Adhesion of *Candida albicans* and *Candida dubliniensis* to acrylic and hydroxyapatite

Mariana Henriques, Joana Azeredo, Rosário Oliveira*

Centro de Engenharia Biológica—CEB, Universidade do Minho, Braga, Portugal

Abstract

The aim of this work was to compare the ability of strains of *Candida albicans* and *Candida dubliniensis* to adhere to acrylic and hydroxyapatite (HAP). In order to interpret the adhesion results, the surface properties of cells and materials were determined. Surface tension components (polar and apolar) and hydrophobicity were calculated through contact angle measurement and the elemental composition was determined by X-ray photoelectron spectroscopy (XPS). The results showed no significant differences in the number of adhered cells of both species to acrylic and hydroxyapatite. This was corroborated by the similarities in their surface properties and elemental composition. For both species, the adhesion to acrylic increased in the presence of artificial saliva due to the increase in the electron-donor capacity of this material. In the absence of artificial saliva, the number of adhered cells to HAP was greater than to acrylic, on account of the higher number of electron-donor groups of HAP. Hydrophobicity played a minor role in the adhesion process of both candidal species. Conversely, Lewis acid–base interactions seamed to govern this phenomenon.
© 2003 Elsevier B.V. All rights reserved.

Keywords: Candida albicans; Candida dubliniensis; Adhesion; Surface properties

1. Introduction

Candida albicans is a commensal pathogen that lives on the skin and mucosal surfaces of the genital and intestinal tracts as well as the oral cavity. [1–3]. This species is one of the most common responsible for nosocomial infections. Recently, Sullivan et al. [4] described a new species of *Candida*, *C. dubliniensis*, isolated from the oral cavity of HIV patients, that is phenotypically and genotypically close to *Candida albicans*. Evidence of the inducibility of a stable fluconazole resistance in vitro in *C. dubliniensis* strains may indicate that it is an emerging pathogen for immunocompromised patients receiving long-term fluconazole prophylaxis [5]. *Candida dubliniensis* is the non-*Candida albicans* species most capable of adhesion to epithelial cells, which may indicate that this species is particularly adapted to colonise the oral cavity [6].

Since the oral cavity is a continuous-flow environment, yeast cells are washed out by saliva and swallowed unless they adhere and replicate. Moreover, growth conditions in the oral cavity are so poor that cells have to adhere to be maintained [2]. So, an essential pre-requisite for the successful colonisation of host tissues by these micro-organisms is the ability to adhere to superficial epithelial cells, or to a closely associated surface [7]. Denture stomatitis is common among denture wearers. Numerous yeasts are commonly found on the palatal surface of the denture [8] and this supports the theory that the upper denture acts as a reservoir for infection. Thus, it is of major importance to study the adhesion of *Candida* spp. to oral surfaces, including prosthetic devices (polymers) and teeth enamel, mainly composed of calcium phosphate, usually simulated, in vitro, by hydroxyapatite. Although the adhesion of *Candida albicans* to oral surfaces has been intensively studied there are no reports, to the authors' knowledge, of the adhesion of *Candida dubliniensis* to oral devices. So, this work aims at comparing the adhesion ability of these two different *Candida* species to acrylic and HAP.

* Corresponding author. Tel.: +351-253-604409; fax: +351-253-678986.

E-mail address: roliveira@deb.uminho.pt (R. Oliveira).

2. Materials and methods

2.1. Yeast growing conditions

Two different strains of *Candida albicans* and *Candida dubliniensis* were used in this work. *Candida albicans* 12A and *Candida albicans* 46B are clinical isolates, kindly provided by the Department of Biology of the University of Minho. *Candida dubliniensis* CBS 7987 and *Candida dubliniensis* CBS 7988 were obtained from Centraalbureau voor Schimmelcultures (CBS). Both species were stored in Sabouraud dextrose agar at 5 °C and sub-cultured every month. For each assay, the yeast cells were sub-cultured in Sabouraud dextrose agar for 24 h and then grown in Sabouraud dextrose broth for 18 h, at 37 °C under agitation, until stationary phase. Cells were harvested by centrifugation (5000 rpm, for 10 min) and washed twice with ultrapure water. The cells were resuspended in a certain volume of water or artificial saliva necessary to achieve the concentration needed for each assay, after being enumerated in a "Neubauer" chamber.

2.2. Artificial saliva preparation

Artificial saliva was prepared according to Gal et al. [9] with the following composition in mg/l: 125.6 NaCl, 963.9 KCl, 189.2 KSCN, 654.5 KH_2PO_4, 200.0 Urea, 763.2 $Na_2SO_4 \cdot 10H_2O$, 178.0 NH_4Cl, 227.8 $CaCl_2 \cdot 2H_2O$ and 630.8 $NaHCO_3$. The pH was adjusted to 6.8, by bubbling carbon dioxide.

2.3. Adhesion surfaces

Two substrata were used in the adhesion assays, hydroxyapatite (batch P120, Plasma Biotal; UK) and self-polymerising acrylic (Vertex, Self Curing, Dentimex BV, The Netherlands).

The hydroxyapatite discs were prepared with hydroxyapatite powder as reported by Lopes et al. [10]. The HAP powder was uniaxially pressed at 288 MPa in steel dishes to produce 14 mm diameter and 2 mm thickness discs using 4 g of powder. Discs were then placed on an alumina tile and fired to 1300 °C at a ramp rate of 4 °C/min. The set temperature was maintained for 1 h, followed by natural cooling inside the furnace. The discs were mechanically polished by several sandpapers with decreasing granulometry to a final 1 μm finish.

The acrylic coupons were prepared as described by Samaranayake and MacFarlane [7]. Briefly, 1.5 g of self-polymerising acrylic powder was mixed with 1 ml of monomer liquid and after mixing the solution was poured onto a surface covered with aluminium foil paper. After 45 s another surface was placed on top of the polymerising mixture. The acrylic sheet, polymerised during 30 min, was cut into 8 mm × 8 mm × 2 mm.

Coupons of both materials, after being washed with water, ethanol and ultrapure sterile water, were immersed in sterile water or sterile artificial saliva, for 24 h at room temperature, and dried at 37 °C for 24 h, before adhesion and surface properties studies (X-ray photoelectron spectroscopy (XPS) and contact angle measurement).

2.4. Surface properties

2.4.1. Contact angle measurement

Cell lawns were prepared according to Henriques et al. [11]. Briefly, a solution of 20 g/l of agar, and 10% (v/v) of glycerol was cast onto a microscope slide (75 mm × 25 mm). An aliquot of 2 ml of 10^9 cells/ml (prepared in water or artificial saliva) was spread over the solidified agar layer, in order to cover the entire surface. This layer was let to dry at 37 °C (for 10–15 h), and two more layers were added, with the same drying period between them.

Contact angles were measured by the sessile drop technique on the cell lawns and on the materials prepared as indicated in 2.3, using a contact angle measurements apparatus (model OCA 15 PLUS, DATAPHYSICS). The measurements were performed at room temperature, using three different liquids: water, formamide and 1-bromonaphtalene. Each assay was performed in triplicate and at least 10 contact angles, per sample, were measured.

2.4.2. X-ray photoelectron spectroscopy

A volume of 200 ml of an aqueous cell suspension (10^9 cells/ml) was vacuum filtered through a nitro-cellulose membrane of 45 μm. The membrane, completely covered with cells, was immediately frozen with liquid nitrogen and then stored at −80 °C until the subsequent step of lyophilization. Freeze drying was performed at 10 Pa, overnight. The samples were placed in a dessicator, at room temperature and immediately analysed by XPS. The XPS analysis was performed using an apparatus ESCALAB 200A, with a VG5250 software and data analysis. The spectrometer used monochromatized Mg Kα X-ray radiation (15,000 eV). The constant pass energy of the analyser was 20 eV and it was calibrated with reference to Ag $3d_{5/2}$ (368.27 eV). The pressure during analysis was under 1×10^{-6} Pa. The spectra, for yeast cells, were recorded following the sequence C 1s, O 1s, N 1s, P 2p. In the case of HAP and acrylic, the spectra sequence was: C 1s, O 1s, N 1s, P 2p, Si 2p, Na 1s, Ca 2p($2p^3 + 2p^1$), K 2p($2p^3 + 2p^1$).

2.4.3. Zeta potential

Cells were diluted in water and artificial saliva to an absorbance of approximately 0.1 (measured at 600 nm). The zeta potential was determined by electrophoretic mobility by means of a Zeta-Meter (Zeta-Meter System, 3.0+).

2.4.4. Roughness

The surface roughness of acrylic and HAP was determined using a roughness tester (Surftest SV 512).

Measurements were done in both longitudinal and transversal directions.

2.5. Adhesion assays

Coupons of acrylic and HAP, prepared as indicated in 2.3, were inserted in a 24-well plate (15 mm diameter each well) and 2 ml of a cell suspension of 10^7 cells/ml, prepared with water or artificial saliva, were added to each well. After 1 h of incubation (100 rpm, at 37 °C) each well was washed twice with ultrapure water, by pipeting carefully only the liquid above the coupon. After the last washing, the liquid was completely removed. The coupons were then stained with SYTO-13 (Molecular Probes) for 5 min, and observed under epifluorescence microscopy (450–490 nm). Twenty-five fields per sample were randomly captured by a video camera connected to the microscope and grabbed by a computer. An image analysis system (SigmaScan Pro 5, SPSS) was used to quantify the adhered yeast cells. Each experiment was repeated six times.

2.6. Statistical analysis

The resulting data were analysed using SPSS (Statistical Package for the Social Sciences). Two-way ANOVA was used to perform multiple analysis of the interaction between all factors. One-way ANOVA with Bonferroni test was used to compare the number of adhered cells of the four strains. All tests were performed with a confidence level of 95%.

3. Results

3.1. Adhesion assays

The number of cells of *Candida albicans* and *Candida dubliniensis* adhered to each type of material immersed in water or in artificial saliva is presented in Table 1.

The two-way ANOVA analysis of the results (Table 1) revealed that adhesion is mainly influenced by the type of material (HAP and acrylic) and the liquid medium where adhesion occurred (artificial saliva or water) ($P = 0.000$ for both cases). For each type of material, no statistical differ-

ence was found between the number of adhered cells of the strains studied ($P = 0.655$).

To better understand the influence of each factor in the process of adhesion, a statistical analysis (one-way ANOVA) was performed with each variable, separately. When water was used as the adhesion medium, the differences in the number of cells adhered to HAP and to acrylic were significantly different (values of P were 0.001, 0.014, 0.000 and 0.008 for each strain, respectively, as ordered in the Table 1), with a higher number adhered to HAP than to acrylic. In the case of artificial saliva, these differences were not significant (values of P for the strains *C. albicans* 12A, *C. albicans* 46B and *C. dubliniensis* 7988 were 0.277, 0.107 and 0.091, respectively), except in the case of *C. dubliniensis* 7987 ($P = 0.018$). Considering the adhesion to acrylic, the number of cells attached in the presence of artificial saliva was significantly greater than in the presence of water ($P = 0.003$ for *C. albicans* 12A and $P = 0.001$ for the other strains). For HAP, the results of the assays performed in water and artificial saliva were not significantly different (P equal to 0.225, 0.308 and 0.086 for strains *C. albicans* 12A, *C. albicans* 46B and *C. dubliniensis* 7987, respectively) except in the case of *C. dubliniensis* 7988 (where $P = 0.022$).

3.2. Cell surface properties

The results obtained by XPS analysis on the C, O, N and P composition of the yeast cells surfaces are expressed in terms of ratios and are presented in Fig. 1. The ratios of cell wall components are similar for all the strains studied.

The contact angles formed by the three liquids (water, formamide and 1-bromonaphethalene) on cell lawns of all strains, pre-contacted with water or artificial saliva, are presented in Table 2. The values indicate that all strains have similar interactions with each of the three liquids tested. The water contact angle can give an indication of the surface hydrophobicity. According to van Oss and Giese [12] a water contact angle lower than 50° corresponds to a hydrophilic surface. Vogler [13] sets the limit at 65°. In this case, the values of the water contact angles are lower than 50°, so cells can be considered hydrophilic. The values of Table 2 were used to calculate cell surface tension parameters (Table 3). Table 3 also presents the values of the degree

Table 1
Number of cells of *C. albicans* 12A, *C. albicans* 46B, *C. dubliniensis* 7987 and *C. dubliniensis* 7988 adhered to acrylic and hydroxyapatite (HAP) in the presence of water and artificial saliva

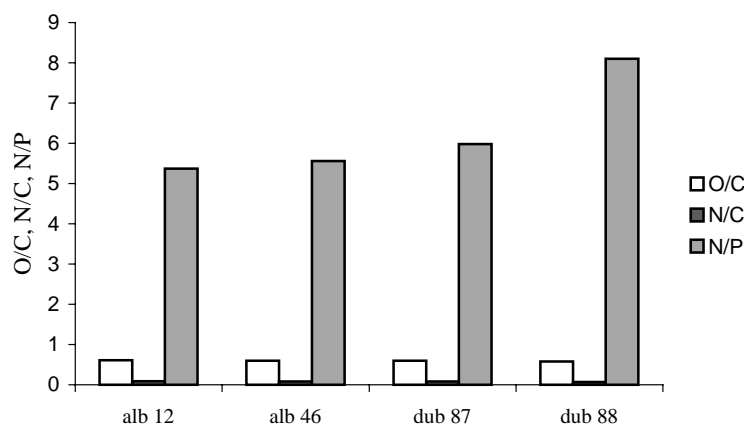| Strain	Mean number of yeasts/mm² (±SEM[a])				
	Water			Artificial saliva	
	Acrylic	HAP		Acrylic	HAP
C. albicans 12A	4958 ± 887	18746 ± 6891		17916 ± 4492	15385 ± 1820
C. albicans 46B	8071 ± 3021	15903 ± 6669		16209 ± 1280	18894 ± 2958
C. dubliniensis 7987	6628 ± 2444	15887 ± 2018		14333 ± 644	18531 ± 2532
C. dubliniensis 7988	6342 ± 1875	12836 ± 3967		15065 ± 3130	19107 ± 3509

[a] SEM: standard error of mean.

Fig. 1. Ratios of O/C, N/C and N/P of cell walls of *C. albicans* 12A (alb 12), *C. albicans* 46B (alb 46), *C. dubliniensis* 7987 (dub 87) and *C. dubliniensis* 7988 (dub 88), obtained by XPS analysis.

Table 2
Values of contact angles measured with water (θ_w), formamide (θ_f) and 1-bromonaphatelene (θ_b) on cells lawns of *C. albicans* 12A, *C. albicans* 46B, *C. dubliniensis* 7987 and *C. dubliniensis* 7988 conditioned with water or artificial saliva[a]

Medium	Cells	θ_w (°) ($\pm$SEM[a])	θ_f (°) ($\pm$SEM[a])	θ_b (°) ($\pm$SEM[a])
Water	*C. albicans* 12A	6 ± 5	18 ± 1	65 ± 5
	C. albicans 46B	17 ± 4	19 ± 3	60 ± 5
	C. dubliniensis 7987	24 ± 2	28 ± 2	44 ± 3
	C. dubliniensis 7988	18 ± 2	18 ± 2	57 ± 2
Artificial saliva	*C. albicans* 12A	15 ± 2	16 ± 1	63 ± 7
	C. albicans 46B	13 ± 1	16 ± 3	83 ± 1
	C. dubliniensis 7987	13 ± 2	14 ± 2	62 ± 5
	C. dubliniensis 7988	10 ± 1	12 ± 2	61 ± 5

[a] SEM: standard error of mean.

of hydrophobicity of yeast cell surfaces (ΔG_{sws}). ΔG_{sws} is expresses the free energy of interaction between two identical surfaces (s) immersed in water (w), as proposed by van Oss and Giese [12]. ΔG_{sws} was determined with the values γ^+, γ^- (electron-acceptor and electron-donor parameters of acid–base component of the surface tension) and γ^{LW} (the Lifshitz van der Waals component of surface tension), which were calculated with the contact angles of water, formamide and 1-bromonaphtalene.

From Table 3, the four strains studied showed similar values of surface tension and a predominantly electron donancy. In the presence of artificial saliva a slightly increase in the electron-acceptor parameter (γ^+) was verified for all strains. As far as hydrophobicity is concerned, all *Candida* strains studied showed positive values of ΔG_{sws} in all conditions assayed, and so can be considered hydrophilic, which is in accordance with the water contact angle values. The ΔG_{sws} values were very similar, with the exception of *C. albicans*

Table 3
Values of the components of surface tension (γ^+, γ^-, γ^{LW}) and degree of hydrophobicity (ΔG_{sws}) of cells (*C. albicans* 12A (alb 12), *C. albicans* 46B (alb 46), *C. dubliniensis* 7987 (dub 87) and *C. dubliniensis* 7988 (dub 88)) conditioned with water or artificial saliva and the average zeta potential of the yeast cells measured in water[a]

Medium	Cells	γ^+ (mJ m^{-2}) ($\pm$SEM[a])	γ^- (mJ m^{-2}) ($\pm$SEM[a])	γ^{LW} (mJ m^{-2}) ($\pm$SEM[a])	ΔG_{sws} (mJ m^{-2}) ($\pm$SEM[a])	ζ (mV) ($\pm$SEM[a])
Water	alb 12	5.04 ± 1.46	48.66 ± 5.59	23.43 ± 23.13	21 ± 3	-14.5 ± 4.2
	alb 46	4.62 ± 1.86	51.22 ± 2.21	25.04 ± 2.38	24 ± 4	-8.3 ± 3.3
	dub 87	3.03 ± 1.31	52.00 ± 2.48	29.98 ± 1.42	27 ± 2	-17.8 ± 3.7
	dub 88	5.91 ± 1.19	49.12 ± 2.39	24.09 ± 1.81	20 ± 3	-9.6 ± 2.7
Artificial saliva	alb 12	6.37 ± 0.53	51.60 ± 1.26	23.53 ± 1.10	21 ± 4	–
	alb 46	13.2 ± 0.64	51.57 ± 1.76	13.61 ± 0.35	10 ± 1	–
	dub 87	6.18 ± 1.53	52.17 ± 0.42	24.20 ± 2.61	22 ± 2	–
	dub 88	6.02 ± 1.74	53.10 ± 1.46	24.61 ± 2.96	23 ± 4	–

[a] SEM: standard error of mean.

Table 4
Values of contact angles measured with water (θ_w), formamide (θ_f) and 1-bromonaphatelene (θ_b) on acrylic and HAP coated with water or artificial saliva[a]

Medium	Cells	θ_w (°) (±SEM[a])	θ_f (°) (±SEM[a])	θ_b (°) (±SEM[a])
Water	Acrylic	75 ± 3	57 ± 3	32 ± 9
	HAP	53 ± 4	57 ± 6	47 ± 3
Artificial saliva	Acrylic	64 ± 4	44 ± 3	36 ± 3
	HAP	23 ± 3	25 ± 83	43 ± 2

[a] SEM: standard error of mean.

46B, that exhibited a lower degree of hydrophilicity, in the presence of artificial saliva.

Table 3 also shows the values of zeta potentials of the four *Candida* strains when immersed in water. Under these conditions, they all displayed negative zeta potentials and the results did not differ statistically. When immersed in artificial saliva, all strains showed no electrophoretic mobility at 50, 100 and 200 mV, which was expected due to the high ionic strength of the medium. Thus, their zeta potentials were considered nulls.

3.3. Materials surface properties

As adhesion involves both cells and materials, the surface properties of the tested substrata were determined. Contact angle values are presented in Table 4, surface tension and hydrophobicity in Table 5, surface elemental composition in Table 6 and roughness in Fig. 2.

The values of water contact angle (Table 4) indicate that HAP is more hydrophilic than acrylic, which can be considered hydrophobic. The water contact angle on the materials conditioned with artificial saliva decreases. Consequently, the ΔG_{sws} value of acrylic becomes less negative when coated with artificial saliva (less hydrophobic) whereas in the case of HAP artificial saliva decreased the ΔG_{sws} values (more hydrophilic) (Table 5). Table 5 also shows that the electron-donor parameter increased in the presence of artificial saliva for both materials. Although that increase is higher for acrylic, γ^- is always higher in HAP (Table 5).

The elemental analysis of acrylic and HAP surfaces conditioned with water or artificial saliva (Table 6) indicated that artificial saliva altered the surface composition of both materials with special relevance for HAP.

There was no difference between the data obtained by the longitudinal and the transversal measurements of roughness

Table 6
Percentage of the different chemical elements detected in the surface of acrylic and HAP, contacted with water or artificial saliva, determined by XPS analysis

Elements (%)	Acrylic		HAP	
	Water	Artificial saliva	Water	Artificial saliva
C 1s	75.75	72.80	23.20	14.05
O 1s	22.48	23.11	45.85	46.16
N 1s	0.76	1.02	0.30	0.39
P 2p	0.04	0.32	12.50	14.18
Si 2p	0.97	1.92	–	–
Na 1s	–	0.83	–	12.09
Ca 2p($2p^3 + 2p^1$)	–	–	18.15	9.87
K 2p($2p^3 + 2p^1$)	–	–	–	3.25

for each type of material (Fig. 2). By the comparison of the roughness of HAP and acrylic, it could be concluded that the former exhibits a surface roughness about two times the latter.

4. Discussion

It is well known that cell surface hydrophobicity plays an important role in adhesion of *Candida* species. Hydrophobic cells bind more readily, to epithelial cells and plastics, than hydrophilic ones [14]. Both *Candida albicans* strains used in this study exhibited hydrophilic cell surfaces under the conditions assayed. The same was observed for the two strains of *Candida dubliniensis*. Moreover, there were no significant differences between *C. albicans* and *C. dubliniensis* hydrophobicity, either conditioned with water or artificial saliva (Table 3).

Other authors [15] found differences in the hydrophobicity of some strains of *C. albicans* and *C. dubliniensis* when grown at 37 °C. The results reported by these authors were based on the hydrophobic microspheres assay method. However, the drawback of this method and others like co-aggregation [16] and microbial adhesion to hydrocarbons [17], is that other forces (as electrostatic), besides hydrophobic ones, may interfere in the interaction between the ligands and the cell surface, and so, surface hydrophobicity is masked [18,19]. Furthermore, the fact that the results obtained in the present study are consistent across two strains of each *Candida* species strongly suggests that these results are not an artefact of chance strain selection.

Table 5
Values of surface tension components (γ^+, γ^-, γ^{LW}) and degree of hydrophobicity (ΔG_{sws}) of acrylic and HAP conditioned with water or artificial saliva

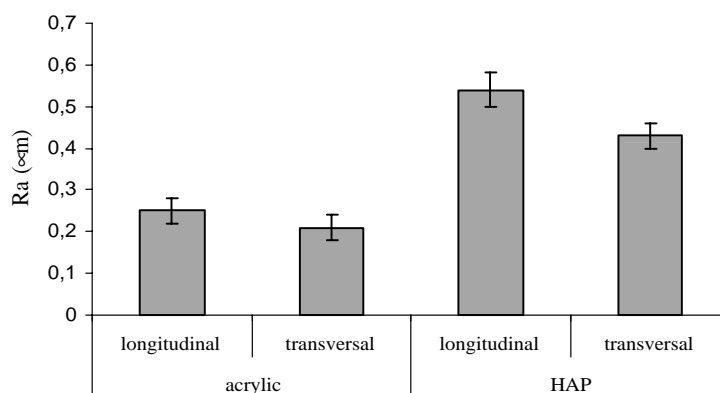|	Surface	γ^+ (mJ/m^2) (±SEM[a])	γ^- (mJ/m^2) (±SEM[a])	γ^{LW} (mJ/m^2) (±SEM[a])	ΔG_{sws} (mJ/m^2) (±SEM[a])
Water	Acrylic	0.91 ± 0.09	4.98 ± 1.37	39.77 ± 3.00	–45 ± 0.2
	HAP	0.05 ± 0.00	38.88 ± 3.76	31.37 ± 1.82	25 ± 3.0
Artificial saliva	Acrylic	0.68 ± 0.10	16.19 ± 3.41	35.84 ± 1.36	–26 ± 4.0
	HAP	1.16 ± 0.53	49.90 ± 3.86	33.37 ± 0.89	30 ± 3.0

[a] SEM: standard error of mean.

Fig. 2. Values of the roughness of acrylic and HAP measured in two different directions (longitudinal and transversal).

According to Rouxhet et al. [20], XPS analysis can provide an indication of the amount of polysaccharides, proteins and hydrocarbons present at the cell surface of yeast strains, which can be determined from the ratios O/C, N/C and N/P. As all the strains present similar cell wall elemental compositions (Fig. 1), the polysaccharides, proteins and hydrocarbons contents are similar and in average the percentages are 66.0 ± 1.0, 27.9 ± 3.0 and 6.1 ± 1.0, respectively. These values are in accordance with the values reported by Chaffin et al. [21] for the cell wall composition of *Candida albicans*.

The XPS results corroborated the similarity of the values of the surface tension, hydrophobicity and zeta potential (Table 3), among all strains.

The liquid medium where adhesion took place strongly influenced the results. In the presence of artificial saliva, the number of cells adhered to acrylic, of the four strains studied, was significantly greater compared to water (Table 1). The environmental conditions, such as pH, ionic strength, temperature and components in solution can influence cell surface characteristics [22], e.g. surface charge and hydrophobicity [23]. Moreover, ionic strength determines the thickness of the electrical double layer which has a direct influence on electrostatic interactions established in the adhesion events [24]. Thus, it is expected that artificial saliva, which has a high ionic strength, influences adhesion to oral surfaces, lowering the electrostatic repulsion. Furthermore, the adsorption of salivary components can also affect adhesion. It has been reported by several authors that adhesion of *Candida albicans* to oral surfaces is influenced by saliva [25–28]. Saliva can enhance the adhesion to acrylic surfaces [27] and polystyrene [28], whereas adhesion to silicone rubber can be discouraged in the presence of saliva [26]. This fact has been attributed to the adsorption of different salivary components, including mucins [29], other salivary proteins [30] and secretory IgA [28].

It must be stressed that the saliva formulation used in this study is a synthetic one without proteins. In almost all studies concerning adhesion of oral micro-organisms to surfaces, the saliva used is obtained from donors [31,32]. Nevertheless, natural saliva varies according to the donor and the time of the day, thus exact duplications are impossible. Furthermore, natural saliva contains proteins such as mucin that can coat the oral surfaces influencing adhesion by specific interactions. So, artificial saliva was used in this study, in order to focus only the physico-chemical interactions.

In the present study, the adsorption of phosphate ions to acrylic surfaces, indicated by XPS analysis (Table 6), caused an increase in the electron donancy of this material (Table 5). Moreover, the cells in the presence of artificial saliva exhibited a slightly increase in their electron-acceptor groups (Table 3). Thus, the increase in the number of adhered cells to acrylic in the presence of artificial saliva can be explained by an increase in the interactions between the electron-donor groups of acrylic and the electron-acceptor groups of cells. In the case of HAP, artificial saliva caused an increase in the number of electron-acceptor groups, perhaps due to the adsorption of sodium and potassium ions, as observed by XPS analysis (Table 6). Probably, in opposition to acrylic, there was no such significant increase in the interactions between the electron-acceptor groups of cells and electron-donating groups of HAP, which can explain the similarity in the number of adhered cells.

The preferential adsorption of phosphate to acrylic and sodium and potassium to HAP can be explained by the fact that acrylic is more electron accepting than HAP and that HAP is more electron donating than acrylic. It has been suggested by other authors [26] that the different behaviours of cell attachment in the presence of artificial saliva may be related to the hydrophobicity of the substratum surfaces which may stimulate the adsorption of different salivary components, a proposal which is true for the present study.

It is curious to notice that the number of cells attached to HAP was greater than to acrylic in the presence of water, although HAP is less hydrophobic than acrylic. Furthermore, the adhesion to acrylic increased in the presence of artificial saliva and in this medium acrylic was less hydrophobic. This indicates that the hydrophobicity of the adhesion substratum played a minor role in the process of adhesion. So it is expected that other types of interactions rather than hydrophobic ones might be governing the phenomenon.

The interactions established between the electron-acceptor groups of the cells and the electron-donating groups of the materials, as hypothesised before, may be determining the adhesion phenomenon.

Studies done by Bollen et al. [33] showed that an increase in the surface roughness of resin strips above an Ra value of 2 μm resulted in a dramatic increase in bacterial colonisation of these surfaces in comparison to smooth strips (Ra = 0.12 μm). Verran and Maryan [34] demonstrated that surface roughness may also facilitate *Candida albicans* retention in silicon prostheses. Taylor et al. [35] verified that an increase in surface roughness (0.15–3.53 μm) enhanced *Candida albicans* retention. Although the values of roughness of both materials are low (Ra < 0.5 μm) the roughness of HAP is higher than that of acrylic (Fig. 2), which can, in part, explain the higher amount of cells adhered to HAP in the absence of artificial saliva. When artificial saliva was present this effect might have been masked.

It should be noted that the acrylic used in the present work was a cold-cure variety of acrylic. However, most dentures are made of a heat-cured acrylic form of resin. Nevertheless, according to Davenport [36] there is no difference in surface roughness between the heat and cold-cure acrylic resin. So, it is expected that they have similar surface properties as they are made by the same chemical compounds.

As the different *Candida* strains showed similar cell wall compositions and cell surface physico-chemical properties they were expected to exhibit the same type of physico-chemical interaction with the adhesion substratum. Accordingly, all strains studied exhibited the same capability to adhere to both types of surfaces. These results proved the similarity of the phenotypic characteristics of *Candida albicans* and *Candida dubliniensis*, with respect to physico-chemical surface properties. The extent of adhesion of *Candida* strains to teeth (HAP) and prostheses (acrylic) is similar in the presence of artificial saliva, which may indicate that both surfaces are equally important reservoirs for candidal infections.

Acknowledgements

References

[1] J. Pla, C. Gil, L. Monteoliva, F. Navarro-García, M. Sanchez, C. Nombela, Yeast 12 (1996) 1677.

[2] R.D. Cannon, W. L, Crit. Rev. Oral Biol. Med. 10 (1999) 359.

[3] R.A. Calderone, W.A. Fonzi, Trends Microbiol. 9 (2001) 327.

[4] D.J. Sullivan, T.J. Westerneng, K.A. Haynes, D.E. Bennett, D.C. Coleman, Microbiology 141 (1995) 1507.

[5] G.P. Moran, D. Sanglard, S.M. Donnelly, D.B. Shanley, D.J. Sullivan, D.C. Coleman, Agents Chemother. 42 (1998) 1819.

[6] G.D. Gilfillan, D.J. Sullivan, K. Haynes, T. Parkinson, D.C. Coleman, N.A.R. Gow, Microbiology 144 (1998) 829.

[7] L.P. Samaranayke, T. W, Arch. Oral Biol. 25 (1980) 603.

[8] J.C. Davenport, Br. Dental J. 129 (1970) 151.

[9] J. Gal, Y. Fovet, M. Adib-Yadzi, Talanta 53 (2001) 1103.

[10] M.A. Lopes, J.C. Knowles, J.D. Santos, F.J. Monteiro, I. Olsen, Biomaterials 21 (2000) 1165.

[11] M. Henriques, K. Gasparetto, J. Azeredo, R. Oliveira, Biotechnol. Lett. 24 (2002) 1111.

[12] C.J. van Oss, R.F. Giese, Clay Miner. 2 (1995) 347.

[13] E.A. Vogler, Adv. Colloid Interf. Sci. 74 (1998) 69.

[14] J. Masuoka, G. Wu, P.M. Glee, K.C. Hazen, FEMS Immunol. Med. Microbiol. 24 (1999) 421.

[15] K.C. Hazen, J.G. Wu, J. Masuoka, Infect. Immun. 69 (2001) 779.

[16] M.A. Jabra-Rizk, W.A. Falkler Jr., W.G. Merz, J.I. Kelley, A.A.M.A. Baqui, T.F. Meiller, J. Clin. Microbiol. 37 (1999) 1464.

[17] Y.H. Samaranayke, P.C. Wu, L.P. Samaranayke, M. So, APMIS 103 (1995) 707.

[18] R.J. Doyle, Microbes Infect. 2 (2000) 391.

[19] M. Aguedo, Y. Waché, V. Mazonyer, A. Sequeira-Le Grand, J. Belin, J. Agric. Food Chem. 51 (2003) 3007.

[20] P.G. Rouxhet, N. Mozes, P.B. Dengis, Y.F. Dufrêne, P.A. Gerin, M.J. Genet, Colloids Surf. B: Biointerf. 2 (1994) 347.

[21] W.L. Chaffin, J.L. López-Ribot, M. Casanova, D. Gozalbo, J.P. Martinez, Microbiol. Mol. Biol. Revi. 62 (1998) 130.

[22] M. Flahaut, D. Sanglard, M. Monod, J. Bille, M. Rossier, J. Clin. Microbiol. 36 (1998) 395.

[23] B. Hsu, C. Huang, Colloids Surf. A: Physicochem. Eng. Aspects 201 (2002) 201.

[24] R. Oliveira, Exp. Therm. Fluid Sci. 14 (1997) 316.

[25] J.M. O'Sullivan, H.F. Jenkinson, R.D. Cannon, Microbiology 146 (2000) 41.

[26] H.J. Busscher, G.I. Geertsema-Doornbusch, H.C. van der Mei, J. Biomed. Mater. Res. 34 (1997) 201.

[27] K.W. Millsap, R. Bos, H.C. van der Mei, H.J. Busscher, Antonie van Leeuwenhoek 75 (1999) 351.

[28] R. San Millan, N. Elguezabal, P. Regulez, M.D. Moragues, G. Quindos, J. Ponton, Microbiology 146 (2000) 2105.

[29] M. Edgerton, F.A. Scannapieco, M.S. Reddy, M.J. Levine, Infect. Immun. 61 (1993) 2644.

[30] R.D. Cannon, A.K. Nand, H.F. Jenkinson, Microbiology 141 (1995) 213.

[31] E. Ueta, T. Tanida, S. Doi, T. Osaki, J. Lab. Clin. Med. 136 (2000) 67.

[32] H. Nikawa, J. Chen, T. Hamada, M. Nishimura, G. Polyzois, J. Oral Rehabil. 28 (2001) 526.

[33] C.M.L. Bollen, P. Lambrechts, M. Quirynen, Dental Mater. 13 (1997) 258.

[34] J. Verran, C.J. Maryan, J. Prosth. Dentistry 77 (1997) 535.

[35] R. Taylor, C. Maryan, J. Verran, J. Prosth. Dentistry 80 (1998) 592.

[36] J.C. Davenport, Br. Dental J. 133 (1972) 101.