Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: http://www.elsevier.com/locate/aob

Effect of experimental photopolymerized coatings on the hydrophobicity of a denture base acrylic resin and on *Candida albicans* adhesion

Andréa Azevedo Lazarin [a], *Ana Lucia Machado* [a,*], *Camila Andrade Zamperini* [a], *Amanda Fucci Wady* [a], *Denise Madalena Palomari Spolidorio* [b], *Carlos Eduardo Vergani* [a]

[a] *Araraquara Dental School, UNESP - Univ. Estadual Paulista, Department of Dental Materials and Prosthodontics, Araraquara, São Paulo, Brazil*
[b] *Araraquara Dental School, UNESP - Univ. Estadual Paulista, Department of Physiology and Pathology, Araraquara, São Paulo, Brazil*

ARTICLE INFO

Article history:
Accepted 7 October 2012

Keywords:
Adhesion
Candida albicans
Hydrophilic
Prosthesis
Surface modification

ABSTRACT

Objective: This study investigated the effect of experimental photopolymerized coatings, containing zwitterionic or hydrophilic monomers, on the hydrophobicity of a denture base acrylic resin and on *Candida albicans* adhesion.

Methods: Acrylic specimens were prepared with rough and smooth surfaces and were either left untreated (control) or coated with one of the following experimental coatings: 2-hydroxyethyl methacrylate (HE); 3-hydroxypropyl methacrylate (HP); and 2-trimethylammonium ethyl methacrylate chloride (T); and sulfobetaine methacrylate (S). The concentrations of these constituent monomers were 25%, 30% or 35%. Half of the specimens in each group (control and experimentals) were coated with saliva and the other half remained uncoated. The surface free energy of all specimens was measured, regardless of the experimental condition. *C. albicans* adhesion was evaluated for all specimens, both saliva conditioned and unconditioned. The adhesion test was performed by incubating specimens in *C. albicans* suspensions (1×10^7 cell/mL) at 37 °C for 90 min. The number of adhered yeasts were evaluated by XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-5-[{phenylamino}carbonyl]-2H-tetrazolium-hydroxide) method.

Results: For rough surfaces, coatings S (30 or 35%) and HP (30%) resulted in lower absorbance values compared to control. These coatings exhibited more hydrophilic surfaces than the control group. Roughness increased the adhesion only in the control group, and saliva did not influence the adhesion. The photoelectron spectroscopy analysis (XPS) confirmed the chemical changes of the experimental specimens, particularly for HP and S coatings.

Conclusions: S and HP coatings reduced significantly the adhesion of *C. albicans* to the acrylic resin and could be considered as a potential preventive treatment for denture stomatitis.

1. Introduction

In spite of its multifactorial etiology, *Candida albicans* infection has often been associated with denture-induced stomatitis.[1] In addition to its high incidence in denture wearers, there is a concern that *Candida* species from the oral cavity may colonize the upper gastrointestinal tract in immunosuppressed patients and lead to septicemia.[2]

* *Corresponding author at*: Departament of Dental Materials and Prosthodontics, Araraquara Dental School, - UNESP - Univ. Estadual Paulista, Rua Humaita, 1680, CEP: 14801-903, Araraquara, SP, Brazil. Tel.: +55 16 33016547; fax: +55 16 33016406.
E-mail addresses: cucci@foar.unesp.br, almachado98@uol.com.br (A.L. Machado).

0003–9969
http://dx.doi.org/10.1016/j.archoralbio.2012.10.005

Candida spp. are more frequently isolated from the fitting surface of dentures when compared to the corresponding region of the oral mucosa.[1] Therefore, the treatment of denture-induced stomatitis should include denture cleansing and disinfection in addition to topic or systemic antifungal drugs. Although these treatments do show some efficacy, they aim at inactivating the microorganisms after denture surface colonization. As the adhesion of microorganisms to denture surfaces is a prerequisite for microbial colonization,[3,4] the development of methods that can reduce *C. albicans* adhesion may represent a significant advance in the prevention of denture-induce stomatitis.

The use of polymers containing zwitterionic groups such as phosphatidylcholines and sulfobetaines,[5–10] which originate from the simulation of biomembranes,[9,11] has been proposed to modify the surface of biomaterials.[12–14] A significant reduction in protein adsorption has been demonstrated[5,8–10,12–18] and attributed to the formation of a hydration layer on the material surface[5–7,9–14,16,17,19] that prevents the conformational alteration of these proteins.[9,11,13,14,19] Previous researchers[7,13,16,20,21] reported that sulfobetaine application on substrate surfaces reduced bacterial adhesion. These results suggest that sulfobetaine-based polymers may be used to modify the surface of acrylic materials used in the fabrication of removable dentures and reduce microbial adhesion.[6] However, the effectiveness of this surface modification on *C. albicans* adhesion remains to be investigated.

Surface modification by deposition of polymer coatings such as parylene has been reported to improve the wettability of a silicone elastomer and reduce *C. albicans* adhesion and aggregation on its surface.[22] Hydrophilic polymers have also been investigated in biomaterial research.[19,23,24] The hydration state of hydrophilic polymers is different from that of zwitterionic polymers, and the free water fraction on polymer surface is lower in the former.[19] Despite these differences, hydrophilic polymers have been used to modify the surface of biomaterials and reduce bacterial adhesion.[23,24] The adsorption of proteins to neutral hydrophilic surfaces is relatively weak, while their adsorption to hydrophobic surfaces tends to be very strong and practically irreversible.[25,26] Therefore, altering the characteristics of the inner surfaces of dentures by increasing their hydrophilicity could reduce colonization by pathogenic microorganisms, including *Candida* spp. It has been reported that substratum surface properties, such as surface free energy, may influence *C. albicans* adhesion to polymers, where hydrophobic interactions play a role.[27–29]

The purpose of this study was to evaluate the effect of experimental photopolymerized coatings, containing zwitterionic or hydrophilic monomers, on the hydrophobicity of a denture base acrylic resin and on *C. albicans* adhesion. The hypotheses were that the coating application would decrease the surface hydrophobicity and reduces *C. albicans* adhesion, and that there would be differences among coatings.

2. Material and methods

2.1. Specimen fabrication

Disc-shaped silicone patterns (13.8 mm × 2 mm) were obtained from metallic matrices. Half of the silicone patterns were inserted between two glass plates and the other half were inserted in dental flasks directly in contact with the stone. These two methods of specimen preparation were used to obtain smooth and rough surfaces that simulate the outer and inner surfaces of the dentures, respectively. The silicone patterns were then removed, and the surfaces were coated with a layer of separating medium (Vipi Film; VIPI Indústria e Comércio Exportação e Importação de Produtos Odontológicos Ltda Pirassununga, SP, Brazil). A colourless microwave-polymerized denture base acrylic resin (Vipi Wave; VIPI Indústria e Comércio Exportação e Importação de Produtos Odontológicos Ltda., Pirassununga, SP, Brazil) was mixed according to the manufacturer's instructions at a mixing ratio of 1 g powder to 0.47 mL of liquid for each specimen. The moulds were filled with the acrylic resin, a trial pack was completed, and excess material was removed. A final pack was performed and held for 15 min. The denture base acrylic resin was processed in a 500 W domestic microwave oven (Brastemp; Brastemp da Amazônia SA, Manaus, AM, Brazil) for 20 min at 20% power followed by 5 min at 90% power. After polymerization, the flasks were allowed to cool at room temperature, the specimens were deflasked, and the excess was trimmed with a sterile bur (Maxi-Cut; Lesfils de August Malleifer SA, Ballaigues, Switzerland). A total of 468 disc-shaped specimens were fabricated by a single operator wearing a mask, gloves and protective clothing.

2.2. Surface roughness measurements

Considering the possible influence of roughness on the adhesion of microorganisms to substrate surfaces,[3,30] the surface roughness of the specimens was measured using a profilometer (Mitutoyo SJ 400; Mitutoyo Corporation, Tokyo, Japan) accurate to 0.01 μm. The cutoff length was 0.8 mm, the transverse length was 2.4 mm, the stylus speed was 0.5 mm/s and the diamond stylus tip radius was 5 μm. Four measurements were made on the surface of each specimen and averaged to obtain the Ra value (μm). All measurements were recorded by a single operator.

2.3. Experimental photopolymerized coatings

After roughness reading, the specimens were randomly assigned to 13 groups of 36 specimens each; 18 specimens had smooth surfaces and 18 specimens had rough surfaces. In the control group (C), the specimens did not receive any surface treatment. In each experimental group, all specimen surfaces were coated with a layer of one of the experimental photopolymerized coatings. Four coating formulations were evaluated: 3 coatings containing hydrophilic monomers: 2-hydroxyethyl methacrylate (HEMA) – HE, 2-hydroxypropyl methacrylate (HPMA) – HP, and 2-trimethylammonium ethyl methacrylate chloride (TMAEMC) – T, and 1 coating containing a zwitterionic monomer (sulfobetaine methacrylate) – S. These monomers were used at concentrations of 25%, 30% and 35% of the total composition in mmol which resulted in 12 experimental coatings (HE25; HE30; HE35; HP25; HP30; HP35; T25; T30; T35; S25; S30; S35). In addition to the above monomers, all coatings contained the monomer methyl methacrylate, two crosslinking agents (triethylene glycol

dimethacrylate (TEGDMA) and bisphenol-A-glycidyl methacrylate (Bis-GMA)) and an initiator agent (4-methyl benzophenone). For the coating S, amino propyl methacrylate was also added. The monomer methyl methacrylate causes the polymer surface to swell,[31] and the adhesion is obtained by interdiffusion of the coatings into the swollen denture base polymer structure, photopolymerization, and formation of interpenetrating polymer network.

The application of the 12 coatings on the specimen surfaces was performed in a sterile laminar flow chamber followed by a 4 min polymerization on each surface in an EDG oven (Strobolux, EDG, São Carlos, São Paulo, SP, Brazil). For the S coating, propane sultone was brushed on specimen surfaces, and the specimens were maintained in an oven at 80 °C for 2 h. Thereafter, all specimens were stored individually in properly labelled plastic bags containing sterile distilled water for 48 h at room temperature for release of uncured residual monomers.[32]

2.4. Exposure of the specimens to human saliva

Half of the specimens in each group (control and experimentals) were exposed to saliva. For this purpose, non-stimulated saliva was collected from 50 healthy male and female adults. Ten millilitres of saliva from each donor were mixed, homogenized and centrifuged at 5000 × *g* for 10 min at 4 °C. The saliva supernatant was prepared at 50% (v/v) in sterile PBS[33] and immediately frozen and stored at −70 °C. The specimens were incubated with the prepared saliva at room temperature for 30 min.[34,35] The other half of the specimens was not exposed to saliva. The research protocol was approved by the Research Ethics Committee of Araraquara Dental School, and all volunteers signed an informed consent form.

2.5. Surface free energy

To characterize the hydrophobicity of the surfaces, the surface free energy of all specimens, regardless of the experimental condition, was calculated from contact angle measurements using the sessile drop method and a contact angle measurement apparatus (System OCA 15 PLUS; Dataphysics). This device has a CCD camera that records the drop image (15 μL) on the specimen surface, and image-analysis software determines the right and left contact angles of the drop after 5 s. The wettability and surface energy of the specimens were evaluated from data obtained in the contact angle measurements. In these analyses, deionized water was used as the polar liquid and diiodomethane (Sigma–Aldrich, St. Louis, MO, USA) as the dispersive (non-polar) compound.[36] Surface free energy components were evaluated by the Owens–Wendt method based on the contact angles of two test liquids with different polarities.[37] For each liquid, both the left and right sides of two drops (on different locations) were obtained for all specimens, and the average was calculated.

The specimens were packed in sealed sterile plastic bags with sterile distilled water and ultrasonicated for 20 min. Then all specimen surfaces were exposed to ultraviolet light in a laminar flow chamber for 20 min for sterilization.[38]

2.6. Microorganism, growth conditions and adhesion to the specimen surface

C. albicans adhesion was evaluated for all specimens, both saliva conditioned and unconditioned. For the preparation of the inoculum, the yeast *C. albicans* ATCC 90028 was seeded in an agar YEPD culture medium (1% yeast extract, 2% peptone, 2% dextrose, 2% agar) and incubated for 48 h at 37 °C. After this period, two loops of the cultivated yeast were transferred to 20 mL of the YNB (yeast nitrogen base) medium (Difco, Detroit, MI, USA) with 50 mM glucose. After incubation for 21 h at 37 °C, the cells were washed twice with sterile phosphate-buffered saline solution (PBS) (pH 7.2) by agitation and centrifugation at 5000 × *g* for 5 min. After washing, the cells were re-suspended in 20 mL of YNB broth with 100 mM sterile glucose. *C. albicans* suspensions were standardized to a concentration of 1×10^7 cell/mL, spectrophotometrically. An aliquot of 3 mL of the standardized *C. albicans* suspension was added to each well of a 12-well microplate containing the specimens and maintained for 90 min at 37 °C in the adhesion phase.[39] Thereafter, the specimens were carefully washed twice with 3 mL of PBS to remove the non-adhered cells. Negative controls were sterile specimens immersed in YNB broth supplemented with glucose at 100 mM. All experiments were performed in triplicate on three different occasions.

2.7. XTT assay

The viability of the *C. albicans* cells adhering to acrylic specimen surfaces was evaluated by XTT (2,3-bis(2-methoxy-4-nitro-5-sulfo-phenyl)-2H-tetrazolium-5-carboxanilide)-reduction assay, which measures the cell metabolic activity. Although XTT is a semi-quantitative colorimetric assay,[40] it correlates well with other quantitative techniques such as ATP and CFU assays[40,41] and, thus, it has been widely used to evaluate fungal adhesion and biofilm formation.[33,40] The XTT solution (Sigma Chemical Co., St. Louis, MO, USA) was prepared using ultra pure water at a concentration of 1 mg/mL, sterilized by filtration and maintained at −70 °C. The menadione solution (Sigma Chemical Co., St. Louis, MO, USA) was prepared in 0.4 mM acetone immediately before each experiment. After washing, the specimens were transferred to 12-well microplates containing, in each well, 2370 μL of PBS supplemented with 200 mM glucose, 600 μL of XTT and 30 μL of menadione. The plates were incubated in the dark for 3 h at 37 °C. The entire contents of each well were transferred to individual tubes and centrifuged at 5000 × *g* for 2 min. The supernatant was then transferred to a 96-well microplate, and the colour change was measured using a microplate reader (Thermo Plate – TP Reader) at 492 nm.

2.8. X-ray photoelectron spectroscopy analysis (XPS)

The chemical composition of the specimen surfaces after the coating application was characterized by XPS (X-ray photoelectron spectroscopy). The XPS analysis was carried out using a commercial spectrometer (UNI-SPECS UHV) to verify surface chemical composition changes in the treated specimens. The Mg Kα line was used ($E = 1253.6$ eV), and the analyzer pass energy was set to 10 eV. The inelastic background of the C 1s, O

1s and N 1s electron core-level spectra was subtracted using Shirley's method. The binding energies of spectra were corrected using the polymer hydrocarbon component fixed at 285.0 eV. The composition of the surface layer was determined from the ratio of the relative peak areas corrected by sensitivity factors of the corresponding elements. Spectra were fitted without placing constraints using multiple Voigt profiles. The width at half maximum (FWHM) varied between 1.6 and 2.0 eV and the accuracy of the peak positions was ±0.1 eV. In the present analysis, 1 specimen from the group control (no surface treatment) and one specimen treated with one of the four experimental coatings formulations were used at the higher concentration.

2.9. *Statistical analysis*

The effect of the two methods used for specimen fabrication on surface roughness was analyzed statistically by the non-parametric Mann–Whitney test. The non-parametric Kruskal–Wallis test was used to compare roughness among groups within each specimen fabrication method. The surface free energy values were analyzed statistically by the three-way ANOVA and Tukey's test. The metabolic activity differences (XTT assay) between the specimens pre-treated or untreated with saliva within each group were analyzed by the non-parametric Kruskal–Wallis test. Since no statistically significant difference was found, the 18 values obtained for each group (pre-treated or untreated with saliva) were grouped and used for group comparisons using the non-parametric Kruskal–Wallis test. A significance level of 5% was used for all analyses.

3. Results

Table 1 shows that the mean roughness values obtained for specimens fabricated between glass plates (smooth surfaces) were lower than 0.23 μm, while for those specimens fabricated in contact with the stone (rough surfaces), the values were significantly different ($p < 0.05$) (higher than 1.73 μm). Within each specimen fabrication method, there were no statistically significant differences ($p > 0.05$) in surface roughness among the groups.

The surface free energy (polar and dispersive components) mean values and standard deviations for control and experimental groups are presented in Table 2. Overall, the coatings application increased the polar component of the surface free energy with statistically significant differences for S25 groups (smooth surface; absence of saliva), S25, S30, S35, HP35 groups (rough surface; absence of saliva) and HP25, HP30, HE25, T25 groups (rough surface; presence of saliva). Compared to the control, the dispersive component was significantly increased in the S35 group (presence of saliva) and decreased in the T35 group (absence of saliva). The total surface free energy was also higher in all the experimental groups compared to the control; the differences were statistically significant for the S25 and S35 groups (smooth surface; absence of saliva), S30, S35 groups (rough surface; absence of saliva) and HP25, HP30, HP35, HE25, T25 groups (rough surface; presence of saliva).

Table 1 – Mean roughness values (Ra-μm) and standard deviations (SD) obtained in the groups ($n = 18$), according to the method used for specimen fabrication.

Groups	Glass		Stone	
Control	0.19	(0.07)[a]	1.95	(0.51)[b]
S25	0.17	(0.08) a	2.13	(0.80)[b]
S30	0.19	(0.09) a	2.29	(0.70)[b]
S35	0.18	(0.07) a	1.95	(0.74)[b]
HP25	0.16	(0.09) a	2.11	(0.54)[b]
HP30	0.20	(0.08) a	2.05	(0.69)[b]
HP35	0.23	(0.06) a	1.73	(0.53)[b]
HE25	0.23	(0.06) a	1.78	(0.56)[b]
HE30	0.17	(0.08) a	1.90	(0.77)[b]
HE35	0.17	(0.07) a	2.09	(0.61)[b]
T25	0.17	(0.08) a	1.93	(0.78)[b]
T30	0.15	(0.07) a	1.74	(0.52)[b]
T35	0.17	(0.08) a	1.94	(0.79)[b]
Kruskal–Wallis test	$p = 0.083$		$p = 0.462$	

Different letters indicate statistically significant difference at 5%.

For the control group, Table 2 also shows that there were no significant differences in polar and dispersive components, as well as the surface free energy, between uncoated and saliva-coated specimens. For the experimental groups, saliva significantly decreased the polar component for S25 group (smooth surface), S25, S30 and S35 groups (rough surfaces), and significantly increased for the HP25, HP30 and HE25 groups (rough surfaces). The dispersive component significantly increased after incubation with saliva for S35 group, regardless of the surface roughness. The total surface free energy of rough surfaces was significantly decreased in the presence of saliva for the S30 group, while for HP25, HE25 and T25 groups, a significant increase was noted.

For specimens fabricated between glass plates (smooth surfaces), there were no statistically significant differences ($p > 0.05$) in absorbance values among the groups (Table 3). This indicates similar *C. albicans* initial biofilm formation. For specimens fabricated in contact with the stone (rough surfaces), S30, S35 and HP30 groups had significantly lower ($p < 0.05$) absorbance values than the control group. When controls were compared, a higher mean absorbance value was observed for rough surfaces ($p < 0.05$). All negative controls exhibited no metabolic activity (data not shown).

Surface compositions evaluated by XPS analysis are shown in Table 4. Spectra of the unmodified surfaces showed peaks for carbon (75.3 at.%), oxygen (23.0 at.%), and silicon (0.3 at.%). After the coatings application, the percentage of the elements changed, particularly for HP and S coatings. HP resulted in a decrease of C 1s and an increase of O 1s and Si 2p; a new peak attributed to phosphor appeared. The S coating which contains sulfobetaine resulted in an increased C 1s peak and Si 2p and a decreased peak for O 1s. An additional peak for the presence of sulphur (0.5 at.%) was also observed.

4. Discussion

In this study, two methods of specimen preparation were used (between glass plates or in contact with stone), and smooth and rough surfaces were obtained. The adhesion of *C. albicans*

Table 2 – Mean polar and dispersive components and surface free energy values (Dyn/cm) and standard deviations (SD) obtained in the groups (n = 9), according to the method used for specimen fabrication.

Groups	Saliva	Polar component				Dispersive component				Total surface free energy			
		Glass		Stone		Glass		Stone		Glass		Stone	
		Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)	
Control	as	6.11(2.33)	a	6.53 (2.94)	a	36.20 (3.00)	bcd	40.83 (3.02)	bcd	42.31 (3.51)	a	47.37 (1.97)	ab
	ps.	5.88 (1.65)	a	6.70 (5.79)	a	36.61 (4.07)	abc	37.13 (2.74)	abc	42.49 (4.04)	a	43.82 (4.79)	a
S25	as	15.80 (5.87)	b	18.01 (8.04)	cd	36.79 (5.08)	abc	36.05 (4.45)	abc	52.58 (6.07)	b	54.06 (8.02)	bcd
	ps	7.03 (2.98)	a	7.98 (4.45)	ab	38.44 (3.49)	cd	41.38 (2.40)	cd	45.48 (2.05)	ab	49.36 (4.65)	abc
S30	as	11.63 (5.43)	ab	20.66 (8.74)	d	36.62 (4.15)	abc	36.47 (4.65)	abc	48.25 (5.45)	ab	57.13 (7.34)	d
	ps	6.55 (3.39)	a	5.80 (2.17)	a	37.96 (3.38)	cd	41.70 (1.59)	cd	44.51 (3.86)	a	47.51 (2.56)	ab
S35	as	13.06 (4.87)	ab	20.61 (5.52)	d	37.34 (3.34)	abc	35.15 (2.74)	abc	50.40 (6.50)	b	55.75 (5.55)	cd
	ps	6.09 (3.47)	a	7.27 (2.24)	ab	41.92 (2.29)	d	41.24 (3.03)	d	48.01 (3.47)	ab	48.50 (3.46)	abc
HP25	as	6.74 (2.74)	a	9.34 (2.57)	ab	37.56 (2.43)	bcd	39.79 (3.14)	bcd	44.30 (2.69)	a	49.13 (2.55)	abc
	ps	10.87 (4.86)	ab	19.02 (7.10)	d	38.46 (3.89)	bcd	39.85 (1.90)	bcd	49.33 (2.69)	ab	58.87 (6.19)	d
HP30	as	7.78 (1.74)	ab	8.99 (3.92)	ab	39.15 (2.19)	cd	39.95 (3.51)	cd	46.93 (2.20)	ab	48.93 (3.16)	abc
	ps	8.82 (3.66)	ab	17.45 (8.24)	cd	37.55 (3.83)	abcd	38.25 (3.61)	abcd	46.37 (4.56)	ab	55.70 (6.32)	cd
HP35	as	8.25 (3.57)	ab	15.51 (2.33)	bcd	37.02 (3.42)	abc	37.42 (3.10)	abc	45.26 (2.68)	ab	52.92 (2.54)	bcd
	ps	7.86 (2.13)	ab	13.32 (5.20)	abcd	38.98 (2.81)	bcd	38.95 (3.31)	bcd	46.84 (3.15)	ab	52.27 (5.74)	bcd
HE25	as	9.31 (2.93)	ab	9.33 (3.46)	ab	37.08 (4.09)	abc	34.79 (3.55)	abc	46.39 (3.36)	ab	44.12 (1.78)	a
	ps	7.18 (3.23)	a	19.37 (3.06)	d	38.40 (3.28)	abc	35.96 (2.36)	abc	45.57 (2.18)	ab	55.33 (3.00)	cd
HE30	as	9.26 (3.83)	ab	8.44 (2.82)	ab	37.36 (4.23)	abcd	38.79 (2.01)	abcd	46.62 (2.82)	ab	47.22 (2.47)	ab
	ps	10.17 (3.69)	ab	10.90 (6.06)	abc	38.31 (2.49)	abcd	38.28 (2.71)	abcd	48.48 (3.02)	ab	49.17 (4.41)	abc
HE35	as	12.34 (4.03)	ab	10.40 (3.01)	abc	36.66 (3.83)	abc	38.02 (3.95)	abc	49.00 (2.55)	ab	48.42 (5.66)	abc
	ps	9.99 (3.76)	ab	13.01 (6.60)	abcd	37.67 (1.81)	abc	36.87 (3.26)	abc	47.65 (4.62)	ab	49.88 (5.66)	abc
T25	as	8.47 (3.05)	ab	12.30 (3.75)	abcd	33.73 (3.26)	ab	36.34 (4.0)	ab	42.21 (4.17)	a	48.64 (5.40)	abc
	ps	12.16 (3.36)	ab	18.07 (3.80)	cd	37.44 (2.23)	abcd	38.87 (3.06)	abcd	49.61 (3.50)	ab	56.94 (1.56)	d
T30	as	9.06 (3.83)	ab	12.56 (5.38)	abcd	35.82 (4.67)	ab	34.03 (4.48)	ab	44.88 (5.17)	ab	46.59 (4.06)	ab
	ps	11.33 (6.98)	ab	7.55 (2.00)	ab	35.59 (4.32)	abcd	40.89 (3.98)	abcd	46.92 (5.48)	ab	48.43 (3.70)	abc
T35	as	9.92 (4.33)	ab	7.39 (3.06)	ab	32.88 (5.88)	a	35.17 (4.74)	a	42.80 (3.32)	a	42.56 (5.31)	a
	ps	11.33 (6.75)	ab	10.92 (5.03)	abc	36.78 (3.24)	abcd	38.91 (3.58)	abcd	48.11 (4.52)	ab	49.83 (3.89)	abc

as: absence of saliva (uncoated specimens); ps: presence of saliva (coated specimens). For polar component, dispersive component and surface free energy, means with the same small letters within the columns are not significantly different at $p \leq .05$.

to the denture base acrylic resin, as determined by the XTT assay, showed that, in control group, there was greater adhesion of *C. albicans* to rough surfaces than to smooth surfaces. This result is in agreement with other studies and may be due to the fact that roughness increases the surface area and may act as niches for microorganisms, thus favouring the adhesion.[3,30,42–44] For the specimens treated with the photopolymerized coatings, significant differences between smooth and rough surfaces were not detected.

It has been reported that the more hydrophobic the surface, the greater is the *C. albicans* cell adherence by area unit.[27] Thus, a commonly used method to reduce the attachment of microorganisms is surface modification with hydrophilic polymers[7,21,24] as attempted in the present study. For instance, coating surfaces with a 2-methacryloyloxyethyl phosphorylcholine (MPC) co-polymer decreased both water contact angles and the adhesion of *C. albicans*.[6] Accordingly, Yoshijima et al.[28] also observed that hydrophilic coatings of denture acrylic surfaces reduced the adhesion of the hydrophobic *C. albicans* hyphae. More recently, it has been also found that coating a denture base material with silica nanoparticles was effective in increasing surface hydrophilicity and decreasing *C. albicans* adherence.[29] Hence, in the present study, the surface free energy of the specimens was calculated.

The total surface free energy is the sum of components arising from dispersive and polar contributions where the polar component describes the hydrophilic character and the dispersive component is associated with the hydrophobic character of the surface. While the dispersive component (or Lifshitz–van der Waals) is influenced by the particle size or specific surface area, the polar component is the result of different forces/interactions such as polar, hydrogen, inductive and acid–base interactions.[45] Thus, while the dispersive component is affected by the surface roughness (or specific surface area), the polar component is dependent on the surface activity, which is related to the surface functional groups such as hydroxyl, carbonyl, and carboxyl.[45] Generally, in this study, the coatings application decreased the water contact angle (data not shown) and increased the polar surface free energy component which may have arisen from a change in the surface polar group concentration in the coated specimens. Only minor significant differences were observed for the dispersive component. Therefore, although the dispersive (or non-polar) component of the surface free energy

Table 3 – Medians (Med), minimum (Min) and maximum (Max) absorbance values (XTT assay - 492 nm) obtained in the groups ($n = 9$), according to the method used for specimen fabrication.

Groups	Saliva	Glass				Stone			
		Med	Min	Max		Med	Min	Max	
Control	as	0.54	0.43	0.97	a	1.23	0.83	1.62	b*
	ps	1.08	0.68	1.23	a	1.33	1.05	1.60	b*
S 25	as	0.83	0.67	1.21	a	0.94	0.46	1.13	ab
	ps	0.94	0.75	1.40	a	0.87	0.66	1.52	ab
S30	as	0.69	0.45	1.34	a	0.65	0.36	1.11	a
	ps	0.91	0.48	1.63	a	0.91	0.72	1.70	a
S35	as	0.80	0.57	1.14	a	0.54	0.38	0.98	a
	ps	0.83	0.57	1.42	a	1.02	0.62	1.62	a
HP 25	as	0.77	0.51	1.10	a	0.80	0.45	1.12	ab
	ps	1.15	0.46	1.53	a	1.16	0.71	1.32	ab
HP 30	as	0.59	0.40	0.95	a	0.72	0.40	0.94	a
	ps	0.87	0.50	1.55	a	1.07	0.59	1.45	a
HP 35	as	0.66	0.31	1.03	a	0.91	0.51	1.19	ab
	ps	1.00	0.61	1.46	a	1.19	0.72	1.77	ab
HE 25	as	0.77	0.45	1.03	a	0.74	0.41	0.87	ab
	ps	0.90	0.56	1.31	a	1.12	0.85	1.40	ab
HE 30	as	0.77	0.55	1.02	a	0.80	0.46	1.19	ab
	ps	0.91	0.58	1.33	a	1.42	0.81	1.50	ab
HE 35	as	0.79	0.33	1.21	a	0.93	0.50	1.61	ab
	ps	0.82	0.55	1.47	a	1.27	0.92	1.74	ab
T 25	as	0.81	0.65	1.22	a	0.99	0.57	1.41	ab
	ps	1.04	0.58	1.66	a	1.25	1.00	1.92	ab
T 30	as	0.85	0.39	1.14	a	1.10	0.82	1.31	ab
	ps	1.01	0.41	1.41	a	1.27	0.85	1.70	ab
T 35	as	0.80	0.59	1.15	a	1.01	0.68	1.39	ab
	ps	0.96	0.45	1.64	a	1.22	1.01	1.95	ab

Groups with the same letters in the columns did not differ significantly at 5%.
as: absence of saliva (uncoated specimens); ps: presence of saliva (coated specimens).
* Groups obtained between glass and stone differed significantly at 5%.

Table 4 – Elemental surface composition (at.%) of the groups evaluated determined by XPS.

Elements (at.%)	Groups				
	Control	HE	HP	T	S
C 1s	75.3	72.7	67.9	71.0	81.8
O 1s	23.0	24.6	23.9	23.3	15.4
Si 2p	0.3	2.0	7.6	4.6	3.1
P 2p	_	0.6	0.6	1.1	–
S 2p	_	_	_	_	0.5

is numerically higher than the polar component, the polar component is the main factor in determining modifications of the total surface free energy. Thus, the values of the surface energy followed the same trend as the polar component. Compared to the control, mean surface free energy values of the rough surfaces coated with S30, S35 and HP30 were significantly higher which indicates increased wettability. These results were expected because it is known that the contact angles are decreased (more hydrophilic) by surface roughness for hydrophilic surfaces.[46]

The effect of saliva on the hydrophobicity of the surfaces was also evaluated. The results showed that incubation with saliva did not significantly alter the polar and dispersive components and surface free energy for the control specimens. For experimental groups, the effect of saliva on the polar component and the total surface free energy varied depending on type of coating, with this effect being more significant for rough surfaces. As observed for the non-coated specimens, significant differences were also found mainly for the polar component of rough surfaces treated with S and HP coatings. However, for the S coating, saliva decreased the polar component, and the values became similar to the polar component of the control group; for the HP coating, an increase in the polar component was observed after incubation with saliva. Thus, the effect of saliva on the surface free energy varied depending on substrate characteristics, particularly the chemical composition and surface roughness. These findings suggest that the nature of the surface-exposed chemical groups after coating applications may influence the formation of the salivary pellicle (adsorbed salivary proteins). Other authors have also reported that small differences in the chemical composition of acrylic resins changed the adsorption of salivary proteins and, consequently the nature of the adsorbed salivary pellicle.[47,48] In this study, this phenomenon was particularly evident for rough surfaces due to a larger surface area and more exposed chemical groups available to interact with saliva.

In the present investigation, XTT assay results showed that, for the specimens fabricated in contact with the stone, the adhesion of *C. albicans* in S30, S35 and HP30 groups was lower as compared with the control. One factor that might have contributed to these findings would be the hydrophilicity of the coated surfaces.[21,27,28] As mentioned before, the rough surfaces coated with S30, S35 and HP30 exhibited significantly higher mean surface free energy values as compared with the control group, suggesting a decreased hydrophobic character. Hence, in this study, the decrease in *C. albicans* adhesion in the S30, S35 and HP30 groups may be partially related to the hydrophilicity of the rough surfaces treated with these coatings. Changes in chemical compositions of the coated acrylic surfaces may also have contributed to the findings as demonstrated by the XPS analysis. There were changes in the carbon and oxygen content with special relevance for S and HP coatings. In addition, surfaces modified with the S coating also exhibited an additional peak for the presence of sulphur. The S coating contains sulfobetaine, a member of the zwitterionic betaine family of compounds,[5,10,11,13–16,18,21,49] which have a mixture of anionic and cationic terminal groups with an overall neutral charge. Surfaces with zwitterionic groups resist non-specific interaction with plasma proteins and cells via a bound hydration layer from solvation of the charged terminal groups in addition to hydrogen bonding.[13,14,17,18,21,50] As observed for the S coating, other studies have shown that surfaces coated with zwitterionic polymers reduced *E. coli*, *S. aureus*, *Streptococcus mutans*, *P. aeruginosa*, *S. epidermidis*, *E. faecalis* and *C. albicans* attachment.[5,6,21]

However, it should be noted that, in addition to S30, S35 and HP30 coatings, other coatings also promoted changes in the surface chemical composition and resulted in hydrophilic surfaces but did not significantly affect the adhesion of *C. albicans* to the denture base acrylic resin.

For all tested conditions, the results revealed that *C. albicans* adhesion was not influenced by saliva. There is no consensus in the literature regarding the effect of saliva in *C. albicans* adhesion. Some authors[4,39,51] found an increase of *C. albicans* adhesion to materials covered with salivary pellicle, while others[30,32,34,52,53] observed a decrease in adhesion. This divergence of results could be attributed to differences among materials used as substrates to test *Candida* adhesion.[4,30,32–35,39,51–54] The chemical nature of the surfaces of the biomaterials influences the formation and composition of the acquired pellicle,[47,55] and consequently the adhesion and formation of biofilms.[56] Furthermore, results may also be influenced by differences in saliva-collection methods, such as the type of collected saliva (stimulated or non-stimulated) and number of donors, and in those procedures for saliva processing, such as the use of filtered or non-filtered saliva, diluted or non-diluted saliva, speed and time of centrifugation, and incubation periods and temperatures.[4,30,32,34,35,39,51–53] In the present study, diluted saliva was prepared in the same manner as Ramage et al.[33] Diluted saliva was used for practical reasons as the saliva volume of hundreds of mL was required in the experiments. Although one could argue that saliva dilution could have contributed to the lack of effect of the pre-conditioning on *Candida* adhesion, other studies where undiluted saliva was used have also shown no significant effect on the adhesion of *C. albicans*.[40,54]

The findings of this study confirm that the interactions among *C. albicans*, substrate and saliva are complex, and that several factors such as the physicochemical properties of the substrates (and conditioning film) and cells may influence this process. Nevertheless, experimental photopolymerized S and HP coatings were able to reduce *C. albicans* adherence and thus warrant further investigations.

5. Conclusions

Experimental S and HP coatings showed promising results and significantly reduced the short-term attachment (90 min) of *C. albicans* to the denture base acrylic resin under evaluation. However, the effect of these coatings on long-term biofilm formation remains to be investigated. In addition, the resistance of these coatings to mechanical (brushing) and chemical (immersion in denture cleansers) denture cleansing methods, as well as their biocompatibility should be analyzed before these materials can be recommended for clinical use.

Competing interest

None declared.

Funding

Source of funding: FAPESP (grants 2006/00435-3 and 2006/06842-0).

Ethical approval

The study was approved by the Ethics committee of Araraquara Dental School, and all subjects volunteered to participate and signed an informed consent form.

Acknowledgements

This study was supported by FAPESP (grants 2006/00435-3 and 2006/06842-0). The authors wish to acknowledge Mr. Jörg Erxleben for preparing the coatings used in this study and Prof. Peter Hammer for his assistance with the XPS analysis.

REFERENCES

1. Dagistan 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**(3):266–71.
2. Epstein JB. Diagnosis and treatment of oropharyngeal candidiasis. *Oral Maxillofacial Surgical Clinics North America* 2003;**15**(1):91–102.
3. Verran J, Maryan CJ. Retention of *Candida albicans* on acrylic resin and silicone of different surface topography. *Journal of Prosthetic Dentistry* 1997;**77**(5):535–9.
4. 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. *Journal of Oral Rehabilitation* 1997;**24**(5):350–7.
5. Lewis AL, Cumming ZL, Goreish HH, Kirkwood LC, Tolhurst LA, Stratford PW. Crosslinkable coatings from phosphorylcholine-based polymers. *Biomaterials* 2001;**22**(2):99–111.
6. Hirota K, Murakami K, Nemoto K, Miyake Y. Coating of a surface with 2-methacryloyloxyethyl phosphorylcholine (MPC) co-polymer significantly reduces retention of human pathogenic microorganisms. *FEMS Microbiology Letters* 2005;**248**(1):37–45.
7. Cheng G, Zhang Z, Chen S, Bryers JD, Jiang S. Inhibition of bacterial adhesion and biofilm formation on zwitterionic surfaces. *Biomaterials* 2007;**28**(29):4192–9.
8. Yang W, Chen S, Cheng G, Vaisocherová H, Xue H, Li W, Zhang J, Jiang S. Film thickness dependence of protein adsorption from blood serum and plasma onto poly(sulfobetaine)-grafted surfaces. *Langmuir* 2008;**24**(17):9211–4.
9. Liu PS, Chen Q, Liu X, Yuan B, Wu SS, Shen J, Lin SC. Grafting of zwitterion from cellulose membranes via ATRP for improving blood compatibility. *Biomacromolecules* 2009;**10**(10):2809–16.
10. Khandwekar AP, Doble M, Patil DP, Shouche YS. The biocompatibility of sulfobetaine engineered poly(ethylene terephthalate) by surface entrapment technique. *Journal of Biomaterials Applications* 2010;**25**(2):119–43.

11. Nakabayashi N, Williams DF. Preparation of non-trombogenic materials using 2-methacryloyloxyethyl phosphorylcholine. *Biomaterials* 2003;**24**(13):2431–5.
12. Tada S, Inaba C, Mizukami K, Fujishita S, Gemmei-Ide M, Kitano H, Mochizuki A, Tanaka M, Matsunaga T. Anti-biofouling properties of polymers with a carboxybetaine moiety. *Macromolecular Bioscience* 2009;**9**(1):63–70.
13. Khandwekar AP, Patil DP, Shouche YS, Doble M. The biocompatibility of sulfobetaine engineered polymethylmethacrylate by surface entrapment technique. *Journal of Materials Science Materials in Medicine* 2010;**21**:635–46.
14. Chang Y, Chang Y, Higuchi A, Shih YJ, Li PT, Chen WY, Tsai EM, Hsiue GH. Bioadhesive control of plasma proteins and blood cells from umbilical cord blood onto the interface grafted with zwitterionic polymer brushes. *Langmuir* 2012;**28**(9):4309–17.
15. Ishihara K, Iwasaki Y, Ebihara S, Shindo Y, Nakabayashi N. Photoinduced graft polymerization of 2-methacryloyloxyethyl phosphorylcholine on polyethylene membrane for obtaining blood cell adhesion resistance. *Colloids and Surface B Biointerfaces* 2000;**18**(3–4):325–35.
16. West SL, Salvage JP, Lobb EJ, Armes SP, Billingham NC, Lewis AL, Hanlon GW, Lloyd AW. The biocompatibility of crosslinkable copolymer coatings containing sulfobetaines and phosphobetaines. *Biomaterials* 2004;**25**(7–8):1195–204.
17. Li G, Cheng G, Xue H, Chen S, Zhang F, Jiang S. Ultra low fouling zwitterionic polymers with a biomimetic adhesive group. *Biomaterials* 2008;**29**(35):4592–7.
18. Kuo WH, Wang MJ, Chien HW, Wei TC, Lee C, Tsai WB. Surface modification with poly(sulfobetaine methacrylate-co-acrylic acid) to reduce fibrinogen adsorption, platelet adhesion, and plasma coagulation. *Biomacromolecules* 2011;**12**(12):4348–56.
19. Ishihara K, Nomura H, Mihara T, Kurita K, Iwasaki Y, Nakabayashi N. Why do phospholipid polymers reduce protein adsorption? *Journal of Biomedical Materials Research* 1998;**39**(2):323–30.
20. Lowe AB, Vamvakaki M, Wassall MA, Wong L, Billingham NC, Armes SP, Lloyd AW. Well-defined sulfobetaine-based statistical copolymers as potential antibioadherent coatings. *Journal of Biomedical Materials Research* 2000;**52**(1):88–94.
21. Kim M, Kim JC, Rho Y, Jung J, Kwon W, Kim H, Ree M. Bacterial adherence on self-assembled films of brush polymers bearing zwitterionic sulfobetaine moieties. *Journal of Material Chemistry* 2012;**22**(37):19418–28. http://dx.doi.org/10.1039/C2JM15912K.
22. Zhou L, Tong Z, Wu G, Feng Z, Bai S, Dong Y, Ni L, Zhao Y. Parylene coating hinders *Candida albicans* adhesion to silicone elastomers and denture bases resin. *Archives of Oral Biology* 2010;**55**(6):401–9.
23. Cagavi F, Akalan N, Celik H, Gür D, Güçiz B. Effect of hydrophilic coating on microorganism colonization in silicone tubing. *Acta Neurochirurgica* 2004;**146**(6):603–10.
24. Cringus-Fundeanu I, Luijten J, van der Mei HC, Busscher HJ, Schouten AJ. Synthesis and characterization of surface-grafted polyacrylamide brushes and their inhibition of microbial adhesion. *Langmuir* 2007;**23**(9):5120–6.
25. Andrade JD, Hlady V, Wei AP. Adsorption of complex proteins at interfaces. *Pure and Applied Chemistry* 1992;**64**(11):1777–81.
26. Sigal GB, Mrksich M, Whitesides GM. Effect of surface wettability on the adsorption of proteins and detergents. *Journal of the American Chemical Society* 1998;**120**(14):3464–73.
27. Klotz SA, Drutz DJ, Zajic JE. Factors governing adherence of *Candida* species to plastic surfaces. *Infection and Immunity* 1985;**50**(1):97–101.
28. Yoshijima Y, Murakami K, Kayama S, Liu D, Hirota K, Ichikawa T, Miyake Y. Effect of substrate surface hydrophobicity on the adhesion of yeast and hyphal *Candida*. *Mycoses* 2010;**53**(3):221–6.
29. Azuma A, Akiba N, Minakuchi S. Hydrophilic surface modification of acrylic denture base material by silica coating and its influence on *Candida albicans* adherence. *Journal of Medical and Dental Sciences* 2012;**59**:1–7.
30. 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. *International Journal of Prosthodontics* 2007;**20**(3):308–10.
31. Arima T, Nikawa H, Hamada T, Harsini. Composition and effect of denture base resin surface primers for reline acrylic resins. *Journal of Prosthetic Dentistry* 1996;**75**(4):457–62.
32. Moura JS, da Silva WJ, Pereira T, Del Bel Cury AA, Rodrigues Garcia RC. Influence of acrylic resin polymerization methods and saliva on the adhesion of four *Candida* species. *Journal of Prosthetic Dentistry* 2006;**96**(3):205–11.
33. Ramage G, Tomsett K, Wickes BL, López-Ribot JL, Redding SW. Denture stomatitis: a role for *Candida* biofilms. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics* 2004;**98**(1):53–9.
34. Waters MG, Williams DW, Jagger RG, Lewis MA. Adhesion of *Candida albicans* to experimental denture soft lining materials. *Journal of Prosthetic Dentistry* 1997;**77**(3):306–12.
35. Zamperini CA, Schiavinato PCS, Machado AL, Giampaolo ET, Pavarina AC, Vergani CE. Effect of different periods of preconditioning with saliva on *Candida albicans* adhesion to a denture base resin by crystal violet staining and XTT assay. *Journal of Investigative and Clinical Dentistry* 2010;**1**(2):114–9.
36. Ma Q, Mei S, Ji K, Zhang Y, Chu PK. Immobilization of Ag nanoparticles/FGF-2 on a modified titanium implant surface and improved human gingival fibroblasts behavior. *Journal of Biomedical Materials Research Part A* 2011;**98**(2):274–86.
37. 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 Biomaterials* 2011;**7**(1):447–57.
38. Sheridan PJ, Koda S, Ewoldsen NO, Lefebvre CA, Lavin MT. Cytotoxicity of denture base resins. *International Journal of Prosthodontics* 1997;**10**(1):73–7.
39. Chandra J, Mukherjee PK, Leidich SD, Faddoul FF, Hoyer LL, Douglas LJ, Ghannoum MA. Antifungal resistance of candidal biofilms formed on denture acrylic *in vitro*. *Journal of Dental Research* 2001;**80**(3):903–8.
40. Jin Y, Samaranayake LP, Samaranayake Y, Yip HK. Biofilm formation of *Candida albicans* is variably affected by saliva and dietary sugars. *Archives of Oral Biology* 2004;**49**(10):789–98.
41. Jin Y, Yip HK, Samaranayake YH, Yau JY, Samaranayake LP. Biofilm-forming ability of *Candida albicans* is unlikely to contribute to high levels of oral yeast carriage in cases of human immunodeficiency virus infection. *Journal of Clinical Microbiology* 2003;**41**(7):2961–7.
42. Quirynen M, Marechal M, Busscher HJ, Weerkamp AH, Darius PL, van Steenberghe D. The influence of surface free energy and surface roughness on early plaque formation. An in vivo study in man. *Journal of Clinical Periodontology* 1990;**17**(3):138–44.
43. Taylor R, Maryan C, Verran J. Retention of oral microorganisms on cobalt-chromium alloy and dental acrylic resin with different surface finishes. *Journal of Prosthetic Dentistry* 1998;**80**(5):592–7.
44. Radford DR, Sweet SP, Challacombe SJ, Walter JD. Adhesion of *Candida albicans* to denture-base materials with different surface finishes. *Journal of Dentistry* 1998;**26**(7):577–83.
45. Park SJ, Seo MK, Nah C. Influence of surface characteristics of carbon blacks on cure and mechanical behaviors of

rubber matrix compoundings. *Journal of Colloids and Interfaces Science* 2005;**291**(1):229–35.
46. Rangel EC, Bento WCA, Kayama ME, Schreiner WH, Cruz NC. Enhancement of polymer hydrophobicity by SF_6 plasma treatment and argon plasma immersion ion implantation. *Surface and Interface Analysis* 2003;**35**(2):179–83.
47. Yildirim MS, Kesimer M, Hasirci N, Kiliç N, Hasanreisoglu U. Adsorption of human salivary mucin MG1 onto glow-discharge plasma treated acrylic resin surfaces. *Journal of Oral Rehabilitation* 2006;**33**(10):775–83.
48. Sipahi C, Anil N, Bayramli E. The effect of acquired salivary pellicle on the surface free energy and wettability of different denture base materials. *Journal of Dentistry* 2001;**29**:197–204.
49. Ishihara K, Ishikawa E, Iwasaki Y, Nakabayashi N. Inhibition of fibroblast cell adhesion on substrate by coating with 2-methacryloyloxyethyl phosphorylcholine polymers. *Journal of Biomaterials Science Polymer Edition* 1999;**10**(10):1047–61.
50. Xu FJ, Neoh KG, Kang ET. Bioactive surfaces and biomaterials via atom transfer radical polymerization. *Progress in Polymer Science* 2009;**34**(8):719–61.
51. Nikawa H, Chen J, Hamada T, Nishimura M, Polyzois G. *Candida albicans* colonization on thermal cycled maxillofacial polymeric materials *in vitro*. *Journal of Oral Rehabilitation* 2001;**28**(6):526–33.
52. Samaranayake LP, McCourtie J, MacFarlane TW. Factors affecting the *in vitro* adherence of *Candida albicans* to acrylic surfaces. *Archives of Oral Biology* 1980;**25**(8–9): 611–5.
53. McCourtie J, MacFarlane TW, Samaranayake LP. Effect of saliva and serum on the adhesion of *Candida* species to chlorhexidine-treated denture acrylic. *Journal of Medical Microbiology* 1986;**21**(3):209–13.
54. Thein ZM, Samaranayake YH, Samaranayake LP. Characteristics of dual species *Candida* biofilms on denture acrylic surfaces. *Archives of Oral Biology* 2007;**52**(12): 1200–8.
55. Yildirim MS, Hasanreisoglu U, Hasirci N, Sultan N. Adherence of *Candida albicans* to glow-discharge modified acrylic denture base polymers. *Journal of Oral Rehabilitation* 2005;**32**(7):518–25.
56. Chandra J, Patel JD, Li J, Zhou G, Mukherjee PK, McCormick TS, Anderson JM, Ghannoum MA. Modification of surface properties of biomaterials influences the ability of *Candida albicans* to form biofilms. *Applied and Environment Microbiology* 2005;**71**(12):8795–801.