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Original Article

In vitro comparison of the effects of microwave irradiation and chemical and mechanical methods on the disinfection of complete dentures contaminated with *Candida albicans*

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ABSTRACT

Background: Dentures can be a source of infection or cross-contamination with microorganisms like *Candida albicans*. The aim of this *in vitro* study was to compare the effects of microwave irradiation, chemical techniques, and a mechanical method (i.e., brushing) on the disinfection of complete dentures contaminated with *C. albicans*.

Materials and Methods: In this experimental study, sixty sterilized dentures were divided into six groups of 10 dentures each. The dentures in Groups 1 and 6 served as negative and positive controls, respectively. The dentures (Groups 2–5) were contaminated with *C. albicans* and subjected to four disinfection procedures: Corega tablets, 2% glutaraldehyde, brushing, and microwave irradiation. Replicate aliquots (25 mL) of the suspension were cultured in Sabouraud dextrose broth. The colonies were counted after 48 h of incubation at 37°C. To confirm long-term disinfection, the Trypticase soy broth (TSB) containers were stored at 37°C for 7 days, and turbidity was visually observed. Data were analyzed with one-way ANOVA and independent-samples *t*-test on SPSS the level of statistical significance was set at 0.05.

Results: The dentures disinfected with microwave irradiation (650 W, 3 min) and glutaraldehyde (2%, 10 min) exhibited no evidence of fungal growth after 48 h of incubation and also no turbidity in the TSB containers after 7 days of incubation. However, the dentures disinfected using the mechanical method and Corega tablets exhibited turbidity after 7 days and fungal growth after 48 h that was significantly more than that in the two other methods ($P = 0.000$) and less than that in the positive control group ($P = 0.000$). The differences between mechanical cleaning and cleansing tablet were not significant ($P = 0.017$).

Conclusion: Base on the results of this study, microwave irradiation (650 W, 3 min) and 2% glutaraldehyde completely disinfected the dentures contaminated with *C. albicans* in the short term and long term.

Key Words: *Candida albicans*, dentures, disinfection, glutaraldehyde, microwave

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INTRODUCTION

Any appliance such as dentures and dental impressions placed in the oral cavity can be a source of infection or cross-contamination[1] with microorganisms such as *Candida albicans*, α-hemolytic streptococci,

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Escherichia coli, *Staphylococcus aureus*,[2] β-hemolytic streptococci, *Klebsiella*, *Pseudomonas*,[3] and *Mycoplasma*.[4]

Several cases of systemic involvement with different fungal species have been reported, including candidal meningitis,[5] respiratory infections, septicemia with *C. albicans*, aspergillosis and mycosis,[6] and penicilliosis[7] that are caused by *Saccharomyces*, *Penicillium*, *Rhodotorula,* and *Aspergillus* fungal species.[8]

In general, the techniques used to clean dentures are divided into three categories: mechanical (e.g., brushing), chemical (e.g., in cream, liquid, or tablet format), and physical.

The denture cleansing tablets are classified into five groups: alkaline peroxide, alkaline hypochlorite, acids, disinfectant agent, and enzyme.

Corega is an alkaline peroxide effervescent tablet. When it is dissolved in water, alkaline hydrogen peroxide is formed by decomposition of sodium perborate. They have a combination of active ingredients.

However, they have a combination of chemical and mechanical effects by active ingredients that attach the organs' constituents and O_2 bubbles to detach debris and stains.

One of the physical techniques is the use of microwave irradiation. In recent years, some attention has been paid to the use of microwaves as an effective and successful disinfection technique with minimum side effects.[9-11] Microwaves do not need a specific reservoir and do not give rise to resistant *C. albicans*.[12]

Webb *et al*. showed that microwaves were more effective than sodium hypochlorite in disinfecting dentures.[13] Moreover, in a study by Banting and Hill, microwave irradiation proved more effective than chlorhexidine in delaying recontamination of denture surfaces with *C. albicans* and infection of soft tissues.[14] Furthermore, Mima *et al*. found that microwaves inactivate *C. albicans*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *S. aureus* in 3 min.[15] Furthermore, Buerger *et al*. reported that disinfection using microwaves had the maximum effect when combined with immersion in water and followed by the use of 1% sodium hypochlorite for 10 min.[16]

Ribeiro *et al*. reported that microwave irradiation at 650 W for 3 min resulted in complete disinfection of dentures and the complete elimination of *C. albicans*, *Staphylococci*, and *Streptococci* from denture surfaces.[17] In addition, a report by Buergers *et al*. revealed that the minimum threshold for the disinfecting effect of microwaves was 60 s.[18]

What triggered the present *in vitro* study is that there is no standardized way of using microwave irradiation[19] and the duration and power of irradiation are a subject of controversy,[20] in addition to the destructive effect of microwave irradiation on acrylic resin. On the other hand, *C. albicans* is regarded as the main cause of denture-related stomatitis.[21] Specifically, this study aimed to evaluate the effect of microwave irradiation for 3 min at 650 W on disinfection of complete dentures contaminated with *C. albicans* and compare it with glutaraldehyde (as gold standard), a cleansing tablet, and brushing (as a conventional technique).

MATERIALS AND METHODS

For the purpose of this experimental study, first, a maxillary denture was fabricated using heat-cured acrylic resin (Mellio Dent, Tehran, Iran) according to the instructions of the manufacturer. Then, 60 identical complete dentures were fabricated following the standardized procedure.[22] All the dentures were sterilized in an autoclave at 121°C under a pressure of 1 atm for 20 min. Subsequently, the sterilized dentures were placed in six groups, with 10 dentures in each group. The dentures in Group 1 were not submitted to any contamination and thus served as negative controls. The rest of the dentures (Groups 2–6) were contaminated with *C. albicans*.

Contamination of dentures

First, the standard strain of *C. albicans*, obtained from the American Type Culture Collection of microorganisms (ATCC: 10231) (23), was cultured for 18–24 h on Sabouraud dextrose agar (SDA) and incubated at 37°C. Then, some *C. albicans* colonies were collected from the SDA culture medium and transferred to Sabouraud dextrose broth (SDB) to adjust the turbidity of the fungal suspension to the 0.5 McFarland standard, which consisted of $1–2 \times 10^8$ CFU/mL of *C. albicans*.

The dentures in Groups 2–6 were then subjected to the contamination process as follows. They were placed in sterile glass containers containing 150 mL of SDB. Then, 1.5 mL of the bacterial suspension

with 0.5 McFarland concentration was added to the glass containers at a concentration of 10^{-1}. Later, the containers were incubated at 37°C for 48 h. At the end of the incubation period, the contaminated dentures were retrieved from the containers and placed on sterile Whatman® filter papers (Merck, Darmstadt, Germany) in sterile plates under a hood to remove their excess moisture.

Disinfection of dentures

The dentures in Groups 2–5 were disinfected using the following techniques:

- Group 2: Use of an alkaline peroxide tablet, Corega® (Stafford Miller, Waterford, Ireland), as a chemical disinfection option. The dentures were placed for 15 min in sterile containers containing 150 mL of distilled water (37°C) and a Corega tablet which had been dissolved according to manufacturer's instructions. Corega antibacterial denture cleansing tablets contain sodium bicarbonate, citric acid, potassium monopersulfate, sodium carbonate peroxide, TAED, sodium benzoate, PEG-180, sodium lauryl sulfoacetate, subtilisin (enzyme), PVP/VA copolymer (film former), and aromatic, and coloring agents (CI 42090, CI 73015, and CI 19140). The sodium carbonate peroxide works through an oxygen-liberating process as active ingredient. H_2O_2 is oxidized to release oxygen, which is related to the observed effervescence and is also supposed to exert a mechanical cleansing effect
- Group 3: Use of 2% glutaraldehyde (2% Behsa Dex, Behsa Pharmaceutical Company, Arak, Iran) as another chemical disinfection alternative. The dentures were placed for 10 min in sterile containers containing 2% glutaraldehyde
- Group 4: Brushing as a mechanical disinfection technique. The dentures were brushed for 5 min in sterile distilled water. A separate toothbrush was used for each denture
- Group 5: Use of microwave irradiation. The dentures were placed in sterile glass containers containing 150 mL of distilled water. These containers were then placed at the center of a microwave oven and exposed to 650-W microwaves for 3 min. To prevent a decrease in the energy output, only one denture was placed in the oven at a time.

It should be reiterated here that the dentures in Group 6 were contaminated and stored in normal saline solution, but they did not undergo any disinfection procedure and were thus used as positive controls.

Colony counting

Colony counting was carried out to determine the number of *C. albicans* on the surfaces of the contaminated dentures in Groups 2–6. To this end, the dentures were placed, using a pair of sterile forceps, in separate sterile glass containers that contained 150 mL of physiological serum. Then, they were vortexed for 1 min to separate microorganisms from the denture surfaces. Vortexing was repeated after an interval of 9 min. Next, 25 mL of the liquid was retrieved from each container and cultured on SDA. These colonies were subsequently subjected to 48 h of incubation at 37°C before they were counted.

To determine the long-term effect of each disinfection option, the dentures were separately placed, following the above-described procedure, in sterile glass containers with 150 mL of SDB and incubated at 37°C for 7 days. At the end of this period, turbidity was evaluated visually.

Statistical analysis of data

The normality of the distribution of data was determined using the Kolmogorov–Smirnov test. Therefore, one-way ANOVA was used to evaluate differences among the groups in terms of the mean colony counts. Independent-samples *t*-test was used for two-by-two comparisons of the groups. For all the tests, the level of statistical significance was set at 0.05. All the statistical analyses were performed using SPSS 21 (IBM Corporation, USA, 2012).

RESULTS

Table 1 presents the results for the colony counts of *C. albicans* in the study groups after 48 h of incubation. It can be seen that the use of 2% glutaraldehyde and microwaves completely disinfected

Table 1: Descriptive statistic for colony number *Candida albicans* after 48 h incubation (CFU/mL)

Group	Mean	SD	Upper bound	Lower bound
Positive control	1.1×10^4	3.9×10^3	1.3×10^4	8.35×10^3
Negative control	0	0	0	0
Mechanical (brushing)	0.8×10^2	0.53×10^2	0.41×10^2	1.18×10^2
Chemical (glutaraldehyde)	0	0	0	0
Microwave	0	0	0	0
Corega tablet	1.3×10^2	0.32×10^2	1.08×10^2	1.55×10^2

SD: Standard deviation

the dentures, and no fungal colonies were detected in the culture media. Statistically, the contamination observed after the use of 2% glutaraldehyde and microwaves were significantly different from that observed in the positive control group ($P = 0.000$).

A mean colony count of 1.3×10^2 CFU/mL was observed in the culture media after the use of Corega tablet. A mean count of 0.8×10^2 CFU/mL was detected in the culture media after the mechanical technique. However, the contamination observed after the use of these two techniques was significantly different from the positive control group ($P = 0.000$). It is also worth noting that the contamination after the use of the mechanical technique and Corega tablets was different insignificantly ($P = 0.017$).

Moreover, the ANOVA results showed significant differences among the study groups in terms of the mean counts of *C. albicans* species ($P = 0.001$).

Evaluation of the turbidity of the culture media in the study groups after 1 week of incubation showed that use of 2% glutaraldehyde and microwaves resulted in no turbidity. Thus, the degree of turbidity observed after the use of these two techniques was fully similar to that detected in the case of the negative control group. An issue that is worth mentioning here is that no significant difference was observed between 2% glutaraldehyde and microwave irradiation in this regard.

The turbidity degree of the culture media in the case of Corega tablets and the mechanical technique was almost similar to that observed in the positive control group.

Finally, for both short- and long-term results, there were no fungal colonies in the culture media in the negative control group, and there was a large number of colonies in the positive control group.

DISCUSSION

In this study, the disinfection effect of different techniques on dentures contaminated with *C. albicans* was evaluated and compared with positive and negative control groups. These techniques were: (1) immersion in a solution containing Corega tablet for 15 min; (2) immersion in glutaraldehyde for 2% in 10 min; (3) brushing for 5 min; and (4) exposure to 650-W microwave irradiation for 3 min.

According to previous studies, immersion of dentures for at least 15 min is necessary for effective reduction of plaque that accumulated during a day;[23-26] however, in relation to glutaraldehyde 10 min of immersion was recommended for complete reduction similar to the gold standard.[27,28]

Since patients are instructed to brush their prosthesis for 2 min, three times after each meal in a day,[24,29,30] in the present study, brushing time was approximately similar to the above time.

The present study showed that microwave irradiation at 650 W for 3 min is highly capable of destroying *C. albicans* on the surfaces of complete dentures and can thus be used as an effective technique for disinfecting dentures and preventing candidal denture stomatitis in patients wearing complete dentures. Indeed, various studies have shown the efficacy of microwave irradiation as a physical method for disinfecting dentures.[31-33] It has also been found that the microwave energy can accelerate structural changes in the cellular walls of microorganisms either thermally[34] or nonthermally.[35]

Like the present study, Silva *et al*.,[33] Neppelenbroek *et al*.[36] and Ahuja *et al*.[37] succeeded in disinfecting dentures through the use of microwave irradiation at 650 W; however, they used microwaves for 6 min. Indeed, a number of studies have shown that the use of 650-W microwave beams for 3 min is adequate for complete disinfection of dentures.[20,38] What is more, it has been found that duration of microwave irradiation can have a detrimental effect on the physical and mechanical properties of acrylic resins.[39-41] This implies that attempts should be made to decrease the duration of radiation to achieve disinfection without such adverse effects.

Another finding of the present study was that microwave irradiation (650 W, 3 min) can inhibit the growth and proliferation of *C. albicans* in the long term. However, microwave irradiation alone cannot remove biofilms of microorganisms from the surface of the denture.[42] Moreover, the fact that the DNA remaining in dead microorganisms can turn into living microorganisms[43] it can cause infection to recur.

It is also important to note that the dentures in the present study were immersed in distilled water during microwave irradiation, a practice which has been found to be a proper technique for eliminating microorganisms.[31,33] More particularly, Dixon *et al*. concluded that materials immersed in water during microwave irradiation are heated in a more homogeneous manner, thereby increasing the

disinfecting effect of microwave irradiation.[31] In addition, Fitzpatrick *et al*. reported that disinfection via microwave irradiation is only possible when the samples are adequately wet due to the fact that water coagulates the principal proteins of microorganisms during the disinfection process.[44] In addition, Silva *et al*.,[33] Neppelenbroek *et al*.,[32] and Dixon *et al*.[31] showed that immersion of acrylic resin samples in water during microwave irradiation helps inactivate microorganisms. Pelczar hypothesized that since sterile distilled water, in which the contaminated samples are immersed, is more hypotonic than the cellular contents of the microorganisms, its osmotic pressure can result in the flow of water into the cells, thus disrupting the cells of microorganisms.[45] In another hypothesis, Jeng *et al*.[46] attributed the disinfecting effect of microwaves to the heat produced by such waves.

The present study also found that although the mechanical technique significantly decreased colony counts after 48 h of incubation, the degree of turbidity detected in the dentures under investigation after 7 days of incubation was not significantly different from that in the positive control group. This finding is consistent with the results reported by Barnabé *et al*.[47]

Another key finding of the present study was that the use of Corega tablets is not an appropriate disinfection technique in the short term and long term, and this concurs with the results reported by Nunes *et al*.[48] and Duyck *et al*.[49] In a study by Aalaei *et al*., too, Corega tablets decreased the colony count, but they did not fully disinfect the dentures.[50] The manufacturer claims this tablet has antibacterial effect not antifungal effect.

Many studies[23,51,52] have evaluated denture cleansing tablets, classifying them into three groups: (1) alkaline peroxide; (2) alkaline peroxide with enzyme; and (3) enzymes. Since their active substrate and mechanism of action is not the same, their findings were different from this study.

Since a large number of microorganisms can contribute to denture stomatitis and risk of oral pathological lesions is high in denture wearers,[53] further research might explore the effect of disinfection methods on microorganisms other than *C. albicans*. However, it is worth noting that due to the differences between *in vivo* and *in vitro* biofilms,[54] *in vivo* conditions cannot be simulated *in vitro*.[55] In addition, due to the existence of DNA molecules in dead microorganisms that can be transferred to live forms[43] when microwave irradiation is used, denture stomatitis is likely to recur in the long term in the oral cavity. Thus, it is advisable to clinically examine microwave irradiation in the long term in an attempt to develop a standard protocol for treating denture stomatitis. Furthermore, it would be a good idea to use different immersion liquids such as disinfection liquids and cleansing tablets to reduce the duration and power of microwave irradiation and for the purpose of reducing damage to the denture.

CONCLUSION

The present study found the chemical technique (use of 2% glutaraldehyde for 10 min) and microwave irradiation (at 650 W for 3 min) to be appropriate options for disinfection and control of *C. albicans* fungal species in dentures both in the short term and the long term. However, since the use of glutaraldehyde is associated with some side effects,[56] microwave irradiation at 650 W for 3 min seems a better technique for removing *C. albicans* from the surface of complete dentures.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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