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ORIGINAL ARTICLE

Efficacy of denture cleansers on denture liners contaminated with *Candida* species

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Abstract As poor denture hygiene is related to *Candida* colonisation, disinfectant solutions have been proposed as an effective method of preventing denture stomatitis. This study assessed the efficacy of denture cleansers on *Candida albicans* and *Candida glabrata* adherence on denture liners. Another aim was to correlate materials' surface roughness (Ra) to *Candida* adherence. Specimens of three denture liners (soft and hard polymethyl methacrylate (PMMA)-based and soft silicone-based) were prepared and had their Ra measured. Specimens were randomly divided to adherence assays with *C. albicans* or *C. glabrata*. After contamination with the fungi, specimens were treated with an enzymatic cleanser solution, a cleanser solution or a 0.5% NaOCl solution by soaking for 3, 15 or 10 min, respectively. Control group specimens were soaked in distilled water for 15 min. Number of remaining *Candida* cells after treatment was determined by light microscopy (×400). Analysis of variance (α=0.05) showed that Ra of the silicone-based liner was lower than that of the PMMA-based liners (p<0.05). The overall results showed high *C. glabrata* adherence (p<0.001), while the lowest levels of remaining *Candida* cells were found for the treatment with 0.5% NaOCl (p=0.0019). No difference among denture cleansers and control was found (p=0.19). There was no correlation between Ra and *C. albicans* or *C. glabrata* adherence in all materials tested. The only treatment able to reduce both *Candida* species adherence on all materials tested was 0.5% NaOCl solution.

Keywords *Candida albicans* · *Candida glabrata* · Denture liners · Denture cleansers · Saliva

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Introduction

Candida species are the main pathogens responsible for the development of denture stomatitis, which is the most common infection in denture wearers [5, 32]. Poorly fitting dentures, the use of denture liners and poor oral hygiene are the most frequent cause of this opportunistic infection [32, 33]. Although *Candida albicans* is the predominant isolate in these infections, other non-*albicans* species, in particular the emerging fungal pathogen *Candida glabrata*, are frequently isolated from acrylic surfaces and the palatal mucosa [13]. Pathogens as *C. glabrata* may exhibit higher denture surface adherence and acquired resistance against antifungal drugs [13]; however, there is no report on the effect of denture cleansers regarding this specific species and denture liners.

The adhesion of *Candida* initially depends on the roughness of the denture surface [30] and the composition of the substratum. Roughness and micro-porosities may cause the surface to harbour micro-organisms that are difficult to remove by mechanical or chemical cleansing [26] thus increasing micro-organisms adherence in vitro [1, 25, 30]. In this way, micro-organism adhesion related to the composition of the substratum should be considered.

The use of denture liners is needed in clinical situations in which patients present thin, sharp or resorbed residual alveolar ridges and chronic tissue irritation from dentures or have received implant treatment [15]. Even though these materials show excellent tissue tolerance, one of the problems associated to these materials is the colonisation of *Candida* spp. on and in the material. Indeed, it is well known that poor oral hygiene is directly related to *Candida* colonisation and, therefore, disinfectant solutions have been proposed as an effective method of preventing denture stomatitis on patients wearing denture manufactured with these materials [16, 19].

Considering that little is known about the effect of denture cleansers on *Candida* species rather than *C. albicans*, the aims of this study were (a) to correlate surface roughness to adherence levels, (b) to compare *C. glabrata* and *C. albicans* behaviour regarding initial adherence to denture liners and (c) to assess the effect of denture cleansers on *Candida* adherence. The null hypotheses tested were that there would be no correlation among Ra and *Candida* adherence; there would be no difference between the two *Candida* species and that *Candida* species counts would not be affected by denture cleansers or substratum type.

Material and methods

All materials used in this study are listed in Table 1.

Experimental design

This in vitro study had a completely randomised and blinded design, with substratum type, treatment with denture cleansers and *Candida* species as factors under study. Surface roughness of the tested substrata and remaining cells of *C. albicans* and *C. glabrata* were the dependent variables.

Specimens were fabricated according to the manufacturer's instructions. After finishing, surface roughness was measured. Next, the specimens were randomly divided into 24 groups (n=8 per group) and submitted to the adherence assay for 2 h with one of the following *Candida* suspensions: *C. albicans* or *C. glabrata*. Specimens received one of the following treatments, according to the designated group: T1—negative control (water); T2—enzymatic cleanser solution; T3—cleanser solution; or T4—0.5% NaOCl solution. After treatment, the remaining adhered cells were counted using a light microscope at ×400 magnification.

Preparation of specimens

Microwave-polymerised PMMA bases (2.5×1.2×0.1 cm) were prepared to be relined by the compression-mould technique, according to the manufacturer's recommendations [18]. The denture liner specimens were prepared according to manufacturer's instructions at room temperature (23±1.0°C and 50±5% relative humidity) under

Table 1 Composition of the materials used in this study

	Product	Chemical composition	Brand and manufacturer
Denture materials	Microwave-cured acrylic resin (PMMA)	*Powder:* methyl methacrylate copolymer, ethacrylate, dibutyl paleoteodine, benzoyl peroxide *Liquid:* methyl methacrylate, topanol, ethylene glycol dimethacrylate	Onda Cryl, Artigos Odontologicos Classico Ltd., Sao Paulo, Brazil
	Soft denture liner (PMMA)	*Powder:* polyethylmethacrylate, zinc undecylenate, pigments *Liquid:* ethyl alcohol, benzyl salicylate, dibutyl phthalate, methylsalicylate, oil mint	CoeSoft, GC America, Alsip, IL, USA
	Hard denture liner (PMMA)	*Powder:* cadmium compound, silica, crystalline—quartz, benzoyl peroxide, polyethylmethacrylate *Liquid:* 2,4-dihydroxy benzophenone, isobutyl methacrylate	Kooliner, GC America, Alsip, IL, USA
	Soft denture liner (silicone-based)	Mixing of several polyalkylsiloxanes	Ufi Gel P, VOCO, Cuxhaven, Germany
Denture cleansers	Enzymatic cleanser solution	Sodium perborate, potassium monopersulfate, proteolytic enzyme, detergent and effervescent base	Polident 3 min, GlaxoSmithKline, Philadelphia, PA, USA
	Cleanser solution	Potassium monopersulfate, sodium perborate, sodium bicarbonate, EDTA, sodium tripolyphosphate, sodium sulfate, flavor, sodium lauryl sulfoacetate, polytetrafluoroethylene, sodium saccharin, FD&C Blue No. 2, and FD&C Green No. 3	Efferdent, Warner Lambert Co., Morris Plains, NJ, USA
	0.5% NaOCl	Water and 0.5% sodium hypochlorite	Proderma Pharmacy, Piracicaba, Brazil

aseptic conditions. Relined specimens were prepared to the same uniform size by inserting the acrylic resin base into a glass mould, pouring the denture liner, placing glass slides over it and firmly fixing both ends, then separating the glass plates after curing, as described elsewhere [8, 20]. They were finished and polished according to manufacturers' recommendation and used immediately.

Surface roughness

Surface roughness of the specimens was measured using a profilometer (Surfcorder SE 1700; Kosaka Laboratory Ltd., Kosaka, Japan) with a 0.01-mm resolution, calibrated at a specimen length of 0.8 mm, 2.4-mm percussion of measure and 0.5 mm/s. Three readings were made for each specimen, and a mean value was calculated [30].

After surface roughness measurements were completed, the specimens were randomly assigned to one of the experimental conditions. The contaminants were removed by sonication in sterilised deionised distilled water for 20 min previous to the adherence assay [14]. All procedures were carried out by a single operator.

Human saliva collection and preparation for the adherence assay

All specimens received a salivary pellicle coating previously to the adherence assay in order to simulate similar conditions as in vivo. Human whole saliva was collected from a single healthy volunteer [6, 18] who had not used antibiotics, mouth rinses or any other medication known to affect salivary composition and flow in the past 3 months and who provided written informed consent previously approved by the Local Ethics Committee. Stimulated saliva was collected and clarified by centrifugation at 10,000×*g* for 10 min at 4°C [17]. The supernatant was placed into sterile Petri dishes where all specimens were placed with the denture liner surface facing down and left for 30 min [18] to form acquired pellicle. After this period, specimens were removed and immediately used in the adherence assay.

Inoculum and growth conditions

A loopful of stock yeast cultures of *C. albicans* (ATCC 90028) and *C. glabrata* (ATCC 2001) were reactivated from their original cultures at −70°C and incubated for 24 h at 37°C. Cells were harvested, suspended in phosphate buffer solution (PBS, Sigma-Aldrich, St Louis, MO, USA) and standardised to 1 to 5×10^6 cells per millilitre, ascertained spectrophotometrically (Bausch and Lomb Spectronic 20, San Pablo, CA, USA) at 530 nm [17, 18].

Adherence assay

The specimens were individually placed vertically [14, 25, 27] into sterile test tubes containing a suspension of 10 mL of Sabouraud broth (Difco) and one of the two *Candida* species and incubated for 2 h at 37°C to promote yeast adherence [18, 23]. Each specimen was subsequently removed and gently washed with PBS (15 s) to remove loose and non-adhered cells before the treatment.

Treatment

Following the adhesion assay, specimens were randomly assigned to four groups of separate treatments: T1—negative control (water); T2—enzymatic cleanser solution (Polident 3 min); T3—denture cleanser solution (Efferdent); or T4—0.5% sodium hypochlorite (NaOCl) solution. Cleaning tablets were placed into 30 mL (40°C) of deionised distilled water [21]. Exposure to the immersion effervescent denture cleansers was controlled to allow all surfaces of the specimen to be in contact with the cleanser. T2 and T3 treatments were prepared and used according to manufacturers' instructions, including treatment times of 3 min for T2 and 15 min for T3. Group T4 specimens were treated for 10 min. The negative control group was not subjected to any treatment as it would be impossible to prepare a common placebo for the two denture cleansers tested. In this group, specimens remained 15 min in deionised distilled water as a reference for the highest time used (T3).

Next, specimens were removed from the test tube, washed with PBS followed by 80% ethanol to fix the yeasts, stained for 1 min with crystal violet (Newprov; Newprov Produtos Laboratoriais, Sao Paulo, Brazil) and washed with PBS [18, 26, 31].

Yeast counts

Adherent yeast cells in 15 different fields for each specimen (0.25 mm^2 per field) were determined using a light microscope (Axiostar 2 Plus, Carl Zeiss, Jena, Germany) at ×400 magnification, and the results were expressed as cell per square millimetre. The majority of the attached yeasts were at the blastospore stage, some with daughter cells and only a few with hyphae or pseudohyphae. The following parameters were used to standardise the counts: a budding yeast was considered as a unit cell if the daughter was smaller than the mother cell, and a hypha was counted as a single cell [27].

Statistical analysis

Statistical analyses were done using SAS software (SAS Institute Inc., version 9.0, Cary, NC, USA) employing a

Table 2 Mean (±SD) surface roughness values (Ra—μm) of the materials

Type of denture liner	Ra (μm)
Soft PMMA-based	3.9±1.4 B
Hard PMMA-based	3.7±1.7 B
Soft silicone-based	0.3±0.2 A

Upper case letters represent statistically different results (analysis of variance; $p<0.05$)

significance level fixed at 5%. Data that violated the assumptions of equality of variances and normal distribution of errors were transformed ($\log_{10}$ for adherence and $Ra^{0.1}$). Data of remaining cells of *Candida* species and Ra were analysed by three-way and one-way analysis of variance, respectively, followed by Tukey test. Relationship between Ra and *Candida* adherence was verified by Pearson's correlation test. The correlation test was applied solely in the control group, as it was not subjected to treatment.

Results

There was no statistical difference in surface roughness regarding the PMMA-based liners. However, the silicone-based denture liner presented the smoothest surface (Table 2, $p<0.0001$). *C. albicans* and *C. glabrata* cells that remained on the materials after treatments ranged from 0.8 to 61.3 and 0.5 to 113.6 cells per square millimetre (minimum to maximum values for each species, respectively), with statistically significant differences. The PMMA-based soft liner exhibited the highest adhered number of cells when compared to the hard PMMA-based ($p<0.05$). The overall colonisation on all materials was significantly decreased when treatment with 0.5% NaOCl was employed ($p<0.001$, Table 3). Except for 0.5% NaOCl, the results showed the same level of remaining cells in all materials tested when compared with the control group ($p>0.05$) for both *Candida* species after treatment with the denture cleansers. Moreover, *C. glabrata* revealed a higher number of remaining cells in all treatments ($p<0.05$) when compared to *C. albicans*, except for 0.5% NaOCl treatment, which was equally effective for both species.

There was no correlation between surface roughness and adherence of *C. glabrata* in all substrata ($p>0.05$). The same trend has occurred for *C. albicans*, where no correlation between Ra and remaining number of cells after treatment was found ($p>0.05$).

The null hypotheses tested were rejected since both species showed different results, and the treatment with 0.5% NaOCl and substratum type and their characteristics influenced the outcomes of this study.

Discussion

This study was the first to evaluate the effect of denture cleansers on *C. glabrata* adherence on different denture liners, which can typically be either acrylic- or silicone-based. These new results are important as denture liners may be easily colonised and deeply infected by *Candida* species. Soft lining materials present a convenient substratum for microbial colonisation due to irregularities and porous surface texture in which micro-organisms, especially yeasts, can be entrapped or harboured, allowing increased adherence [24].

Although these materials may present different surface properties [10, 11], they can be similar concerning fungal adherence and its removal from the surface after using denture cleansers as treatment, as shown in this study. Moreover, in this study, the PMMA-based liners showed the highest values of surface roughness and the silicone-based denture liner the lowest, presenting the smoothest surface.

Soaking dentures in disinfectant solutions or denture cleansers has been shown to be an effective method to prevent *Candida* contamination [2, 12]. In this study, one disinfection solution (NaOCl) and two different denture cleansers have been chosen due to their different composition and immersion times. Differences between cleansers were small and are likely to be clinically irrelevant.

Table 3 Mean (±SD) *C. albicans* and *C. glabrata* recovered (cell per square millimetre) values according to treatments

		Control	Polident	Efferdent	0.5% NaOCl
Soft PMMA-based	*C. albicans*	21.8±20.6Aa*	17.4±10.0Aa*	10.0±9.6Aa*	6.3±4.4Ca§
	C. glabrata	33.6±28.0Ba*	31.3±23.7Ba*	31.6±41.3Ba*	8.2±4.6Ca§
Hard PMMA-based	*C. albicans*	11.1±10.3Ab*	10.5±8.3Ab*	6.6±5.1Ab*	3.4±2.4Cb§
	C. glabrata	21.5±20.5Bb*	16.5±11.3Bb*	15.2±14.0Bb*	4.4±3.3Cb§
Soft silicone-based	*C. albicans*	8.7±9.2Aab*	8.3±6.8Aab*	6.73±3.4Aab*	4.5±3.6Cab§
	C. glabrata	27.1±20.8Bab*	37.7±25.9Bab*	23.51±15.6Bab*	7.63±6.3Cab§

Distinct upper case letters show statistical differences between *Candida* species within materials. Distinct lower case letters show differences among materials. Different symbols show statistical differences among treatments (ANOVA; $p<0.05$).

Although it is reported that alkaline peroxide solutions are effective against *Candida* colonisation [28], our study has shown that the tested cleansers are not effective on preventing initial *Candida* adherence to denture liners when compared to the immersion in water. On the other hand, soaking dentures in sodium hypochlorite, which is considered fungicidal, showed its efficacy since it decreased *Candida* counts in comparison to the other treatments. These results are in accordance with those from Ghalichebaf et al. [7] and Webb et al. [32]. While its effectiveness is already proven regarding *C. albicans*, how sodium hypochlorite affects other *Candida* species is still not fully understood. Our study has shown that even though *C. glabrata* presented higher adherence rates when compared with *C. albicans*, sodium hypochlorite is equally effective in diminishing colonisation of *C. glabrata*.

The use of sodium hypochlorite has not been recommended due to the possibility of affecting physical properties of materials [3, 32]. However, no detrimental effect on denture base or denture lining materials occurred when hypochlorite cleansers were used, especially those having lower percentages of hypochlorite [32]. Furthermore, although previous studies have reported that 5.25% hypochlorite solutions would be effective on disinfecting dental acrylic [4, 34], our results revealed that soaking the specimens in 0.5% sodium hypochlorite for 10 min reduced the number of micro-organisms in comparison to the denture cleansers tested.

The degree of adhesion of a certain species to biological surfaces may indicate their pathogenic potential. This is likely why *C. glabrata* showed higher counts than *C. albicans* in most of the experimental conditions and materials, concurring with previous studies where the same trend has occurred [9, 14], regardless of the fact that these studies did not assess the effect of denture cleansers. These different adherence results may be explained by the complexity and phenotypic heterogeneity of the *Candida* species population expressed in different hydrophobicity, secretion of extracellular proteinases, hyphal formation and thigmotropism [14], which directly influence *Candida* adherence rates.

A limitation of our study was that we have only assessed newly fabricated denture liner specimens. It has been shown that soft lining materials show changes in their physical properties with age [22, 29], and studies on how *Candida* grows when ageing of the material occurs is mandatory. Moreover, the results of this study should be interpreted with care since the nutrient-rich environment of the oral cavity does not fully match the in vitro nature of our study. However, they point towards important evidence on how different *Candida* species behave in the presence of various denture cleansers and concerning several denture liners regularly used in clinical practice.

It is important to highlight that the presence of a denture material associated to the use of a denture cleaning protocol, which could somewhat be more favourable than others to avoid the oral cavity recolonisation is mandatory. Hence, further studies with larger number of strains and studies on biofilms formed on these surfaces and the action of denture cleanser solutions are nevertheless important. Although our results should be interpreted with care since the nutrient-rich environment of the oral cavity does not fully matches the in vitro nature of our study, these results are an important clue on how denture cleansers act regarding *Candida* adherence and especially considering different substrata.

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Conflict of interest The authors declare that they have no conflict of interest.

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