

Original Article

A comparison of the efficacy of mechanical, chemical, and microwave radiation methods in disinfecting complete dentures

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ABSTRACT

Background: Poor denture hygiene can be a potential source of pathogens. The aim of this study was to compare the efficacy of microwave radiation with that of chemical and mechanical techniques in disinfecting complete dentures contaminated with *Staphylococcus aureus* and *Pseudomonas aeruginosa*. **Materials and Methods:** Seventy-two sterilized mandibular dentures were separately contaminated with *S. aureus* ($n = 32$) and *P. aeruginosa* ($n = 32$) and then incubated at 37°C for 48 h. The contaminated dentures were disinfected as follows: chemical disinfection with Corega tablets; chemical disinfection with 2% glutaraldehyde; mechanical disinfection by brushing the denture; and physical disinfection by 650-W microwaves irradiation for 3 min with six samples in each subgroup. Six dentures served as negative control group, and six contaminated dentures with no disinfection served as the positive control group. 10^{-3} – 10^{-6} dilutions were cultured in the nutrient agar, and the colonies were counted after incubation at 37°C for 48 h. To evaluate the lasting time of disinfection, the containers with nutrient agar and dentures were stored for 7 days at 37°C to evaluate turbidity. Data were analyzed using Kruskal–Wallis and Mann–Whitney U-test ($\alpha = 0.05$).

Results: There was no evidence of bacterial growth in 48 h and turbidity after 7 days of incubation of dentures disinfected by microwaves, glutaraldehyde, and Corega tablets, which was statistically significant compared to the positive controls ($P < 0.001$). In mechanically disinfected dentures (brushing), bacterial growth was detected after 48 h which was statistically significant compared to the positive controls ($P < 0.001$) and turbidity was seen in all the nutrient agar plates.

Conclusion: Microwave irradiation, 2% glutaraldehyde, and Corega tablets disinfected complete dentures contaminated with *S. aureus* and *P. aeruginosa* which lasted for a long and a short terms.

Key Words: Glutaraldehyde, microwaves, *Pseudomonas aeruginosa*, *Staphylococcus aureus*

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INTRODUCTION

Plaque and poor denture hygiene result in denture stomatitis.^[1,2] Furthermore, dentures can be a potential source of pathogens leading to halitosis, bacterial endocarditis, pneumonia, gastrointestinal infections, and chronic pulmonary obstruction.^[3]

Different techniques are used to disinfect dentures. These are generally divided into chemical, mechanical, and physical methods. At present, a wide range of chemical denture disinfectants are available, including glutaraldehyde,^[4] chlorhexidine,^[5,6] and

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sodium perborate.^[7] Chemical disinfectants exert the minimum scratch on denture surfaces so as not to increase plaque accumulation and staining.^[8] An easy chemical technique to clean dentures is the use of denture-cleansing tablets, which comprise different constituents, and their mechanism of action depends on the release of oxygen.^[9]

Mechanical techniques include the use of ultrasound and brushing. The ultrasonic methods are usually supplemented with the use of a chemical solution. Different studies have yielded varying results in relation to the efficacy of the ultrasonic technique depending on whether chemical agents be used as supplements or not.^[9-11]

In the brushing technique, a toothbrush is used together with some toothpaste to clean the dentures. This technique is widely used due to its ease and low cost.^[12] However, various studies have shown that cleaning dentures using a mechanical technique, especially in patients with motion problems and muscular deficiencies, is not as effective as chemical techniques.^[9,12,13] There is the risk of abrading the acrylic resin and damaging the surface of relining materials.

The physical technique includes microwave irradiation.^[14,15] Microwave irradiation is one of the techniques used to disinfect the dentures of patients with candidiasis.^[16,17] Dovigo *et al.*^[15] showed the disinfecting effect of microwaves on complete dentures contaminated with *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* bacterial species, *in vitro*. Ribeiro *et al.*^[18] reported that microwave irradiation for 3 min can prevent cross-contamination.

Cruz *et al.*^[9] reported that brushing alone cannot sufficiently maintain the hygiene of dentures and that other adjunctive methods are necessary to achieve better results. In that study, the use of peroxide solutions, ultrasound technique, and a combination of these two techniques yielded similar results.

Sesma *et al.*^[19] showed that microwave plus brushing (MW + B) and a combination of microwave irradiation with denture cleanser and brushing (MW + DC + B) were effective in decreasing microorganism counts, with no significant differences ($P = 0.553$); however, it was found that only the MW + DC + B technique can completely eliminate microorganisms from denture surfaces.^[19]

Mojarrad *et al.*^[20] showed that the use of both 2% glutaraldehyde for 10 min and microwave irradiation at an output power of 650 W for 3 min completely removed *Candida* colonies from denture surfaces; however, brushing and denture-cleansing tablets did not fully remove the *Candida* colonies.

Access to microwave equipment is easy in the majority of homes, and the elderly have difficulty observing denture hygiene. Since *Candida* is the main cause of denture stomatitis,^[21,22] most studies have focused on the effect of microwave irradiation on this microorganism.^[16,17,20,24-29] However, the role of *S. aureus* and *P. aeruginosa* has also been documented.^[30] The purpose of the present study was to compare the efficacy of microwave irradiation with that of conventional mechanical and chemical techniques in disinfecting complete dentures contaminated with *S. aureus* and *P. aeruginosa*. The null hypothesis was that the mechanical technique (brushing), microwave irradiation, and chemical technique (2% glutaraldehyde and Corega cleansing tablets) were not different in terms of their disinfecting effect on *S. aureus* and *P. aeruginosa*.

MATERIALS AND METHODS

Specimens and sterilization

Seventy-two complete mandibular dentures were fabricated using the standard method^[12] for the purpose of this experimental *in vitro* study. The samples were sterilized in an autoclave at 121°C and under a pressure of 1 atm for 20 min.

Half of the dentures were used to study *S. aureus*, and the other half to examine *P. aeruginosa*. For each bacterial species, the dentures were placed in six groups of six dentures each: four experimental groups (corresponding to the four cleansing techniques under investigation), one negative group ($n = 6$), and one positive control group ($n = 6$).

Contamination of the specimens

First, the standard strains of *S. aureus* (ATCC = 25,929) and *P. aeruginosa* (ATCC = 27,853) were cultured on nutrient agar plates and incubated for 24 h. Then, a suspension was prepared from the newly prepared bacterial species at 0.5 McFarland concentration.

To contaminate the dentures (except in the negative control group), 1.5 mL of the prepared suspension was added to the glass containers that contained the dentures in the nutrient broth medium (Becton,

Dickinson and Company, New Jersey, USA). This was followed by incubation at 37°C for 48 h. Subsequently, the dentures were retrieved from the glass containers in the vicinity of a flame and were placed in sterile plates that contained sterile Whatman® filter papers (Merck, Darmstadt, Germany) under a hood to remove their excess moisture.

Experimental and control groups

The specimens contaminated with each bacterial species were randomly assigned to the disinfection methods as follows:

- Mechanical technique: All the denture surfaces in this group were brushed with a soft toothbrush for 5 min using sterile distilled water. A separate toothbrush was used for each denture. Then, the dentures were separately placed in a container with distilled water for 5 min
- Chemical technique A: The dentures in this group were immersed in 2% glutaraldehyde (2% Behsadex, Behsa Pharmaceutical Company, Arak, Iran) in sterile containers for 10 min
- Chemical technique B: Corega® tablets (Stafford Miller, Waterford, Ireland) were used to sterilize the dentures in this group. The dentures were immersed in 200 mL of distilled water (37°C), containing one denture-cleansing tablet in association with sodium bicarbonate, for 15 min according to the manufacturer's instructions
- Physical technique: After the dentures in this group were dried, they were placed in glass containers with 150 mL of sterile distilled water. These containers were then put in microwave equipment and were exposed to 650-W microwave irradiation for 3 min. To prevent a decrease in microwave energy, only one sample was placed in the equipment at a time
- Negative control: The purpose of this group was to determine the sterilization of specimens and the accuracy of tests. For each microorganism, six sterilized specimens were placed in a container with sterilized water
- Positive control: For each microorganism, six contaminated specimens were put in a container with sterilized water and did not undergo any disinfection procedure.

Microbial counting

All samples were transferred into sterile glass containers containing 150 mL of sterile physiological serum after they were dried and vortexed for 1 min. After a rest interval of 9 min, the procedure was

repeated to detach microorganisms from denture surfaces.

At the next step, 25 µL of the physiological serum was retrieved from the glass containers and was cultured in the agar medium. After 48 h of incubation at 37°C, the colonies were counted.

Long-term evaluation

After the above procedures, the dentures were separately retrieved with sterile forceps, were placed in sterile glass containers with 150 mL of Sabouraud dextrose broth (Becton), and were incubated at 37°C for 7 days. At the end of this period, the turbidity of the glass containers was evaluated visually.

Analysis of data

Kruskal–Wallis was used to evaluate the differences in colony counts between the studied groups. Mann–Whitney U-test as the *post hoc* test was used for two-by-two comparisons. SPSS 21 (IBM Corporation, USA, 2012) and Microsoft Excel were used for statistical analysis. The statistical significance was set at $P < 0.05$.

RESULTS

There were significant differences between colony counts of the studied groups ($P < 0.001$). The use of glutaraldehyde, denture-cleansing tablets, and microwaves resulted in the complete disinfection of the dentures, and no colonies of the two bacterial species were detected in the culture media [Table 1].

However, the mechanical technique of brushing was unable to completely disinfect the dentures, resulting in *S. aureus* and *P. aeruginosa* mean colony counts of 1.96×10^3 and 1.56×10^3 colony-forming unit (CFU)/mL in the culture media, respectively [Table 1]. The bacterial contamination after this technique was less than that in the positive control group significantly ($P < 0.001$).

As for the long-term effect of disinfection, when the chemical, Corega tablet, and microwave techniques were used, no turbidity was observed in the culture media containing *S. aureus* and *P. aeruginosa*, indicating that these methods completely disinfected the dentures in the long run. However, with the mechanical technique, culture media turbidity was almost similar to that in the positive control group. Finally, there was no turbidity and no bacterial colonies in the culture media of the negative control group, and there was a high rate of turbidity and a

Table 1: Mean±standard deviation (95% confidence interval) of *Pseudomonas aeruginosa* and *Staphylococcus aureus* colony counts after 48 h incubation (CFU/mL)

| Microorganisms | Studied groups | | | | | |
|-------------------------------|--|------------------|---|----------------|-----------|---------------|
| | Positive control | Negative control | Brushing | Glutaraldehyde | Microwave | Corega tablet |
| <i>Pseudomonas aeruginosa</i> | 1.36×10 ⁶ ±1.42×10 ⁵ | 0 | 1.56×10 ³ ±3.1×10 ² | 0 | 0 | 0 |
| | 1.21×10 ⁶ –1.50×10 ⁶ | - | 1.23×10 ³ , 1.89×10 ³ | - | - | - |
| <i>Staphylococcus aureus</i> | 9.76×10 ⁶ ±1.5×10 ⁵ | 0 | 1.96×10 ³ ±2.5×10 ² | 0 | 0 | 0 |
| | 8.18×10 ⁵ –11.3×10 ⁵ | - | 1.69×10 ³ –2.22×10 ³ | - | - | - |

large number of colonies in the positive control group.

DISCUSSION

Various studies have shown the efficacy of microwave irradiation.^[16,17,20,24-29] However, there is no standard way of using microwaves,^[31] and there is much controversy regarding the duration and power of its irradiation^[26,31] as a physical technique in disinfecting complete dentures. The results of the present study showed that microwave irradiation at a power of 650 W for 3 min has a high efficacy to eliminate *S. aureus* and *P. aeruginosa* bacterial species from the surfaces of complete dentures, and this technique can be used as an effective method for disinfecting dentures and preventing the transmission of infection in denture-wearing patients.

In this study, *S. aureus* (ATCC = 25923) and *P. aeruginosa* (ATCC = 27853) were used because they are considered featured pathogenic microorganisms.^[32]

The efficacy of microwave irradiation is consistent with the previous studies on complete dentures^[17,18,28] and acrylic resin samples.^[23,24,33] However, considering the greater surface area of complete dentures than that of acrylic resin samples, and given the fact that the number of microbial colonies on the surface of acrylic resin samples is proportional to the overall surface area, the present study used complete dentures instead of acrylic resin samples.

Moreover, previous research has shown that microwave irradiation at an output power of 650 W for 6 min can have detrimental effects on the physical and mechanical properties of acrylic resin samples.^[34-38] Therefore, the duration of irradiation should be lowered to achieve disinfection without negative effects on acrylic resin.^[39]

Fitzpatrick *et al.*^[40] reported that sterilization with microwaves is possible only when the samples are adequately wet because water act as a medium

for the coagulation of the principal proteins of microorganisms during sterilization. In addition, studies^[23,24,28] showed that immersion of contaminated acrylic resin samples in water during microwave irradiation helps inactivate microorganisms.

Pelczar *et al.*^[41] reported that since distilled water, in which the contaminated samples are immersed, is hypotonic relative to the cellular contents of microorganisms, the osmotic pressure of water can result in the flow of water into the cells and disruption of microorganism.

Several studies have shown the destructive effect of microwave irradiation on microorganisms; however, the exact mechanism of action of microwaves has not been elucidated. Fitzpatrick *et al.*^[40] and Jeng *et al.*^[42] have attributed the destructive effect of microwave irradiation to the thermal effect of microwaves. However, it has been reported that microwave irradiation affects the metabolic activity of *S. aureus* in a manner that cannot be explained by only thermal effects, indicating that nonthermal effects might be involved as well.^[42] A simple explanation for the nonthermal effect of microwave irradiation might be the selective absorption of microwaves by biological molecules such as nucleotides, proteins, and lipopolysaccharide-binding proteins of cell walls.^[15]

Barnabé *et al.*^[43] studied the effect of three different disinfection techniques (i.e., mechanical and chemical techniques and a combination of them) on *Escherichia coli*, *S. aureus*, *Enterococcus faecalis*, *Candida albicans*, *Streptococcus mutans*, and *P. aeruginosa*. It was concluded that these techniques yield different results depending on the type of the microbial biofilms on acrylic resin samples. In addition, Polyzois *et al.*^[44] showed that immersing dentures in 2% glutaraldehyde for up to 12 h had no effect on the flexural strength of dentures. However, they found denture hardness was completely affected. Moreover, their study showed that the extent of changes in the flexural strength and hardness of dentures after being

placed in a microwave set was so mild that it was clinically acceptable and was similar to the changes in the positive controls immersed only in water.^[44] It was not found such a similarity in color stability^[45] and surface roughness.^[46]

In the present study, the use of Corega tablets resulted in the elimination of *S. aureus* and *P. aeruginosa*. In a study by Aalaei et al.,^[47] Corega tablets reduced bacterial infection more significantly than water and saline solution, which had equal effects on reducing CFU counts.^[47] Nonetheless, it should be noted that the quantity and type of microorganisms vary *in vivo* and *in vitro* settings. There is the possibility of recurrent denture-related stomatitis because the biofilm of microorganisms continues to exist after microwave irradiation. Hence, it is suggested that a longitudinal clinical study is carried out for the purpose of developing a standard protocol for the treatment of denture-related stomatitis. Another recommendation is to use different immersion liquids such as disinfection liquids together with cleansing tablets to lower the duration and power of microwave irradiation and reduce the damage to the denture.

CONCLUSION

The use of 2% glutaraldehyde for 10 min, 650 W microwave irradiation for 3 min, and denture-cleansing tablets were effective in removing *S. aureus* and *P. aeruginosa* from complete dentures, with long-term and stable disinfecting effects, but the mechanical technique was ineffective in this regard.

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Conflicts of interest

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

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