Effectiveness of Microwave Irradiation on the Disinfection of Complete Dentures

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Purpose: The purpose of this study was to evaluate the effectiveness of microwave irradiation on the disinfection of simulated complete dentures. ***Materials and Methods:*** Eighty dentures were fabricated in a standardized procedure and subjected to ethylene oxide sterilization. The dentures were individually inoculated (10^7 cfu/mL) with tryptic soy broth (TSB) media containing one of the tested microorganisms (*Candida albicans*, *Streptoccus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*). After 48 hours of incubation at 37°C, 40 dentures were individually immersed in 200 mL of water and submitted to microwave irradiation at 650 W for 6 minutes. Forty nonirradiated dentures were used as positive controls. Replicate aliquots (25 μL) of suspensions were plated at dilutions of 10^{-3} to 10^{-6} on plates of selective media appropriate for each organism. All plates were incubated at 37°C for 48 hours. TSB beakers with the microwaved dentures were incubated at 37°C for 7 more days. After incubation, the number of colony-forming units was counted and the data were statistically analyzed by Kruskal-Wallis test ($\alpha = .05$). ***Results:*** No evidence of growth was observed at 48 hours for *S aureus*, *B subtilis*, and *C albicans*. Dentures contaminated with *P aeruginosa* showed small growth on 2 plates. After 7 days incubation at 37°C, no growth was visible in the TSB beakers of *S aureus* and *C albicans*. Turbidity was observed in 3 broth beakers, 2 from *P aeruginosa* and 1 from *B subtilis*. ***Conclusion:*** Microwave irradiation for 6 minutes at 650 W produced sterilization of complete dentures contaminated with *S aureus* and *C albicans* and disinfection of those contaminated with *P aeruginosa* and *B subtilis*. *(Int J Prosthodont 2006;19:288–293)*

Removable prostheses may be potential sources of infection,[1–6] which has led to renewed interest in sterilization and disinfection of dentures. Sterilization is the process by which all forms of microorganisms, including viruses, bacteria, fungi, and spores, are destroyed. Disinfection is the destruction of most but not necessarily all microorganisms; particularly the highly resistant microbial spores may survive.[7] Performing a sterilization or disinfection of any dental prosthesis before it is transferred to a dental laboratory and immediately before it is returned to the patient provides a measure of infection control for all parties.[8,9]

Several studies have focused on the materials and methods necessary to ensure proper disinfection control of dental prostheses.[8–11] When selecting a disinfection procedure, the effect of the disinfectant on the denture must be carefully considered. Although chemical disinfection has been largely recommended,[9–11] it is possible that certain components of the disinfectant solutions may penetrate the material and not be completely eliminated by rinsing. Consequently, these

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components may be unintentionally introduced to the oral cavity. In addition, the use of disinfectants has been considered time consuming or inappropriate.[8,11] It also has been demonstrated that the hardness,[12,13] flexural strength,[14] and color stability[15] of denture base resins can be significantly affected by disinfectant solutions such as glutaraldehyde, chlorhexidine, phenolic-based, alcohol-based, and hypochlorite disinfectants. Furthermore, it is well documented that some of these disinfectant solutions can promote tarnish and corrosion of metal denture components and bleaching of acrylic resin.[11,16]

Recently, the use of microwave energy to disinfect dentures has been suggested to overcome the problems associated with chemical disinfection.[17–19] Microwave irradiation may be used for decontamination of food,[20] microbiologic laboratory materials,[21] dental instruments,[22,23] nitrous oxide nasal hoods,[24] contact lenses,[25,26] household sponges,[27] clinical waste,[28] material used in clinical laboratories,[29] and *Candida*-contaminated underwear.[30] However, little information is available concerning the efficacy of microwave irradiation on the disinfection of denture base materials.[17–19] An earlier study[31] investigating the effectiveness of microwave sterilization demonstrated that acrylic resin specimens contaminated with individual suspension of 3 bacteria (*Pseudomonas aeruginosa*, *Streptococcus aureus*, and *Bacillus subtilis*) and 1 fungus (*C albicans*) showed sterilization of all microorganisms after 6 minutes of microwave irradiation at 650 W. Nevertheless, the microwave exposure was performed on specimens with small dimensions (10 mm in length and 1 mm in thickness), that had been processed against acetate sheet and glass slab. This procedure resulted in a mirror-like finish of the specimens, which is less likely to facilitate microbial retention than a surface with a higher roughness such as the tissue surface of a denture base and any unpolished areas, where the irregularities of the acrylic resin are more pronounced.[32,33] Therefore, a more clinically relevant in vitro approach is necessary to predicting the effectiveness of microwave disinfection.

Thus, this study tested the hypothesis that simulated complete dentures contaminated with 3 bacteria (*P aeruginosa*, *S aureus*, and *B subtilis*) and 1 fungus (*C albicans*) could be sterilized by microwave irradiation.

Materials and Methods

Simulated Denture Base Production

A stainless steel master die simulating an edentulous maxilla was duplicated via a high-viscosity silicone mold (RTV 3120, Daltomare) to produce 80 dental stone casts (Herodent, Vigodent). On 1 prepared cast, a simulated maxillary complete denture base was waxed and acrylic resin denture teeth were arranged accordingly. This waxed-up denture was duplicated using the high-viscosity silicone and 80 identical simulated maxillary dentures were produced. This was accomplished by placing the acrylic artificial teeth (Dental Vip) in the silicone mold, pouring the melted wax, and fully seating a duplicate cast in the mold. After bench cooling at room temperature for 30 minutes, the wax-simulated dentures were removed from the silicone mold and conventionally invested in metal dental flasks (Jon 5.5, Jon Produtos Odontológicos) with dental stone. After the stone was set, the flasks were placed in boiling water to soften the baseplate wax. The flasks were separated, the wax was removed, and the stone and teeth were cleaned with boiling water and liquid detergent (ODD, Bombril-Cirio). Two coats of sodium alginate (Isolak; Clássico Dental Products) were used as a mold separator. Poly(methyl methacrylate) denture base resin (Lucitone 550, Dentsply International) was prepared according to the manufacturer's directions by mixing 21 g polymer powder with 10 mL monomer liquid. The denture base resin at dough stage was packed into the molds and the flasks closed under pressure using a hydraulic press (Dental Vip). The flasks were placed in an automatic polymerization tank (P-100, Termotron Equipamentos) at 73ºC for 90 minutes followed by 30 minutes at 100ºC boiling water. After polymerization, the flasks were bench cooled for 30 minutes and placed in running tap water for 15 minutes. The flasks were opened and the dentures recovered carefully. The dentures were trimmed using metal burs (Maxi-Cut; Dentsply-Maillefer) and finished with a handheld micromotor (Kavo, Biberach/Riss) using 360, 400-, 600-, and 1,200-grit abrasive papers (Norton, Saint-Gobain Abrasivos). Finally, the dentures were polished on a wet rag wheel with slurry of coarse pumice followed by tin oxide. After polishing, all dentures were individually stored in a 200-mL beaker of distilled water at 37 $\pm$ 1ºC for 48 $\pm$ 2 hours.[34]

Sterilization of Dentures

After 48 $\pm$ 2 hours of storage in water, all dentures were sterilized with ethylene oxide (ACECIL, Comércio e Esterilização a Óxido de Etileno). To confirm the effectiveness of this procedure, 2 additional dentures were tested as negative controls. Fifteen days after sterilization,[10] dentures were added individually to 200 mL of tryptic soy broth (TSB) (Acumedia Manufacturers) in a 600-mL sterile beaker, which was sealed with foil. The beakers were then incubated at 37ºC for 7 days. At 48 hours and 7 days, the broths were evaluated for microbial growth (turbidity). No turbidity in the broth beakers was observed at 48 hours and 7 days.

Table 1 Mean Colony-Forming Units Per Milliliter (cfu/mL) for Each Microorganism

Microorganism	Positive control dentures	Microwaved dentures
P aeruginosa	2.30×10^9	15*
S aureus	5.07×10^9	0
C albicans	2.56×10^7	0
B subtilis	5.89×10^9	0*

*Turbidity was observed on broth beakers after 7 days of incubation.

Contamination and Microwave Disinfection Procedures

The recently published *Handbook of Disinfectants and Antiseptics*[35] recommended that gram-positive *S aureus*, gram-negative *P aeruginosa*, resistant spore *B subtilis*, and fungus *C albicans* be used as indicators or surrogate pathogen organisms, based on peer-reviewed scientific data. Organisms of this study were American Type Culture Collection (ATCC) strains of *S aureus* (25923), *P aeruginosa* (27853), *C albicans* (60193), and *B subtilis* (6633), similar to a preliminary study.[31]

On day 1, bacterial (*S aureus*, *P aeruginosa*, and *B subtilis*) and yeast (*C albicans*) isolates were individually inoculated to a turbidity of 0.5 of the McFarland standard, corresponding to 10^7 organisms/mL in 10 mL of TSB and incubated for 24 hours at 37ºC. The following day, 15 µL of inoculated TSB were transferred to each 600-mL sterile beaker containing the 200 mL of sterile TSB. Each sterile denture to be tested was aseptically placed into the beakers, sealed with foil, and incubated for 24 hours at 37ºC. After incubation, 40 dentures were selected for microwaving and 40 dentures were not microwaved (positive controls). The beakers containing positive control dentures were vortexed vigorously in a shaker incubator (Model MA-562, Marconi Equipamentos para Laboratório) for 1 minute and allowed to stand for 9 minutes, followed by a short vortex to resuspend any organisms present. To determine the number of microorganisms in the 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilutions, replicate specimens (25 µL) of the suspension were transferred to plates of 4 selective media (Acumedia Manufacturers): Mannitol salt agar for *S aureus*, Miller Hinton for *P aeruginosa*, Sabouraud agar containing 5 µg/mL gentamicin for *C albicans*, and tryptic soy agar for *B subtilis*. The plates were incubated at 37ºC for 48 hours.

Each microwaved denture was aseptically transferred to a 600-mL beaker containing 200 mL of sterile distilled water. Each beaker was placed on the rotational plate in an unmodified domestic microwave oven (Model Sensor Crisp 38, Brastemp, Double Emission System) and irradiated at 650 W for 6 minutes.[31] Dentures were then individually placed in sterile beakers containing 200 mL of TSB and treated identically to positive control dentures.

After incubation for 48 hours, bacterial and yeast colony counts of each plated denture were quantified using a digital colony counter (Phoenix CP 600 Plus, Phoenix Ind e Com de Equipamentos Científicos). The colony-forming units per milliliter (cfu/mL) were then calculated. To verify the long-term effectiveness of microwave sterilization, the TSB beakers with microwaved specimens were incubated at 37ºC for 7 more days. Cultures were interpreted by a single microbiologist (DMPS) as positive or negative growth.

Since the cfu/mL values among the positive control dentures had an inhomogeinity distribution, a Kruskal-Wallis 1-way analysis of variance (ANOVA) at 95% confidence level ($\alpha = .05$) on ranks was used. If significant differences in the cfu/mL numbers were found, pairwise multiple comparison procedures (Dunn method) were performed to analyze the data.

Results

Simulated complete dentures contaminated with individual suspensions of *S aureus* and *C albicans* showed consistent sterilization after 6 minutes of irradiation at 650 W. After microwave irradiation, no evidence of growth was observed at 48 hours for *S aureus*, *B subtilis*, and *C albicans* on plates (Table 1). For microwaved dentures contaminated with *P aeruginosa*, a few microorganisms grew on 2 plates, but these cfu/mL values were dramatically lower than those of positive control dentures (Table 1). After 7 days incubation at 37ºC, the results demonstrated that no growth was visible in the TSB beakers of *S aureus* and *C albicans*. Turbidity was observed in 3 broth beakers, 2 from *P aeruginosa* and 1 from *B subtilis* (Table 1).

Positive control dentures contaminated with individual suspensions showed substantial microbial growth on plates at 48 hours of incubation. There was no significant difference ($P > .05$) in cfu/mL mean values between *P aeruginosa*, *B subtilis*, and *S aureus* in positive control dentures. The mean numbers of cfu/mL for *P aeruginosa*, *B subtilis*, and *S aureus* were significantly ($P < .05$) higher than those observed for *C albicans* (Table 1).

Discussion

The present study demonstrated that simulated acrylic dentures contaminated with *C albicans* and *S aureus* showed sterilization after 6 minutes of microwave irradiation at 650 W. In addition, this procedure promoted effective disinfection of dentures contaminated with

P aeruginosa and *B subtilis*. These findings generally agreed with previous studies on microwave disinfection of complete dentures,[17,18,36] hard and soft chairside reline resins,[19,31] and dental handpieces and burs.[17,22,23] Rohrer and Bulard[17] observed that acrylic resin dentures contaminated with individual suspensions of 4 aerobic bacteria and 1 fungus showed sterilization of all microorganisms after 10 minutes of microwave irradiation (720 W). Microwave irradiation at 350 W for 6 minutes was also recommended over soaking in sodium hypochlorite as a more effective method of sterilization for dentures inoculated with *C albicans* and *Streptococcus gordonii*.[18] Moreover, microwave irradiation at 850 W for 1 minute in a dry state has proved to be an effective method to disinfect complete maxillary dentures as an adjunct to antifungal medication on the treatment of denture stomatitis.[36]

Although in most of the recommended microwave regimens the disinfection is performed in dry conditions,[17,18,36] a study regarding microwave disinfection of acrylic resins contaminated with *C albicans* demonstrated that all acrylic resin specimens immersed in water and irradiated for 5 minutes were effectively sterilized, while dry specimens irradiated for 5 minutes were only disinfected.[19] This procedure was considered adequate to kill organisms even within the pores of the acrylic resins. Accordingly, Neppelenbroek et al[31] observed that immersed specimens of 3 hard chairside reline resins showed consistent sterilization of 4 pathogenic microorganisms after 6 minutes of microwave exposure at 650 W. The authors attributed the favorable results to water immersion during microwave irradiation. Other studies have also demonstrated that wetting of contaminated sponges[27] and underwear[30] before microwave irradiation was necessary to obtain adequate disinfection. Although the lethal action of microwaves on various microorganisms is well established, the mechanism of destruction is not completely understood. Some investigators believe that the only lethal effects are those resulting from the heat generated during microwave irradiation (thermal effects).[37] However, destruction of microorganisms by microwave irradiation at temperatures lower than the thermal destruction point has been observed by other studies, which suggests that destruction of the organisms probably resulted from the interaction of the electromagnetic field with the molecules of the cells and the surrounding liquid medium, creating effects that could not be caused by thermal action alone (non-thermal effects).[20,38–43] Depending on the relative chemical composition of microbial cells and the composition and volume of their surrounding medium, the cells may also be selectively heated by microwave irradiation.[40,43] Microwave ovens heat materials containing water by making the molecules vibrate. Since cells contain water molecules,[26] it can be assumed that they are vulnerable to microwave irradiation. Moreover, microorganisms generally contain high intracellular concentrations of ionizable compounds, which may absorb microwave thermal heat at a much greater rate than a surrounding liquid medium such as distilled water.[41] In addition, mechanical disruption would occur if the oscillations of the cells in the electromagnetic field were rapid enough and of sufficient displacement to exceed the elastic limitations of the cell wall.[38] Whether the nature of the lethality of the microwave irradiation for microorganisms in the present study was molecular, mechanical, or selective heating remains to be investigated.

The present study confirms the findings of Neppelenbroek et al[31] concerning sterilization of *C albicans* and *S aureus* but is in disagreement concerning sterilization of *P aeruginosa* and *B subtilis*. These differences could be attributable to the distinct processing of the specimens. In the present study, the dentures were processed against dental stone, whereas the specimens from Neppelenbroek et al[31] were processed against acetate sheet and glass slab. Therefore, the roughness of the tissue surface of the dentures in this study is probably higher. Surface roughness is known to be a factor in the entrapment of microorganisms on surfaces and their protection from shear forces.[32] Moreover, the dimensions of the specimens used in the study of Neppelenbroek et al[31] were significantly smaller (10 mm in length and 1 mm in thickness) than those of the dentures used in the present study. The higher roughness associated with the greater area of the dentures increased the surface available for colonization, thus contributing to the growth of viable colonies of *P aeruginosa* on 2 plates at 48 hours and the turbidity in 2 beakers of *P aeruginosa* and 1 beaker of *B subtilis* at 7 days. However, the observed microbial growth could be considered irrelevant compared with that of the positive control dentures.

The selection of the microorganisms used in the present study was based on peer-reviewed scientific data regarding concepts of indicator and surrogate pathogen organisms, as well as their intrinsic microbial resistance.[35] Gram-positive *S aureus,* gram-negative *P aeruginosa,* fungus *C albicans*, and bacterial spore *B subtilis* have been recommended as indicator and surrogate pathogens to validate the effectiveness of disinfection procedures. Therefore, based on the results of the present study, microwave irradiation of immersed dentures for 6 minutes at 650 W might be considered suitable for disinfection of complete dentures. Considering that microwave irradiation produced sterilization of dentures contaminated with *C albicans,* which is believed to be the most important factor in the etiology of denture stomatitis, a pathogenic condition

observed in approximately 48% to 67% of healthy denture weares,[36] the disinfection protocol used in this investigation may contribute to the treatment of denture stomatitis. Clinical studies should be performed to confirm this hypothesis.

On positive control specimens, the mean colony count for *C albicans* was significantly lower than those for *S aureus*, *P aeruginosa*, and *B subtilis*. Larger yeast cells (5 to 10 μm) required larger surface defects to enhance their retention compared with smaller bacteria (0.5 to 3.0 μm).[44,45] Zissis et al[46] observed that the surface roughness of 4 denture base materials ranged from 3.4 to 7.6 μm. Therefore, the surface topography of the dentures tested in this study probably restricted the retention of the yeast (*C albicans*) and aided the retention of the small bacteria (*S aureus, P aeruginosa*, and *B subtilis*).

No apparent deformation or color change was observed on the microwaved prostheses. Limited information is available regarding the effect of microwave disinfection on the physical and mechanical properties of the denture base materials with specimens immersed in water during irradiation. A previous study[47] demonstrated that 2 or 7 cycles of microwave disinfection (650 W for 6 minutes) did not adversely affect the flexural strength of 4 hard chairside reline resins and 1 denture base resin. It has also been demonstrated that the Vickers hardness of 5 brands of acrylic resin denture teeth was not significantly affected by 2 cycles of microwave disinfection (650 W for 6 minutes).[48] The effect of the microwave disinfection protocol used in the present study on the dimensional stability of acrylic dentures needs further investigation. However, microwave irradiation performed in dry conditions demonstrated a clinically insignificant influence on the dimensional stability of acrylic dentures[17] and acrylic resins.[49,50]

This investigation demonstrated that microwave irradiation may be a reliable alternative for the disinfection of acrylic resin complete dentures. Caution is advisable when removable partial dentures are disinfected, because their metallic components may cause the microwave to spark and scorch the denture base material.[36] To overcome this problem, Rohrer and Bulard[17] enclosed the removable partial dentures with metallic components in plastic autoclave bags during microwave irradiation, which was performed in a dry state.

Conclusions

Within the limitations of this in vitro study, the following conclusions were drawn:

1. Microwave irradiation for 6 minutes at 650 W resulted in sterilization of complete dentures contaminated with *S aureus* and *C albicans* and disinfection of dentures contaminated with *P aeruginosa* and *B subtilis*.
2. Lower mean numbers of cfu/mL of the *C albicans* were observed for positive control dentures when compared with other microorganisms.

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