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ORIGINAL ARTICLE

Effects of different denture cleaning methods to remove *Candida albicans* from acrylic resin denture based material

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Abstract *Background/purpose:* Different denture cleaning methods have different effects. The purpose of this study was to evaluate the efficiency of six different denture cleaning methods to remove *Candida albicans* that had accumulated on denture-based materials.
Materials and methods: We generated 140 identical acrylic resin specimens and soaked them in a suspension of *C albicans*. The reduction in microorganism counts after application of the different denture cleaning methods was calculated. Six cleaning methods were evaluated: a mechanical method of brushing with a toothbrush, a chemical method of soaking in a commercial cleansing tablet solution, a combined method of brushing and soaking in a commercial cleansing tablet solution, a chemical method of soaking in a commercial mouthwash solution, irradiation in an ultraviolet (UV)-light e-box, and soaking in distilled water. The effectiveness of the denture-cleaning methods in reducing *C albicans* was evaluated following a single cleaning event.
Results: The denture cleaning techniques had considerably different efficacies in reducing *C albicans*. There was no significant difference among the effectiveness levels of cleaning by brushing, soaking in a commercial cleansing tablet solution, or a combination of both in removing *C albicans*. Similarly, the effectiveness levels of soaking in a commercial mouthwash solution or irradiation in a UV-light e-box were statistically similar.

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Conclusion: Compared to other methods, brushing, soaking in a commercial cleansing tablet solution, or a combination of both methods can significantly reduce the adherence of *C albicans* to denture samples. Compared to soaking in distilled water, soaking in a commercial mouthwash solution or irradiation with UV-light had more significant cleaning effects, but these methods were not as effective as the aforementioned three methods.

Introduction

In Taiwan, approximately 12.6% of people aged 65 years and above are completely edentulous (11.9% in the age range 65—74 years, World Health Organization Index age group).[1] Owing to decreased dexterity with age, the majority of elderly people with dentures fail to keep them clean and generally have a poor sense of oral hygiene. According to a study by Dikbas et al,[2] only 11.9% of patients had clean dentures. According to a study by Kulak-Ozkan,[3] 28.6% of patients reported that they cleaned their dentures once a day, and 45.7% cleaned their dentures more than once a day. Contaminated dentures are either a direct cause or a contributing factor to mucosal diseases such as denture stomatitis. Budtz-Jorgensen and Bertram[4] found a significant correlation between poor denture cleanliness and denture stomatitis, and Kossioni[5] confirmed the high prevalence of denture stomatitis in denture users. Proper care and cleanliness of dentures and mucosal tissues of the edentulous mouth are vital for good health, particularly in the elderly.

Mechanical and chemical methods are typically advised for patients to remove plaque and debris from their dentures. The most frequently used manual method for cleaning dentures is water and a toothbrush.[6] However, toothbrushes are ineffective against microbial activity on denture biofilms and can only remove large debris.[7,8] According to a study by Veres et al,[9] 60—90% of patients with dentures use mechanical cleaning in association with products such as toothpaste, soap, or water. An inappropriate cleaning method involves the use of a toothbrush and toothpaste to remove large particles, which may affect the texture of the denture material and also result in the formation of plaque or the inhibition of plaque removal.[10] By contrast, soaking in disinfectant solutions[11—13] with chemical agents was shown to be an effective procedure to decrease the number of contaminating organisms, although some chemical agents used for denture cleaning are known to damage acrylic resin[14,15] and metal alloy materials. Recent scientific developments indicated that microwaving, ultraviolet C (UVC) light, and ozonated water can be effective in controlling infection. Arita et al[16] suggested that ozonated water may be useful in reducing the number of *Candida albicans* on denture plates. The use of microwave energy to disinfect dentures was suggested to overcome problems associated with chemical disinfection.[17,18] Andersen et al[19] suggested that disinfection with UVC light might notably reduce environmental bacterial contamination, and the product is currently being sold for denture cleaning. Although several techniques were shown to disinfect dentures, no comparative study has been performed to determine the most effective denture cleaning method.[20]

In this study, we applied standardized procedures to test the effectiveness of six different cleaning methods in decreasing the number of *C albicans* on denture-based material. Our null hypothesis was that the colony-forming units (cfu) obtained from the sample surfaces would be the same following cleaning by all six methods.

Materials and methods

Specimen fabrication

We produced 140 identical denture-based acrylic resin denture samples (40 × 12 × 3 mm) using a stainless steel mold. Heat-processed acrylic resin (Lucitone 199, standard powder, original shade, Densply, Pennsylvania, USA) was mixed according to the manufacturer's recommendations and packed into a stainless steel mold. A hydraulic press at 1200 psi was used to pack the denture-based resin; subsequently, the excess resin was removed, and finally, 800 psi was applied for 15 minutes. Samples were then polymerized by a conventional heat method with metal flasks in an automatic polymerization water tank at 70°C for 8 hours, followed by 100°C for 1 hour. All samples were cooled at room temperature overnight. Samples were then deflasked, and excess resin was sequentially removed with a low-speed carbide bur, a green stone, and a big silicone bur. One of the resin surfaces was finished and polished following standard procedures with a rag wheel and fine, wet pumice powder. Finally, all samples were immersed in distilled water at room temperature for over 50 hours[18,21] to eliminate any residual monomers.

Contamination of specimens

C albicans was separated from the clinical environment, cultured in Sabouraud dextrose agar, and incubated at 37°C for 48 hours in an orbital shaker. *C albicans* was identified by conventional methods including a germ tube, chlamydospore formation, and sugar assimilation tests. These methods are commonly used in clinical microbiological laboratories. Moreover, we also used selective CHROMagar *Candida* medium to select and identify the clinically isolated *C albicans*. After incubation, cells were harvested and adjusted to a suspension of 10^7 cfu/mL to be used as the fungal solution.

Before contamination, samples were disinfected in 70% alcohol for 10 min, washed with sterile, distilled water, and then sterilized with ethylene oxide gas. Each sample

was placed in a 10-mL test tube with 6 mL of a *C albicans* suspension ($6-7 \times 10^7$ cfu/mL) and incubated at 180 Hz at 37°C for 2 h. Each sample was removed with sterile forceps and washed with 5 mL of sterile water. Each sample was placed into 1 mL Sabouraud dextrose broth for 10 minutes and then vortexed for 30 seconds. To verify that *C albicans* was present on the samples, specimens of the Sabouraud dextrose broth were streaked on Sabouraud dextrose agar and incubated at 37°C for 24 hours. The numbers of cfu of *C albicans* were calculated on Sabouraud dextrose agar.

Experimental and control groups

Contaminated samples ($n = 20$) were randomly assigned to one of the following cleaning methods.

1. Method A: contaminated samples were transferred to a sterilized basket and then brushed on all faces with a new soft-bristle toothbrush (Colgate Extra Clean, Colgate-Palmolive, Guangzhou, China) and washed with sterile distilled water 30 times.
2. Method B: contaminated samples were transferred to a sterilized basket and immersed in a container with 250 mL of distilled sterilized water and one tablet of a commercial enzymatic denture cleaner (Polident, GlaxoSmithKline, Dublin, Ireland) at 40°C for 15 minutes.
3. Method C: contaminated samples were first cleaned by Method B for 15 minutes and then cleaned by Method A.
4. Method D: contaminated samples were transferred to a sterilized basket and immersed in a container with 250 mL of 0.2% chlorhexidine gluconate (Parmason Shining, Taipei, Taiwan) at room temperature for 15 minutes.
5. Method E: contaminated samples were transferred to a sterilized basket and placed in a commercial UV light e-box (e-box, ADH Health Products, Seoul, Korea) for 10 minutes on each surface.
6. Method F: contaminated samples were transferred to a sterilized basket and immersed in 250 mL of distilled, sterilized water for 15 minutes.
7. Control group: contaminated samples were transferred to a sterilized basket and received no further treatment.

Statistical methods

After the cleaning methods were applied, each sample was flushed with distilled, sterilized water. Numbers of cfu of *C albicans* were calculated, and these data were grouped and analyzed according to the different cleaning methods. As cfu/mL values were highly skewed to the right, the normality assumption did not hold for the hypothesis testing. The nonparametric Kruskal–Wallis test was used to compare differences in cfu/mL. Wilcoxon rank-sum tests were used to conduct *post hoc* comparisons among the different cleaning methods versus the control group with the Bonferroni procedure (type I error rate = 0.05/6 = 0.0083). Values of cfu/mL were further categorized into four groups based on the microorganism counts, and Chi-square tests were used to compare the distributions.

Table 1 Growth of microorganisms on resin specimens after different cleaning methods.

Method	Samples	Mean	SD	P[a]
Method C (brush + Polident)	20	340	387	<0.0001
Method B (Polident)	20	360	391	<0.0001
Method A (brush)	20	870	837	<0.0001
Method D (0.2% CHX)	20	6000	3464	0.0001
Method E (UV light)	20	6750	2826	0.0003
Method F (distilled water)	20	11,850	5019	0.5058
Control group	20	14,100	6889	

[a] *Post hoc* comparisons among the different cleaning types versus the control group. Kruskal–Wallis test, $P < 0.0001$.
CHX = chlorhexidine.

Results

We examined the effects of different cleaning methods on the adherence of *C albicans* to resin plates (Table 1), which showed that the combined method (toothbrush and chemical immersion) was most effective in reducing the growth of *C albicans*, but was not completely aseptic. Means of the different cleaning methods were compared by a nonparametric method, which indicated significant differences among these groups. *Post hoc* comparisons were conducted using Wilcoxon rank-sum tests with the Bonferroni procedure, and results showed significant differences between Methods A, B, C, D, E, and F versus the control group.

Values of cfu/mL were further categorized into four groups, and Chi-square tests were used to compare the distributions (Table 2). The control group had the highest colony count of *C albicans*, and immersion in distilled, sterilized water was ineffective at removing *C albicans* from the denture samples. Among the groups, there was no significant difference among Methods A, B, and C or between Methods D and E, although Methods A, B, and C were overall more effective than Methods D and E at removing *C albicans*. Method C (brushing and chemical immersion) was determined to be the best technique to achieve maximal dental hygiene.

Discussion

In this study, six different denture hygiene methods are presented, which differed in their ability to remove *C albicans* from denture samples, thus supporting a rejection of the null hypothesis for *C albicans* removal.

In Taiwan, 12.6% of adults aged 65 years and above are edentulous. Increased longevity presents a huge challenge for health systems to address the needs of older people. Taking proper care of dentures and mucosal tissues in the edentulous mouth has a positive effect on overall health. Denture plaque may act as a reservoir of potential respiratory pathogens to expedite colonization of the oropharynx in the elderly.[22] A denture biofilm is a dense microbial layer comprising microorganisms and their metabolites. Biofilms formed from *Candida* species are

Table 2 A hygiene method according to an ordinal scale of microbial contamination.[a]

% in each hygiene method	cfu/mL < 1200	1200 ≤ cfu/mL < 8000	8000 ≤ cfu/mL < 15,000	15,000 ≤ cfu/mL
Method C (brush + Polident)	90	10	0	0
Method B (Polident)	95	5	0	0
Method A (brush)	65	35	0	0
Method D (0.2% CHX)	0	70	30	0
Method E (UV light)	0	55	45	0
Method F (distilled water)	0	20	60	20
Control group	0	15	40	45

[a] Chi-square test, $P < 0.0001$.
CHX = chlorhexidine.

a causative factor in denture stomatitis.[23] As such, *in vitro* assays have focused on the development of improved antimicrobial techniques for *C albicans* removal.

Denture cleanliness is essential to prevent malodor, poor esthetics, and the accumulation of plaque/calculus and biofilms. Several denture cleaning methods are clinically used to reduce plaque and biofilms and are generally divided into mechanical and chemical techniques. Mechanical methods include the use of toothbrushes, nail-brushes, magnetic stirrers, agitators, sonic vibrators, and ultrasonic cleansers.[16] Chemical methods include soaking in a household solution (e.g., diluted sodium hypochlorite) or commercial solutions, and exposure to ozonated water or microwave radiation. In this study, we compared the effectiveness of brushing, soaking in commercial solutions, and UV irradiation, which are simple and convenient techniques for removing *C albicans*.

Our data indicated that the combination of brushing and chemical immersion was more effective than chemical and mechanical methods alone for *C albicans* removal from denture samples, which is in contrast to data presented by Paranhos et al,[24] who demonstrated that the effectiveness of mechanical and combined methods was similar and more effective than chemical methods alone. In another clinical study by Paranhos et al,[25] a combination of different methods was used to achieve optimal removal of biofilms from the internal surface of upper complete dentures. Overall, results from all three studies indicate that there was no significant difference between the effectiveness of the chemical, mechanical, and combined methods in reducing *C albicans*.

Ellepola and Samaranayake[26] reported the usefulness of chlorhexidine as an adjunct to conventional antimycotic therapy in managing oral *Candida* infections, although our data indicated that soaking in 0.2% chlorhexidine was ineffective compared to the combined method (brushing and chemical immersion) in reducing the growth of *C albicans*. A study by Sena et al[27] on endodontic-irrigating substances showed that mechanical agitation with 5.25% NaOCl or 2% chlorhexidine improved the antimicrobial properties of the biofilm study model. Vianna et al[28] found that antimicrobial action is related to the type, concentration, and presentation form of the irrigants, as well as the microbial susceptibility. In this study, we soaked samples in 0.2% chlorhexidine; this concentration is lower than what was applied in the above study (2% chlorhexidine), where improved antimicrobial activity was noted. In a study by Theraud et al,[29] the efficacies of five antiseptics, three surface disinfectants, and UV radiation were evaluated against a wide range of clinical and environmental yeast isolates. Only a high concentration of chlorhexidine (0.5%) and hypochlorite exhibited fungicidal activity against yeast biofilms, whereas hydrogen peroxide, 0.25% Ecodiol, and UV treatment were ineffective.

Conclusions

Our results indicate that brushing, soaking in a commercial cleansing tablet solution, or a combination of these methods can significantly reduce the adherence of *C albicans* to denture-based materials. Methods such as soaking in a commercial mouthwash or irradiating with UV light were significantly better than distilled water at cleaning dentures, but were not as effective as the other three methods.

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