylic resin to reduce the *C. glabrata* adhesion. The WCA, DCA and SFE measurements made immediately after the treatments indicated that, since the changes in contact angle values is related with the plasma atmosphere and the power applied, it was possible to regulate surface hydrophilicity as planned.[14,20]

Immediately after the treatments, WCA, DCA and SFE values indicated that, for Ar/50 W and AAt/130 W groups, the surfaces became more hydrophilic. This decrease in hydrophobicity can be attributed to energetic electrons generated during the procedure that collide on the acrylic surface. These collisions can result in chemical bonds breakage creating free radicals in the surface.[15,39] The reactions between the free radicals and species from material or atmosphere can increase the surface free energy that is reflected in a decrease of the contact angle values.[15,39] Similar results were also reported in other investigations, in which plasma treatments were made to modify polymeric surfaces.[14,17–20,38,43]

The XPS analyses revealed chemical modifications caused by the two plasma treatments on acrylic resin surface. The Ar (argon) is a chemically inert element and therefore did not react with the polymer surface and, therefore, this plasma treatment caused small changes in the chemical composition. These changes can be attributed to chemical bonds breakage (C-H, mainly) induced by energetic-ion bombardment (Ag+).[36] Subsequently, the rearrangement of chemical bonds (formation of unsaturated carbon bonds) and the incorporation of species from the environment (such as atmospheric oxygen) after exposure to atmosphere air may occur. Therefore, as demonstrated by XPS analyses, the introducing of polar groups (new oxygen-containing groups) on the surface, mainly C = O, did occur indirectly.[36] In addition, the increase in the C = O bonds was only from 47.96% to 50.35%. Conversely, XPS results showed larger chemical changes in the AAt/130 W-treated specimen which can be attributed, mainly, to oxygen and nitrogen contained in the atmospheric air that chemically react with the polymer.[35] Oxygen incorporation increased from 24.58% to 34.94% and the C = O bonds from 47.96% to 64.79%. As C = O bond is the key factor in the reduction of surface hydrophobicity,[36] these changes may help explain the lowest water contact angle of the AAt/130 W-treated specimen.

Immediately after plasma treatments, the SFE increased with the decrease of the hydrophobicity, following the same trend presented by the polar components (data not shown). In this study, although the dispersive component of the SFE was numerically higher than the polar component, the latter was the main factor determining the modifications of the SFE. This was probably due to the alteration of polar groups caused by the plasma treatments.

It has been reported that the surface roughness is also an important factor that can affect the adhesion of *Candida* to the material surfaces.[34] Thus, in this study, the specimens were made between glass slides to obtain smooth and standardised surfaces. As can be observed in Table 3, the results demonstrated that there were no significant differences in the mean roughness values among all groups evaluated before plasma treatments. After plasma treatments, the images obtained by nanoindenter demonstrated that there was significant alteration on surface topography, particularly after the Ar/50 W plasma treatment. These results demonstrate that the acrylic resin topography is sensitive to treatment conditions. The specimens exposed to Ar/50 W plasma were very smooth compared with control and AAt/130 W specimens. This finding was confirmed when a greater scan length was evaluated by profilometer. The ''polishing effect'' on the acrylic resin surface caused by Ar/50 W plasma treatment can be attributed to breaking of micro- and nanoscale peaks caused mainly by bombardment of energetic particles present in the Ar/50 W treatment.[36] Some studies [44,45] have showed that *Candida* adhered in greater amounts on rough surfaces when compared with smooth acrylic surfaces. Thus, it is likely that the smoothing of the surface caused by Ar/50 W plasma may have contributed to the decrease on *C. glabrata* adhesion (in the absence of saliva).

After immersion in water for 48 h, the hydrophobicity of the specimens submitted to plasma treatments came close to that observed for the control group specimens. The temporal evolution observed in plasma treated polymer surfaces is ascribed to the removal of polar groups from the surface by different phenomena. In one of them, hydrophilic groups incorporated on the surface are emitted causing chemical modifications of the region. Other possible explanation could be the increase in the surface energy caused by plasma treatment.[15,39] Under this condition, it has been observed that the polymers have a tendency to recover their hydrophobicity,[14,20] which has been attributed to the rotation of polar groups around the polymeric backbone into the material bulk.[39] This last mechanism is allowed by the high flexibility of the carbon backbones in polymers.

It is well known that the combination of hydrophobicity and roughness should lead a more complex interpretation of the surface thermodynamical properties. The surface roughness influences the contact area between the drop and the surface, intensifying the electrostatic forces. Thus, if a hydrophilic surface has its roughness increased, it becomes even more hydrophilic. On the other hand, surface roughness increase the contact angle of hydrophobic materials.[46] Hence, the evolution of the contact angle with time can be attributed to a combination of chemical and morphological changes on acrylic resin surface.

Despite the alteration in the SFE values observed in experimental groups, the WCA measurements performed immediately after treatments and at periods of 5, 15, 30, 60 and 120 min, demonstrated that the Ar/50 W treated specimens showed slower hydrophobic recovery compared to AAt/130 W-treated specimens. Moreover, after 48 h of immersion in water (in absence of saliva), they remained equal to each other and significantly more hydrophilic compared with control. Since hydrophobic interactions are involved in the adhesion process,[4–8] the hydrophilic surface observed in Ar/50W group could have inhibited the adherence of *C. glabrata*. Although the characteristic of cell surface hydrophobicity (CSH) is not species specific,[4,47] *C. glabrata* has been considered a relatively hydrophobic *Candida*.[8,48] When compared with *C. albicans*, *C. glabrata* presented higher CSH and higher tendency to adhere to acrylic surface.[8]In addition, the closer the surface free energy of the surface and the microorganism, it is higher that the probability of *Candida* adherence.[6] Therefore, the results obtained suggest that not only topographical modifications but also a decrease in surface hydrophobicity (chemical composition changes) caused by plasma treatments may contribute to the decrease on *C. glabrata* adhesion.

It has been demonstrated that the salivary coating is an important factor in determining the wettability properties of denture materials. In this study, the preconditioning with saliva altered the wettability of all groups, which have also been observed by other authors.[18,49] The levelling of the differences in WCA, DCA and SFE among the groups and the presence of receptors in the salivary pellicle may have contributed to the results of the adhesion assay, in which *C. glabrata* adhesion to untreated and plasma treated surfaces was similar. It should be noted, however, that there are only a few studies dealing with interactions between salivary pellicle and *Candida glabrata*.[31,33,34,50] Hence, further studies are needed on this subject.

In conclusion, the Ar/50 W plasma treatment reduced the adhesion of *C. glabrata* to the denture base resin compared with control. However, although statistically significant, the observed difference may not be clinically relevant. Moreover, in the presence of saliva, there were no significant differences among all groups. Nevertheless, the plasma treatments showed potential to modify the chemical composition, hydrophobicity and topography of denture base acrylic resin surface and deserve further investigation, especially to tailor the parameters to inhibit the temporal recovery of the contact angle and prolong the increased wettability.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

1 Dağistan S, Aktas AE, Caglayan F, Ayyildiz A, Bilge M. Differential diagnosis of denture-induced stomatitis, *Candida*, and their variations in patients using complete denture: a clinical and mycological study. *Mycoses* 2009; **52**: 266–71.

2 Coco BJ, Bagg J, Cross LJ, Jose A, Cross J, Ramage G. Mixed *Candida albicans* and *Candida glabrata* populations associated with the pathogenesis of denture stomatitis. *Oral Microbiol Immunol* 2008; **23**: 377–83.

3 Li L, Redding S, Dongari-Bagtzoglou A. *Candida glabrata*: an emerging oral opportunistic pathogen. *J Dent Res* 2007; **86**: 204–15.
4 Yoshijima Y, Murakami K, Kayama S *et al.* Effect of substrate surface hydrophobicity on the adherence of yeast and hyphal *Candida*. *Mycoses* 2010; **53**: 221–6.
5 Klotz SA, Drutz DJ, Zajic JE. Factors governing adherence of *Candida* species to plastic surfaces. *Infect Immun* 1985; **50**: 97–101.
6 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.
7 Samaranayake YH, Wu PC, Samaranayake LP, So M, Yuen KY. Adhesion and colonisation of *Candida krusei* on host surfaces. *J Med Microbiol* 1994; **41**: 250–8.
8 Luo G, Samaranayake LP. *Candida glabrata*, an emerging fungal pathogen, exhibits superior relative cell surface hydrophobicity and adhesion to denture acrylic surfaces compared with *Candida albicans*. *APMIS* 2002; **110**: 601–10.
9 Park SE, Periathamby AR, Loza JC. Effect of surface-charged poly[methyl methacrylate] on the adhesion of *Candida albicans*. *J Prosthodont* 2003; **12**: 249–54.
10 Dhir G, Berzins DW, Dhuru VB, Periathamby AR, Dentino A. Physical properties of denture base resins potentially resistant to *Candida* adhesion. *J Prosthodont* 2007; **16**: 465–72.
11 Park SE, Blissett R, Susarla SM, Weber HP. *Candida albicans* adherence to surface-modified denture resin surfaces. *J Prosthodont* 2008; **17**: 365–9.
12 Puri G, Berzins DW, Dhuru VB *et al.* Effect of phosphate group addition on the properties of denture base resins. *J Prosthet Dent* 2008; **100**: 302–8.
13 Redding S, Bhatt B, Rawls HR, Siegel G, Scott K, Lopez-Ribot J. Inhibition of *Candida albicans* biofilm formation on denture material. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009; **107**: 669–72.
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 Hauser J, Zietlow J, Köller M *et al.* Enhanced cell adhesion to silicone implant material through plasma surface modification. *J Mater Sci- Mater Med* 2009; **20**: 2541–8.
16 Bazaka K, Jacob MV, Crawford RJ, Ivanova EP. Plasma-assisted surface modification of organic biopolymers to prevent bacterial attachment. *Acta Biomater* 2011; **7**: 2015–28.
17 Li Y, Kuai P, Huo P, Liu C. Fabrication of CuO nanofibers via the plasma decomposition of $Cu[OH]_2$. *Mater Lett* 2009; **63**: 188–90.
18 Yildirim MS, Hasanreisoglu U, Hasirci n, Sultan N. Adherence of *Candida albicans* to glow-discharge modified acrylic denture base polymers. *J Oral Rehabil* 2005; **32**: 518–25.
19 Yildirim MS, Kesimer M, Hasirci N, Kiliç N, Hasanreisoğlu U. Adsorption of human salivary mucin MG1 onto glow-discharge plasma treated acrylic resin surfaces. *J Oral Rehabil* 2006; **33**: 775–83.
20 Zamperini CA, Machado AL, Vergani CE, Pavarina AC, Rangel EC, da Cruz NC. Evaluation of fungal adherence to plasma-modified polymethylmethacrylate. *Mycoses* 2011; **54**: 344–51.
21 Nikawa H, Hayashi S, Nikawa Y, Hamada T, Samaranayake LP. Interactions between denture lining material, protein pellicles and *Candida albicans*. *Arch Oral Biol* 1993; **38**: 631–4.
22 Nikawa H, Yamamoto T, Hamada T, Rahardjo MB, Murata H, Nakanoda S. Antifungal effect of zeolite-incorporated tissue conditioner against *Candida albicans* growth and/or acid production. *J Oral Rehabil* 1997; **24**: 350–7.
23 Nikawa H, Jin C, Hamada T, Murata H. Interactions between thermal cycled resilient denture lining materials, salivary and serum pellicles and *Candida albicans* in vitro. Part I. Effects on fungal growth. *J Oral Rehabil* 2000; **27**: 41–51.
24 Nikawa H, Chen J, Hamada T, Nishimura M, Polyzois G. *Candida albicans* colonization on thermal cycled maxillofacial polymeric materials in vitro. *J Oral Rehabil* 2001; **28**: 526–33.
25 Chandra J, Mukherjee PK, Leidich SD *et al.* Antifungal resistance of candidal biofilms formed on denture acrylic in vitro. *J Dent Res* 2001; **80**: 903–8.
26 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.
27 Tari BF, Nalbant D, Dogruman Al F, Kustimur S. Surface roughness and adherence of *Candida albicans* on soft lining materials as influenced by accelerated aging. *J Contemp Dent Pract* 2007; **8**: 18–25.
28 Thein ZM, Samaranayake YH, Samaranayake LP. Characteristics of dual species *Candida* biofilms on denture acrylic surfaces. *Arch Oral Biol* 2007; **52**: 1200–8.
29 Karaagaclioglu L, Can G, Yilmaz B, Ayhan N, Semiz O, Levent H. The adherence of *Candida albicans* to acrylic resin reinforced with different fibers. *J Mater Sci- Mater Med* 2008; **19**: 959–63.
30 Samaranayake LP, McCourtie J, MacFarlane TW. Factors affecting th in vitro adherence of *Candida albicans* to acrylic surfaces. *Arch Oral Biol* 1980; **25**: 611–5.
31 McCourtie J, MacFarlane TW, Samaranayake LP. Effect of saliva and serum on the adherence of *Candida* species to chlorhexidine-treated denture acrylic. *J Med Microbiol* 1986; **21**: 209–13.
32 Waters MG, Williams DW, Jagger RG, Lewis MA. Adherence of *Candida albicans* to experimental denture soft lining materials. *J Prosthet Dent* 1997; **77**: 306–12.
33 Moura JS, da Silva WJ, Pereira T, Del Bel Cury AA, Rodrigues Garcia RC. Influence of acrylic resin polymerization methods and saliva on the adherence of four *Candida* species. *J Prosthet Dent* 2006; **96**: 205–11.
34 Pereira-Cenci T, Cury AA, Cenci MS, Rodrigues-Garcia RC. In vitro *Candida* colonization on acrylic resins and denture liners: influence of surface free energy, roughness, saliva, and adhering bacteria. *Int J Prosthodont* 2007; **20**: 308–10.
35 Riccardi C, Barni R, Selli E *et al.* Surface modification of poly(ethylene terephthalate) fibers induced by radio frequency air plasma treatment. *Appl Surface Sci* 2003; **211**: 386–97.
36 Lai J, Sunderland B, Xue J *et al.* Study on hydrophilicity of polymer surfaces improved by plasma treatment. *Appl Surface Sci* 2006; **252**: 3375–9.
37 Polukoshko KM, Brudvik JS, Nicholls JI, Smith DE. Evaluation of heat-cured resin bases following the addition of denture teeth using a second heat cure. *J Prosthet Dent* 1992; **67**: 556–62.
38 Moisan M, Barbeau J, Crevier MC, Pelletier J, Philip N, Saoudi B. Plasma sterilization. Methods and mechanisms. *Pure Appl Chem* 2002; **74**: 349–58.

39 Rangel EC, Gadioli GZ, Cruz NC. Investigations on the stability of plasma modified silicone surfaces. *Plasmas Polym* 2004; **9**: 35–48.
40 Dong Y, Li X, Tian L, Bell T, Sammons RL, Dong H. Towards long-lasting antibacterial stainless steel surfaces by combining double glow plasma silvering with active screen plasma nitriding. *Acta Biomater* 2011; **7**: 447–57.
41 Peros WJ, Gibbons RJ. Influence of growth medium on adsorption of *Streptococcus mutans*, *Actinomyces viscosus*, and *Actinomyces naeslundii* to saliva-treated hydroxyapatite surfaces. *Infect Immun* 1981; **32**: 111–7.
42 Ferreira MA, Pereira-Cenci T, Rodrigues de Vasconcelos LM, Rodrigues-Garcia RC, Del Bel Cury AA. Efficacy of denture cleansers on denture liners contaminated with *Candida* species. *Clin Oral Investig* 2009; **13**: 237–42.
43 Ozden N, Akaltan F, Suzer S, Akovali G. Time-related wettability characteristic of acrylic resin surfaces treated by glow discharge. *J Prosthet Dent* 1999; **82**: 680–4.
44 Verran J, Maryan CJ. Retention of *Candida albicans* on acrylic resin and silicone of different surface topography. *J Prosthet Dent* 1997; **77**: 535–9.
45 Radford DR, Sweet SP, Challacombe SJ, Walter JD. Adherence of *Candida albicans* to denture-base materials with different surface finishes. *J Dent* 1998; **26**: 577–83.
46 Elkin B, Mayer J, Schindler B, Vohrer U. Wettability, chemical and morphological data of hydrophobic layers by plasma polymerization on smooth substrates. *Surface Coatings Technol* 1999; **119**: 836–40.
47 Panagoda GJ, Ellepola AN, Samaranayake LP. Adhesion of *Candida parapsilosis* to epithelial and acrylic surfaces correlates with cell surface hydrophobicity. *Mycoses* 2001; **44**: 29–35.
48 Minagi S, Miyake Y, Fujioka Y, Tsuru H, Suginaka H. Cell-surface hydrophobicity of *Candida* species as determined by the contact-angle and hydrocarbon-adherence methods. *J Gen Microbiol* 1986; **132**: 1111–5.
49 Sipahi C, Anil N, Bayramli E. The effect of acquired salivary pellicle on the surface free energy and wettability of different denture base materials. *J Dent* 2001; **29**: 197–204.
50 Pereira-Cenci T, Deng DM, Kraneveld EA *et al.* The effect of *Streptococcus mutans* and *Candida glabrata* on *Candida albicans* biofilms formed on different surfaces. *Arch Oral Biol* 2008; **53**: 755–64.