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Effectiveness of Different Cleaning Agents against the Colonization of *Candida* spp and the *in Vitro* Detection of the Adherence of These Yeast Cells to Denture Acrylic Surfaces

A. Dilek Nalbant,[1] Ayse Kalkanci,[2] Banu Filiz,[1] and Semra Kustimur[2]

[1]Department of Prosthetic Dentistry, Faculty of Dentistry, University of Gazi, Ankara; [2]Department of Microbiology, Gazi University Faculty of Medicine, Ankara, Turkey.

Purpose: The aim of this study is to examine the effect Klorhex and Fittydent, which are used as cleaning agents on the adhesion of *Candida* on the surfaces of acrylic denture and palatal mucosa. In addition, ability of yeasts to adhere to acrylic strips was evaluated after applying these agents *in vitro*. **Materials and Methods:** Each group of 15 patients cleaned their dentures with either Klorhex or with Fittydent. The control group cleaned their dentures with water. **Results:** It was found that 62.2% of the patients had colonies of *Candida* species on their palatal mucosa which was reduced to 51.1% after using these cleaning agents. The colonization rate with *Candida* spp on their dentures was reduces from 82.2% to 68.8% using these cleaning agents. The mean adhesion value of the *Candida* strains isolated from the acrylic strips were found to be 75 cell/strip prior to applying the Klorhex and Fittydent and 37.5 cell/strip and 15 cell/strip after applying these agents, respectively. **Conclusion:** These results showed that Klorhex and Fittydent have a certain preventive effect on the colonization rate of *Candida* spp on the surface of these dentures, the palatal mucosa, as well as on the acrylic strips *in vitro*.

Key Words: *Candida*, colonization, adhesion, acrylic

INTRODUCTION

Candidiosis induced by *Candida* albicans is the most common fungal infection of the oral cavity in humans. The predisposing factors to infections with *Candida* species can be divided into two major categories, systemic factors and local factors. Some of the systemic factors include age, endocrine diseases, systemic steroids and antibiotics, concurrent infections and deficiency states. Local factors include reduced salivation, smoking, topical antibiotics or steroid treatment, coexistent oral mucosal diseases and specifically the wearing of dentures.[1,2] There is reliable evidence showing that unclean dentures and insufficient hygiene care are significant predisposing factors.[1] *Candida* associated denture stomatitis is a common infection observed in elderly denture wearers because the acrylic denture fitting surfaces act as a reservoir for infection. *Candida* species are frequently isolated both from acrylic denture surfaces and from the palatal mucosa.[2] The tissue surfaces of the dentures usually show microporosites, which harbor the microorganisms that are difficult to remove by mechanical or chemical cleaning. *In vitro* studies indicate that the microbial contamination of denture acrylic resin occurs quite rapidly and the yeast cells adhere strongly to denture base materials.[1,3-6]

It is well known that the removal of denture plaque is essential for maintaining the health of oral soft tissue.[7,8] Studies comparing the efficacy of the proposed denture cleansing techniques, either mechanical or chemical, used a variety of methods to evaluate the control of plaque. Among elderly patients the most popular method for removing denture plaque is brushing with an abrasive paste and water. However, effective plaque removal requires a degree of manual dexterity that is often lacking particularly among elderly individuals.[9-11] Therefore, chemical clean-

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Reprint address: requests to Dr. A. Dilek Nalbant, Department of Prosthetic Dentistry, Faculty of Dentistry, University of Gazi, Ankara, Turkey. Tel: 90-312-2034183, Fax: 90-312-2239226 E-mail: dnalbant@gazi.edu.tr

ing with immersion denture cleaners is suggested as the first choice for plaque control in these patients.[11,12] Candidal infections have been successfully treated with chlorhexidine gluconate because it is an effective oral disinfectant.[13,14] Fittydent is a chemical cleaning agent that is also used to remove denture plaque.[9]

Several studies have been performed to investigate the adhesion of *Candida* albicans on acrylic surfaces. However, many of them are *in vitro* studies, which have proposed complex and difficult techniques. Researchers have problems in quantifying the candidal adherence to surfaces due to the co-aggregation of adhered yeasts and to the laborious, time-consuming removal methods.[3,5,14] The aim of this study was to examine the adherence of *Candida* on the surfaces of acrylic denture prosthetic materials and the palatal mucosa in a group of patients and examine the *in vitro* adhesion abilities of these yeasts to acrylic strips. This study describes a simple microscopic examination, which was presented previously.[15-17] In addition, the difference in the ability of yeast cells to adhere to acrylic surfaces were evaluated before and after using cleaning agents.

MATERIALS AND METHODS

Study population

The study protocol was reviewed and approved by the Faculty of Medicine, Gazi University ethics committee. Forty-five complete denture wearers attending the Department of Prosthetic Dentistry of Gazi University, Faculty of Dentistry were enrolled in the study. The group consisted of 19 males and 26 females aged between 43 - 91 with a mean age of 63.7. All the subjects underwent a routine dental check up and none of them complained of any mucosal lesions. The exclusion criteria were; 1) subjects taking antifungal agents or antiseptic mouthwashes, 2) subjects taking medication known to predispose them to oral candidiosis, such as antibiotics or steroid therapy, 3) subjects with a medical history of any disease or medical condition that predisposed them to oral candidiosis or promoted subjects oral carriage of *Candida* species.

The patients were randomly divided into three groups wearing complete dentures. Each group of 15 patients had their dentures cleaned either with Klorhex® (0.2% of chlorhexidine solution-belongs to Bisbiquanides groupe) (Drogsan Doğa kaynakları İlac Hammaddeleri Sanayi ve Tic. A. Ş., Ankara, Turkey) or with Fittydent® (Sodium perborate, sodium bicarbonate) (Mag. Hoeveler and Co. Gmbh, Geinberg, Germany). The control group cleaned their dentures with water. For the microbiological examinations, the posterior mid-palatal part of the palatal mucosa and the corresponding area of the fitting denture base were swabbed with sterile cotton swabs. These swabs were then transferred to a Sabouraud dextrose broth in the Department of Prosthetic Dentistry. The specimens were obtained from the same part of the denture and palatal mucosa before and seven days after using the cleaning agents. In the first group, the dentures were soaked in a 200 mL Klorhex bath, while the dentures in the second group were soaked in a 200 mL solution of distilled water with one tablet of effervescent Fittydent. The dentures in the control group were washed overnight in 200 mL of water.

Culture and the identification

Cotton swabs were streaked onto the Sabouraud dextrose agar (SDA) plates in the Research Laboratory of Gazi University Faculty of Medicine, Department of Microbiology. The plates were incubated at 37℃ for 48 hours. The yeast colonies were identified using the identification criteria such as germ tube production, the fermentation of carbohydrates in ID32 C strips (bioMerieux, Marcy l'Etoile, France). The yeast cultures were stored in 0.9% NaCl at - 20℃ until they were tested.

Preperation of yeast suspensions

The stock cultures in 0.9% NaCl were transferred onto the SDA plates in order to re-grow the yeast colonies. A loopful of stock culture was incubated on a Sabouraud dextrose broth for 18 hours at 37℃ in a horizontal shaker in order to grow the yeast cells in the stationary phase.[17] The culture was centrifuged at 1,700 × g for 10 minutes and the deposit was washed once with 0.15 M

phosphate buffered saline (PBS, pH 7.2). A final yeast suspension of approximately 4×10^7 yeast cells/mL was prepared by adding 100 μL of the deposit to 10 mL of PBS. The number of the yeast cells per mL in all the experiments was counted spectrophotometrically.

Preperation of acrylic strips

Twenty acrylic resin disks (20 × 1 mm) were prepared. Each disk was cut from a pink base-plate wax sheet. Twenty wax disks were invested in a denture flask using a standard processing technique.[18,19] The wax was eliminated and heat-curing acrylic resin (Meliodent. Heraus Kulzer, Germany) was packed into the mold cavity. The denture acrylic poly (methyl-methacrylate) powder and the monomer liquid were mixed according to the manufacturer's recomendations. All the disks were polymerized with the same batch of acrylic resin using a standard heat-curing resin cycle (10 hours at 90℃). The disks were polished after they had been deflasked and prepared. They were sterilized by ethylene oxide and aired for 3 days in order to remove all the ethylene oxide.

Adhesion assay

Using strict aseptic techniques, the acrylic strips were placed in tubes containing 4×10^7 yeast cells/mL of PBS. The tubes were placed in a shaker incubator for 3 hours at 37℃ with gentle agitation. The strips were then washed in sterile distilled water, air-dried, and stained using a gram stain. After drying at room temperature the strips were mounted on glass slides and the adherent yeasts were quantified using optical microscopy at a × 40 magnification. Four fields were randomly counted in each strip, and all the experiments were repeated on three separate occasions with duplicate determinations on each occasion.

The adhesion of the *Candida* strains isolated from the denture surfaces to the acrylic strips was examined. The adhesion assay was also repeated for the acrylic strips with the adhered *Candida* strains isolated from the denture surfaces, after the strips were incubated in 2 mL of a Klorhex solution at a concentration of 0.2% and a Fittydent solution at room temperature overnight.

Statistical analysis

The Friedman non-parametric two-way analysis of the variance was used to compare the levels of the *Candida* species at the palatal mucosa and the denture surfaces before and after using the cleaning agents.[20] The effect of each cleaning agent on the adhesion of the yeast cells to the denture acrylic was analyzed statistically with a Wilcoxon signed rank test.

RESULTS

The prevalence of *Candida* species on the surfaces of the dentures and palatal mucosa before using the cleaning agents and the effect of these agents on this colonization are documented in Table 1. The *Candida* species were isolated from the palatal mucosa of 28 patients (62.2%), and from the denture surfaces of 37 patients (82.2%). The *Candida* species were isolated both from the palatal mucosa and denture surfaces of 16 patients (36.3%). The detection of more than 10 colonies of *Candida* species on the agar plate was evaluated as a "positive" culture result. The distribution of the 28 *Candida* species isolated from the palatal mucosa were as follows; 25 *Candida* albicans, 2 *Candida* glabrata, 1 *C. albicans* plus *C. glabrata*. The distribution of the 37 *Candida* species isolated from the denture surfaces were as follows: 35 *C. albicans*, 1 *C. tropicalis*, 1 *C. glabrata*.

It was found that 62.2% of the patients had *Candida* spp colonies on their palatal mucosa, which was reduced to 51.1% after using the cleaning agents. The cleaning agents reduced the colonization rate of *Candida* spp on their dentures from 82.2% to 68.8%. The number of the yeast colonies was reduced in nine (60%) of the patients using Klorhex, while they were reduced in seven (46.6%) patients using Fittydent. On the other hand, the colonization rates were unchanged in the control group.

Figs. 1 and 2 show a boxplot of the colonization value of the *Candida* species on palatal mucosa and corresponding denture surface before and after using the cleaning agents. According to the

Table 1. Growth of the Different Yeast Species Cultured from the Palatal Mucosa and the Corresponding Denture Surfaces before and after Using the Cleaning Agents

	Before				After			
	Palate No. of carriers (%)		Denture No. of carriers (%)		Palate No. of carriers (%)		Denture No. of carriers (%)	
	Test group (n = 30)	Control group (n = 15)	Test group (n = 30)	Control group (n = 15)	Test group (n = 30)	Control group (n = 15)	Test group (n = 30)	Control group (n = 15)
C. albicans	18 (40)	8 (17.8)	25 (55.5)	10 (22.2)	9 (20)	11 (24.4)	16 (35.5)	11 (24.4)
C. tropicalis	-	-	-	1 (2.2)	-	-	-	1 (2.2)
C. glabrata	2 (4.4)	-	-	1 (2.2)	3 (6.6)	-	1 (2.2)	1 (2.2)
C. krusei	-	-	-	-	-	-	1 (2.2)	-
No growth	10 (22.2)	7 (15.5)	5 (11.1)	3 (6.6)	18 (40)	4 (8.9)	12 (26.7)	3 (6.6)

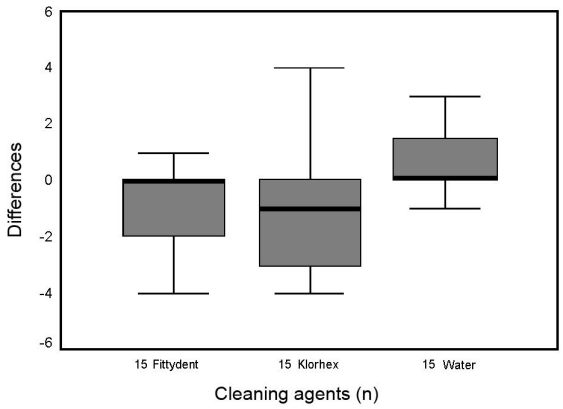

Fig. 1. Boxplot of the colonization value of the Candida species on the palatal mucosa before and after using the cleaning agents. According to the Non parametric Friedman two-way analysis of variance; Water (i), Klorhex (j); $|R_i-R_j|=12$; critical value > 10.641; significance. There was no significant difference between the water and Fittydent; Fittydent and Klorhex.

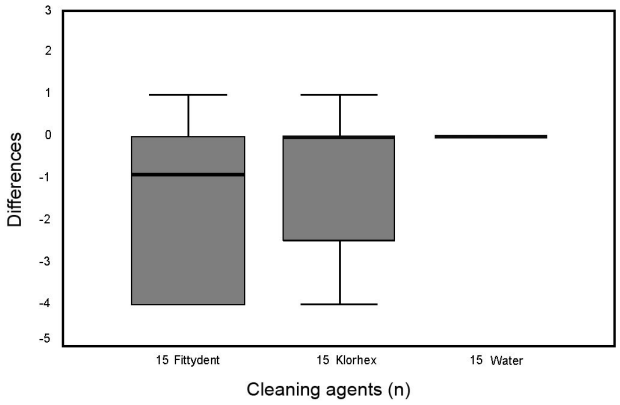

Fig. 2. Boxplot of the colonization value of Candida species on the denture surfaces before and after using the cleaning agents. According to the Non parametric Friedman two-way analysis of variance; Water (i), Fittydent (j); $|R_i-R_j|=10.95$; critical value > 10.730; significance. There was no significant difference between water and Klorhex; Klorhex and Fittydent.

Friedman test results, significant differences in the colonization of *Candida* species were found between the water and Klorhex on the palatal mucosa as well as between water and Fittydent for the denture surface (Figs. 1 and 2).

The adhesion ability of the *Candida* strains were evaluated using *Candida* spp isolated from the denture surfaces. The value of the adhesion of *Candida* species is shown in Table 2. After soaking the dentures in Fittydent overnight, the adhesion scores changed for all of the strains tested. No adhesive cells were observed after applying the Fittydent on five of the acrylic strips. The mean adhesion value of the *Candida* strains isolated from the denture surfaces were calculated as 15 cells/strip after applying the Fittydent, while it was 75 cells/strip before application. The mean adhesion value of *Candida* strains was 37.5 cells/strip after applying the Klorhex. According to the Wilcoxon signed rank test results, the adherence of the *Candida* species in the Klorhex and Fittydent groups was similar (Table 3).

Table 2. Selected Candida Strains and Their Adherence Values to the Acrylic Strips

Species	Adhesion scores		
	Before	After fittydent	After klorhex
C. albicans	++	NA	+
C. albicans	++++	++	++
C. albicans	+++	NA	++
C. albicans	++	+	+
C. albicans	++++	+	++
C. albicans	++	NA	+
C. albicans	++	NA	+
C. albicans	++++	NA	+
C. albicans	++++	+	++
C. glabrata	+++	+	++

NA, no adhesion; +, 1 - 25 cells; ++, 25 - 50 cells; +++, 50 - 75 cells; ++++, 75 - 100 cells.

Table 3. Mean Adhesion Values of the Selected Candida Strains before and after Using Klorhex and Fittydent and the Results of the Wilcoxon Signed Rank Test

Mean adhesion values		Wilcoxon signed rank test	
K_1	75 cells/strip	K_1 - K_2	
K_2	37.5 cells/strip	-2.842a	
		0.004	$(K_1 - K_2) - (F_1 - F_2)$
			-1.897[a]
F_1	75 cells/strip	F_1 - F_2	0.058
F_2	15 cells/strip	-2.848[a]	
		0.004	

[a]Based on negative ranges.

*Significant at level $\alpha = 0.05$.

Mean adherence values of selected *Candida* strains isolated from denture surfaces before using Klorhex, K_1; after using Klorhex, K_2. Mean adherence values of selected *Candida* strains isolated from denture surfaces before using Fittydent, F_1; after using Fittydent, F_2.

DISCUSSION

The prevalence of oral candidal carriage is varies widely in different populations. *Candida* species have been isolated from the oral cavity in 24 - 60% of the general population.[2,21-25] Yeast colonization of the mucosa, gingiva, tongue-buccal mucosa, the surface of orthodontic brackets and dentures has been investigated in different populations in Turkey.[26-29] It is well known that a significant proportion of elderly denture wearers suffer from *Candida*-associated denture stomatitis.[18,30,31] The colonization of *Candida* species on the oral mucosa and the surfaces of the denture materials may be the first step of an infection.[3,21] This study found 62.2% of the patients were colonized with *Candida* species on their palatal mucosa, while 82.2% of them were colonized on their denture surfaces. Both the palatal mucosa and denture surfaces were colonized in 36.3% of the patients.

The results in all these previous reports correlate with the results in the present study. Therefore, the importance of the oral carriage of the

yeasts needs to be discussed in all populations. In this study, *Candida* albicans was the most common species isolated from the oral cavity (Table 1). *C. tropicalis, C. galabrata, C. krusei* were also isolated, as reported in previous studies.[2,18,23-25,30]

Mechanical methods and some chemical agents have been used to eliminate denture plaque.[7,32] Chlorhexidine is one of these chemical agents with a powerful antimicrobial effect against most oral bacteria, and can be used on prescription both as a mouthrinse and denture soak.[33,34] Fittydent is another chemical that is used as a cleaning agent for prosthesis.[9]

The results of this study showed that both Klorhex and Fittydent have a certain preventive effect on the colonization rate of *Candida* species on the surface of the dentures and on the palatal mucosa. *Candida* spp were isolated from the palatal mucosa as 51.1% after using these cleaning agents. The number of colonies on the surface of dentures was also affected, and was reduced by up to 68.8% after using both with Klorhex or Fittydent. In contrast, the colonization rates were unchanged in the control group. A statistically significant difference was found between the water and Klorhex application on the surface of palatal mucosa, and the water and Fittydent application on the surface of dentures (Figs. 1 and 2).

In vivo and *in vitro* studies have shown clorhexidine to be effective against fungi.[13,35] In a clinical study, a denture cleaner showed a 51% reduction in plaque levels at day 2 and 42% at day 14 when compared with water.[7] Dills et al.[32] reported that brushing was not as effective as effervescent soaking. Kulak et al.[9] showed that sodium hypochloride and Savlon are significantly more effective in the decontamination of denture surfaces than Fittydent or other cleaners .

In this study Klorhex and Fittydent were also tested for their ability to inhibit candidal adhesion to acrylic surfaces *in vitro*. These results indicate that the exposure of denture acrylic to Klorhex and Fittydent effectively inhibited the adherence of all ten *C. albicans* strains to acrylic *in vitro*. However, of these two agents, Fittydent appears to be more effective in modulating the adhesion to denture acrylic, as it significantly inhibited the adhesion of almost all the *Candida* isolates. Nonetheless, although not statistically significant, both agents induced a substantial inhibition of the adhesion of the isolates.

The adhesion of *Candida* spp to the epithelial cells and to the acrylic surfaces has been examined in different studies. Panagoda et al.[36] used an image analysis system (IBAS 2000; Kontron, Berlin, Germany). This semi-automated system allows the rapid quantification of adherent yeasts by scanning only the surface area occupied with adherent cells.[37] Waters et al.[38] used another *in vitro* model to compare the adherence of several *C. albicans* strains to denture base materials and experimental soft lining materials. They used acridine orange to examine the adhesion of the cells microscopically. This method is similar to one used in this study, as it requires a fluorescent microscope, and is more suitable, cheaper and practical for scanning.

No additional factors such as galactose, sucrose were used in this adhesion assay. Saliva or serum was not used as augmentative factors for the adhesion ability of the yeast cells. The solution used in the adhesion assay consisted of phosphate buffered saline only. A simple environment was preferred because the aim was to evaluate this virulence factor independently from the host factors. Moreover, several studies have been performed to examine the adhesion of *C. albicans* on acrylic surfaces or epithelial cells in the presence of bacteria, saliva and serum or carbohydrates.[5,6,14,39,40]

The effect of antiseptic solutions and denture cleaners on the adhesion of yeast cells to acrylic surfaces has been investigated in previous studies.[6,19] Mc Courtie et al.[14] reported that a treatment of acrylic with chlorhexidine reduced the adherence by 19 - 86%. In another study, the same researchers found that adherence was reduced to 20% after a pretreatment with chlorhexidine.[41] In this study, the adhesion decreased from an average of 75 cells/strip to 37.5 cells/strip after Klorhex and 15 cells/strip after Fittydent.

These results suggest that the application of Klorhex and Fittydent modulates the candidal adherence to denture acrylic *in vitro*, and these cleaning agents affect the colonization rate of *C. albicans* on the surfaces of dentures and palatal mucosa *in vivo*.

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