JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 12 (2001) 399–405

Conditioning film and environmental effects on the adherence of *Candida* spp. to silicone and poly(vinylchloride) biomaterials

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The reported incidence of colonization of oropharyngeal medical devices with *Candida* spp. has increased in recent years, although few studies that have systematically examined the adherence of yeast cells to such biomaterials, the primary step in the process of colonization. This study, therefore, examined the effects of oropharyngeal atmospheric conditions (5% v/v carbon dioxide) and the presence of a salivary conditioning film on both the surface properties and adherence of *Candida albicans, Candida krusei* and *Candida tropicalis* to PVC and silicone. Furthermore, the effects of the salivary conditioning film on the surface properties of these biomaterials are reported. Growth of the three *Candida* spp. in an atmosphere containing 5% v/v CO_2 significantly increased their cell surface hydrophobicity and reduced the zeta potential of *C. albicans* and *C. krusei* yet increased the zeta potential of *C. tropicalis* ($p < 0.05$). Furthermore, growth in 5% v/v CO_2 decreased the adherence of *C. tropicalis* and *C. albicans* to both PVC and silicone, however, increased adherence of *C. krusei* ($p < 0.05$). Pre-treatment of the microorganisms with pooled human saliva significantly decreased their cell surface hydrophobicity and increased their adherence to either biomaterial in comparison to yeast cells that had been pre-treated with PBS ($p < 0.05$). Saliva treatment of the microorganisms had no consistent effect on microbial zeta potential. Interestingly, adherence of the three, saliva-treated *Candida* spp. to saliva-treated silicone and PVC was significantly lower than whenever the microorganisms and biomaterials had been treated with PBS ($p < 0.05$). Treatment of silicone and PVC with saliva significantly altered the surface properties, notably reducing both the advancing and receding contact angles and, additionally, the microrugosity. These effects may contribute to the decreased adherence of saliva-treated microorganisms to these biomaterials. In conclusion, this study has demonstrated the effects of physiological conditions within the oral cavity on the adherence of selected *Candida* spp. to biomaterials employed as oropharyngeal medical devices. In particular, this study has ominously shown that these materials act as substrates for yeast colonization, highlighting the need for advancements in biomaterial design. Furthermore, it is important that physiological conditions should be employed whenever biocompatibility of oropharyngeal biomaterials is under investigation.
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Introduction

A number of medical devices manufactured from poly(vinyl chloride) (PVC) are routinely used in the oropharynx of patients. For example, the endotracheal (ET) tube is used in the mechanical ventilation of patients in intensive care. In addition, the silicone voice prosthesis is implanted after laryngectomy for voice rehabilitation [1]. Both devices share the same host physiological environment, i.e. a gaseous atmosphere of 5% carbon dioxide and a salivary liquid medium [2].

A common sequel to the introduction of medical devices into the oropharynx involves the adherence of microorganisms, a step that is accepted to represent the initial step in the process of colonisation [3, 4]. The importance of this colonisation process may not be underestimated and indeed, in a recent study, the causal relationship between microbial biofilm on the ET tube and ventilator-associated pneumonia (VAP) has been illustrated using molecular (PCR and RapD) techniques [5]. Apart from the expected opportunistic bacterial pathogens, yeasts avail the opportunity to forge a possible niche on the newly implanted foreign body. Furthermore, *Candida* spp., most notably *C. albicans*, have emerged as major nosocomial pathogens with nosocomial candidaemia accounting for 10–15% of all hospital-acquired bloodstream infections [6]. *Candida* spp. have been isolated from ET tubes retrieved from intensive care patients [7–9], and voice prostheses

explanted from laryngectomy patients [10, 11]. In addition, urinary catheters, prosthetic heart valves, cardiac pacemakers and cerebrospinal fluid shunts are also prone to candidal colonization [12]. In common with microbial biofilm described on ET tubes [7, 8], biofilm development has been reported on voice prostheses [1, 13] and has been implicated in failure of the device [10].

Microbial colonization of ET tubes and laryngeal implants is a multi-factorial process that is influenced by a number of physicochemical factors including, microbial cell surface hydrophobicity and zeta potential and the surface energy and rugosity of the biomaterial [4, 14, 15]. Furthermore, medical devices located within the oropharynx are bathed with saliva, which is reported to alter the surface properties of medical device biomaterials [16] and, additionally, will be exposed to an elevated level of carbon dioxide [17]. These factors may have a significant influence on subsequent microbial colonization. Therefore, this study examined the influence of host environmental conditions in the oropharynx and trachea on the cell surface characteristics of *C. albicans*, *C. tropicalis* and *C. krusei*, and their adhesion to the medical device biomaterials PVC and silicone.

Materials and methods

Chemicals

Sodium chloride, sodium dihydrogen orthophosphate and disodium hydrogen orthophosphate were purchased from BDH Chemicals Ltd., Poole, Dorset, UK.

Xylene was obtained from Aldrich Chemical Co., Gillingham, Dorset, UK.

PVC emulsion (medical grade) was a gift from Rusch Manufacturing Ltd., Lurgan, Northern Ireland.

Medical grade silicone elastomer (NuSil MED-6382) was purchased from Polymer Systems Technology Ltd., High Wycombe, Buckinghamshire, UK.

Sabouraud liquid medium (SLM) and Sabouraud dextrose agar (SDA) were obtained from Oxoid, Basingstoke, UK.

All other chemicals were of AnalaR®, or equivalent, quality and were purchased from Sigma Chemicals Ltd., Poole, Dorset, UK.

Candidal isolates

Type cultures of *C. albicans* (NCYC 1467), originally isolated from a case of denture stomatitis, *Candida krusei* (NCYC 1398) and *Candida tropicalis* (NCYC 1393) were also employed. The microorganisms were maintained on beads (Protect Bacterial Preserve System, Technical Services Consultants Ltd., UK) and on Sabouraud dextrose agar in 10% glycerol at $-70\,°C$. Stationary phase yeast suspensions were grown by transferring isolates into pre-warmed Sabouraud Liquid Medium and incubating at 37 °C for 16 h in either an atmosphere of air or 5% CO_2/95% air.

Preparation of biomaterials

PVC sheets of thickness 2.0 ± 0.01 mm were prepared as previously described by heating PVC emulsion for 10 min, at 160 °C [15, 16]. Silicone sheets were prepared by thoroughly mixing equal masses of liquid silicone elastomer and chloroform, followed by addition of polymer catalyst (0.5% w/w). A fixed mass of this liquid was then poured into a glass petri dish and placed in a fume cupboard for 18 h. Disks, diameter 6 mm, of both biomaterials were cut from the sheets for incubation in the adhesion assay.

Saliva treatment of microorganisms and biomaterials

Unstimulated saliva was collected from six healthy volunteers (who were not taking any oral medication or antibiotics), pooled and diluted 1 : 1 with sterile phosphate buffered saline (PBS, pH 7.3), as previously reported [15, 16]. Stationary phase yeast cells (*ca.* 5×10^6 cfu ml^{-1} or disks of either PVC or silicone were then suspended in pooled saliva or PBS (as a control) and shaken in an orbital incubator (100 oscillations/minute) at 37 °C for 30 min. Prior to inclusion in the adherence assay, saliva was removed either by decantation or centrifugation.

Determination of Candidal zeta potential

The zeta potentials of the various *Candida* spp., were measured using a Malvern Zetasizer IV (Malvern Instruments, Malvern, UK), as previously described [16, 18]. Suspensions of stationary phase yeast cells (either grown in air or in an enriched 5% carbon dioxide atmosphere and either treated with PBS or saliva) were prepared in phosphate buffer solution and then injected into a ZET 5104 capillary cell. Ten measurements of zeta potential were performed for individual samples (field strength 10–20 V cm^{-1}, electrode spacing 50 mm, dielectric constant 78.54) and three individual replicate samples were examined in each case.

Determination of candidal cell surface hydrophobicity (CSH)

The MATH test [19, 20] was employed to measure the cell surface hydrophobicity (CSH) of the yeast isolates. In brief, stationary phase yeast cells of *C. albicans*, *C. krusei* and *C. tropicalis*, which had been grown either in air or in an atmosphere of 5% CO_2/95% air and had been pre-treated either with pooled human saliva or PBS, were suspended in phosphate buffer to a defined optical density (OD 0.7 at 600 nm). To a defined volume of yeast suspension (4.8 mL), 1.0 ml of xylene was added. The biphasic system mixed using a vortex mixer for 10 s and allowed to equilibrate for 10 min, following which the OD of the aqueous phase was determined at 600 nm. The yeast CSH was calculated as follows [20]:

$$\text{CSH} = \frac{[(\text{OD of yeast suspension initially}) - (\text{OD of yeast suspension after mixing})]}{(\text{OD of yeast suspension initially})}$$

Candidal adherence to PVC and silicone

PBS-treated (control) and saliva-treated PVC and silicone disks were secured to the base of sterile McCartney bottles with vacuum grease. To each bottle

was added 15 ml of yeast suspension (5×10^6 cfu ml^{-1}) that had been grown either in air or an atmosphere of 5% CO_2/95% air and treated with either PBS or pooled human saliva. The bottles were then incubated at 37 °C in an orbital incubator (100 oscillations min^{-1}) for 8 h, after which, the yeast suspension was decanted from the bottles and the disks removed using sterile forceps. Each disc was vortexed at low speed for 30 s in two 5 ml volumes of sterile PBS, pH 7.4 in order to remove non-adherent yeast cells. Selected final rinsings were plated onto SDA to ensure successful removal of non-adherent yeasts, indicated by no growth on SDA following incubation. The disks were then fixed in 5% w/v glutaraldehyde in cacodylate buffer (pH 7.4) for 2 h, after which time they were stained with crystal violet for 15 s and rinsed with de-ionized water. The surface of the disks was then observed under a magnification of 400 using light microscopy and surface coverage (%) by yeasts determined by image analysis with a Bio-Foss Automated Microbiology System 3 analyzer (Foss Electric Ltd., UK). Surface coverage was calculated for 10 randomly selected areas on each of the disks.

At each time point, after the removal of non-adherent cells, disks were also placed in a 10 ml volume of sterile PBS, pH 7.4. To remove adherent microorganisms, the disks were sonicated for a 5 min period in a low output sonicator and vortexed for 30 s. This period of sonication was shown not to have an adverse effect on candidal viability [15]. Disks were removed and the viable count of adherent yeasts per cm^2, expressed as a % of the initial inoculum, determined by serial dilution. All experiments were performed using, at least, three replicate samples.

Dynamic contact angle analysis of biomaterials

The advancing and receding contact angles of silicone and PVC, which had been treated with either PBS or pooled human saliva, were quantified, in quadruplicate, using a dynamic contact angle analyzer (DCA 312, Cahn Instruments) at 25 °C. Reagent grade 1 water was used as the wetting medium [21]. Prior to analysis, all treated biomaterials were allowed to dry at room temperature for 1 h. Salivary proteins were not observed to desorb during the course of the analysis.

Characterization of biomaterial microrugosity by atomic force microscopy

The surface microrugosity of PBS-treated and saliva-treated PVC and silicone disks was measured by atomic force microscopy (AFM) using a Burleigh Personal AFM (Burleigh Instruments, New York, USA) as previously reported [4, 21]. Microrugosity was calculated as the root mean square of the vertical dimension (Z_{rms}) using the software supplied. At least 50 measurements of surface microrugosity were performed for each treatment of the two biomaterials.

Statistical analysis

A two way analysis of variance was employed to analyze the effects of atmospheric growth conditions and saliva treatment on yeast cell surface hydrophobicity, cell surface charge and adherence to untreated, PBS-treated and saliva-treated biomaterials. *Post hoc* comparison of the means from independent groups was performed using Fisher's least significant difference. Changes in dynamic contact angle and surface microrugosity of PVC and silicone following treatment with PBS or saliva were examined using an unpaired two-tailed *t*-test. In all cases, $p < 0.05$ denoted significance.

Results

The influences of growth conditions on the adherence of *Candida* spp. to PVC and silicone are illustrated in Tables I and II, respectively. *Candida* spp. cultured in an atmosphere of air, or one enriched with CO_2, exhibited a significantly increased adherence to PVC and silicone following microbial treatment with saliva. The maximum adherence occurred with air grown, saliva-treated *C. albicans* to PVC (6.41 ± 0.31% of the original inoculum). Growth in an enriched carbon dioxide atmosphere (5% v/v) significantly increased the adherence of *C. krusei* to both PVC and silicone, however, adherence of *C. albicans* or *C. tropicalis* to these biomaterials was significantly reduced following growth in a carbon dioxide enriched atmosphere. Table III presents the effects of treatment of both microorganisms and biomaterial (PVC or silicone) with either PBS or saliva on their subsequent adherence. For all *Candida* spp. treatment of both yeast cell and biomaterial with saliva significantly reduced their adherence, in comparison to their PBS-treated counterparts.

The effects of growth and pre-treatment conditions on the cell surface hydrophobicity of the three *Candida* spp. are shown in Table IV. Differences in the cell surface hydrophobicities of the three air-grown microorganisms were observed. According to the classification schemes by Martin *et al*. [22], Schneider and Riley [23] and, more recently, Jones *et al*. [20], *C. krusei* possessed moderately hydrophobic properties whereas *C. albicans* and *C. tropicalis* were highly hydrophilic. A rank order of

TABLE I The effects of growth in a CO_2-enriched atmosphere (5% v/v CO_2) and saliva pre-treatment on the adherence of *Candida albicans, Candida krusei* and *Candida tropicalis* to PBS-treated medical grade PVC

Growth atmosphere	Cell treatment	Mean (± s.d.) adherence (% of original inoculum) to PVC		
		Candida albicans	*Candida krusei*	*Candida tropicalis*
Air	PBS	2.21 ± 0.14	0.90 ± 0.11	1.09 ± 0.10
Air	Saliva	6.41 ± 0.31	1.35 ± 0.05	2.91 ± 0.16
5% v/v CO_2	PBS	2.05 ± 0.11	1.24 ± 0.04	1.05 ± 0.05
5% v/v CO_2	Saliva	3.04 ± 0.13	1.56 ± 0.12	2.01 ± 0.11

TABLE II The effects of growth in a CO_2-enriched atmosphere (5% v/v CO_2) and saliva pre-treatment on the adherence of *Candida albicans, Candida krusei* and *Candida tropicalis* to PBS-treated medical grade silicone

Growth atmosphere	Cell treatment	Mean ($\pm$ s.d.) adherence (% of original inoculum) to silicone		
		Candida albicans	*Candida krusei*	*Candida tropicalis*
Air	PBS	1.74 ± 0.14	1.71 ± 0.18	1.81 ± 0.06
Air	Saliva	2.31 ± 0.06	2.91 ± 0.09	3.41 ± 0.22
5% v/v CO_2	PBS	1.41 ± 0.14	2.01 ± 0.19	1.43 ± 0.10
5% v/v CO_2	Saliva	4.01 ± 0.19	4.91 ± 0.33	4.32 ± 0.24

cell surface hydrophobicity for the three microorganisms was apparent, with *C. krusei* being the most hydrophobic, followed by *C. tropicalis* and finally *C. albicans*. Growth in a carbon dioxide enriched atmosphere significantly increased cell surface hydrophobicity of the three microorganisms. Conversely, treatment of air-grown yeasts with saliva significantly decreased the cell surface hydrophobicity of *C. krusei* and *C. tropicalis*. In light of the highly hydrophilic nature of *C. albicans* as recorded using the MATH test (0.00 ± 0.01), treatment with saliva did not significantly alter the cell surface hydrophobicity. Similarly, treatment of 5% CO_2 grown *C. albicans* and *C. tropicalis* with saliva significantly decreased their cell surface hydrophobicity, however, treatment of 5% CO_2 grown *C. krusei* with saliva did not alter the cell surface hydrophobicity of this microorganism.

Table V illustrates the effects of growth atmosphere and saliva treatment on the cell surface charge (zeta potential), of each *Candida* spp. Prior to saliva treatment, air-grown *C. albicans* displayed a significantly more negative zeta potential whereas the reverse was true for 5% CO_2-grown cells. PBS-treated cells were significantly more negative if grown in 5% CO_2, whereas saliva-treated cells were significantly less negative when grown in an atmosphere of 5% CO_2. *C. tropicalis* grown in air exhibited a significant increase (i.e. less negative) in zeta potential when treated with saliva whereas the surface charge of cells grown in 5% CO_2 became significantly more negative. Relative to air-grown yeasts, PBS-treated cells which had been cultured in 5% CO_2 were significantly less negative, and saliva-treated cells grown in 5% CO_2 were significantly more negative. Finally, saliva treatment of air-grown *C. krusei* had no significant effect on surface charge but made 5% CO_2-grown cells significantly more negative. *C. krusei* grown in an atmosphere of 5% CO_2 was significantly more negative after either PBS or saliva treatment, relative to air-grown cells.

The effects of saliva treatment on the surface characteristics of PVC and silicone are presented in Table VI. Following exposure to saliva, a significant decrease in both advancing and receding contact angles was recorded for each biomaterial, indicating a greater hydrophilic nature. Saliva treatment was also observed to significantly reduce the microrugosity of the surface of each biomaterial.

Discussion

The incidence of candidal colonization of oropharyngeal medical devices has increased over the part decade and is now accepted to be a major reason for medical device failure. Furthermore, there is considerable morbidity associated with candidal colonization of oropharyngeal medical devices. As a result of this, a clinical need has arisen for the identification and use of biomaterials that offer resistance to candidal, and indeed microbial colonization, in general, of oropharyngeal devices. However, one important step in the design and development of such novel materials is an understanding of the process of candidal colonization of oropharyngeal biomaterials and the effects of physiological conditions within the oral cavity on this process. Therefore, in this study we have examined the effects of two key factors, namely, atmospheric growth conditions and the presence of saliva, on the adherence of three *Candida* spp. to two oropharyngeal biomaterials. These microorganisms were selected as those implicated in oropharyngeal medical device failure [10, 11, 24].

A growth atmosphere supplemented with 5% CO_2 has been reported to alter bacterial adherence to polymers and modify microbial sensitivity to antimicrobial agents [15, 25, 26]. With regard to yeasts, elevated levels of CO_2 have been found to influence adherence of *C. albicans* to vaginal epithelial cells [27]. In the present study, decreased adherence of both *C. albicans* and *C. tropicalis* to untreated PVC and silicone was observed when the yeasts were cultured in a CO_2-enriched atmosphere. In contrast, adherence of *C. krusei* to PVC displayed a significant increase following cell growth in a CO_2-enriched atmosphere. One obvious explanation

TABLE III Adherence (% of original inoculum) of pre-treated yeast cells (PBS or saliva), grown in a CO_2-enriched atmosphere (5% v/v CO_2), to pre-treated (PBS or saliva) medical grade PVC and silicone

Biomaterial	Cell treatment	Biomaterial treatment	Mean ($\pm$ s.d.) adherence (% of original inoculum)		
			Candida albicans	*Candida krusei*	*Candida tropicalis*
PVC	PBS	PBS	21.8 ± 0.18	6.01 ± 0.39	3.79 ± 0.20
PVC	Saliva	Saliva	1.19 ± 0.11	4.21 ± 0.31	2.32 ± 0.13
Silicone	PBS	PBS	2.68 ± 0.18	3.46 ± 0.16	2.03 ± 0.18
Silicone	Saliva	Saliva	0.93 ± 0.11	0.99 ± 0.10	0.91 ± 0.05

TABLE IV The effects of growth in a CO_2-enriched atmosphere (5% v/v CO_2) and saliva pre-treatment on the cell surface hydrophobicity of *Candida albicans*, *Candida krusei* and *Candida tropicalis*

Growth atmosphere	Cell treatment	Mean (± s.d.) cell surface hydrophobicity (%)		
		Candida albicans	*Candida krusei*	*Candida tropicalis*
Air	PBS	0.00 ± 0.01	49.06 ± 2.53	7.54 ± 0.12
Air	Saliva	0.00 ± 0.01	42.10 ± 1.21	0.26 ± 0.01
5% v/v CO_2	PBS	9.13 ± 0.05	54.64 ± 0.73	14.87 ± 0.24
5% v/v CO_2	Saliva	6.27 ± 0.02	54.76 ± 1.77	0.00 ± 0.01

for these observations may involve biochemical changes that accompany growth in the CO_2-enriched atmosphere. Growth in an atmosphere containing 5% v/v CO_2 has been reported to alter the expression of surface molecules that are either involved in or inhibit microbial adherence [15, 28]. Therefore, in the case of *C. albicans* and *C. tropicalis*, the production of molecules that are normally involved in adherence may have been suppressed or, alternatively, molecules may have been expressed that possess a lower affinity for PVC and silicone. Conversely, the opposite may be true for *C. krusei*. Furthermore, growth in a CO_2-enriched atmosphere significantly altered the cell surface of the yeast species. In this regard growth of each organism in a CO_2-enriched atmosphere rendered the cell surface more hydrophobic although no correlation of this observation with effects on adherence to PVC was recorded.

Following residence within the oral cavity, both medical devices and microorganisms will be coated with a conditioning film that has been derived from saliva [16]. In this study, adsorption of salivary components onto both microbial and biomaterial surfaces was highlighted by alterations in the various cell surface hydrophobicities and advancing/receding contact angles. Saliva is a biochemically complex medium that contains several macromolecules including, glycoproteins, enzymes and immunoglobulins [29] and consequently, the nature of the adsorbed film on the surface of microorganisms and biomaterial may be structurally complex. Furthermore, it has been shown that the nature of the substrate (microbial or biomaterial) influences the process of macromolecular adsorption [15, 16]. The exact role of the adsorbed conditioning film on the process of microbial adherence to biomaterials is unclear, with some reports advocating increased adherence [31], and others decreased adherence [32]. For example, McCourtie *et al*. [33] found that pre-treatment of acrylic surfaces with saliva for 30 min led to a reduced adherence by *C. albicans*, *C. tropicalis* and *C. glabrata*. Whereas Edgerton *et al*. [34] reported a significant increase in *C. albicans* adherence following treatment of poly(methylmethacrylate) with mixed human submandibular and sublingual saliva. Similarly, enhancement of yeast adherence involving the salivary conditioning film was illustrated by an approximately ten-fold increase in candidal biofilm activity on acrylic strips pre-treated with saliva [35]. The increased adherence to PVC and silicone following saliva treatment of the *Candida* spp. investigated in this study may be accredited to the deposition of salivary macromolecules onto the surface of the yeast cell that promote attachment to the biomaterial surfaces. Similarly, enhanced adherence of *Escherichia coli* and *Enterobacter faecalis* to artificial urine-coated polyurethane has been reported [18]. No primary relationship was observed between the CSH of saliva-coated organisms and adherence to either PVC or silicone. For example, saliva treatment of *C. albicans* and *C. tropicalis* decreased CSH and increased adherence to PVC and silicone. However, the presence of a salivary-coating increased the adherence of *C. krusei* without significant alterations in the CSH. Similarly, the lack of correlation concerning the effects of cell surface charge on yeast adherence to the biomaterials under examination infers that this physicochemical parameter contributed to the adherence interaction in a relatively minor way.

The adherence of the *Candida* spp. under investigation in this study to either PVC or silicones increased following treatment of the yeast cell with saliva. It was therefore of interest to observe that the adherence of saliva-treated yeast cells to saliva-treated biomaterials was significantly reduced in comparison. One explanation for this phenomenon involves the role of the conditioning film on the surface of the biomaterials [16]. Thus, molecules within this conditioning film that have been adsorbed onto the biomaterial surface may effectively block sites of interaction of the saliva-coated (but not PBS-treated) yeast cells on the surface of the biomaterial. Furthermore, the presence of the macromolecular conditioning film on the biomaterial surface was observed to reduce both the surface free energy (advancing and receding contact angles) and, additionally, the biomaterial surface microrugosity. These parameters have been reported to play significant roles

TABLE V The effects of growth in a CO_2-enriched atmosphere (5% v/v CO_2) and saliva pre-treatment on the cell surface charge (zeta potential, mV) of *Candida albicans*, *Candida krusei* and *Candida tropicalis*

Growth atmosphere	Cell treatment	Mean (± s.d.) zeta potential (mV)		
		Candida albicans	*Candida krusei*	*Candida tropicalis*
Air	PBS	− 17.08 ± 0.03	− 15.00 ± 0.41	− 29.68 ± 0.27
Air	Saliva	− 19.61 ± 0.33	− 14.71 ± 0.22	− 25.11 ± 0.20
5% v/v CO_2	PBS	− 19.39 ± 0.28	− 17.01 ± 0.14	− 26.05 ± 0.21
5% v/v CO_2	Saliva	− 18.86 ± 0.34	− 19.57 ± 0.88	− 30.92 ± 1.21

TABLE VI The effect of saliva treatment on the advancing and receding contact angles and surface roughness of medical grade silicone and PVC

Biomaterial	Treatment	Mean (± s.d.) advancing contact angle°	Mean (± s.d.) receding contact angle°	Mean (± s.d.) microrugosity (nm)
PVC	Untreated	93.99 ± 0.58	68.01 ± 0.23	42.08 ± 1.58
PVC	PBS	92.19 ± 0.22	67.99 ± 1.24	43.69 ± 1.43
PVC	Saliva	81.11 ± 0.69	50.01 ± 0.55	21.21 ± 3.23
Silicone	Untreated	110.80 ± 0.58	76.92 ± 0.62	61.08 ± 7.62
Silicone	PBS	112.74 ± 0.28	71.90 ± 0.94	51.06 ± 6.31
Silicone	Saliva	74.82 ± 2.58	42.97 ± 0.56	38.13 ± 3.57

in the process of microbial colonization of surfaces. For example, Weerkamp *et al*. [36] reported that the presence of a salivary conditioning film reduced biomaterial surface energy and, subsequently, altered adherence to this substratum. In addition, alterations in biomaterial microrugosity have been reported to significantly affect microbial adherence. The adherence of *Staphylococcus epidermidis* to polyurethane peritoneal catheters was reported to be greater whenever the microrugosity of the biomaterial was increased [37]. Therefore, the effects of a salivary conditioning film on the surface free energy and microrugosity of PVC and silicone may explain, at least in part, the observations concerning the subsequent adherence of salivary-coated yeast cells.

In conclusion, this study has highlighted the role of oropharyngeal environmental factors, namely a CO_2-enriched atmosphere and saliva, on the adherence of *C. albicans*, *C. tropicalis* and *C. krusei* to silicone and PVC from which many oropharyngeal medical devices are fabricated. In particular, this study has ominously shown that these materials act as substrates for yeast colonization, confirming that advances in biomaterial design are required to prevent colonization of PVC endotracheal tubes and silicone voice box prostheses. Interestingly, following treatment with saliva, reduced adherence of yeasts to both biomaterials was observed, indicating a role for saliva in the suppression of microbial colonization. Furthermore, growth of yeast cells in a (CO_2-enriched) atmosphere, was observed to significantly reduce subsequent adherence to each biomaterial. Therefore, when evaluating the *in vitro* resistance of biomaterials to microbial colonization, it is important to ensure that a physiological (CO_2-enriched) atmosphere is employed, as these conditions are more relevant to the potential clinical performance of the biomaterial.

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*Received 2 June
and accepted 28 July 2000*