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Microwave Disinfection of Complete Dentures Contaminated *In Vitro* with Selected Bacteria

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Abstract

Purpose: This study evaluated the effectiveness of microwave irradiation for disinfection of simulated complete dentures.
Materials and Methods: Seventy dentures were fabricated in a standardized procedure, subjected to ethylene oxide sterilization, individually inoculated (10^7 cfu/mL) with *Staphylococcus aureus* (n = 20), *Pseudomona aeruginosa* (n = 20), and *Bacillus subtilis* (n = 30) and incubated for 24 hours at 37°C. After that, 40 dentures were selected for microwaving. For each microorganism, 10 dentures were submitted to microwave irradiation at 650 W for 3 minutes. In addition, 10 dentures contaminated with *B. subtilis* were irradiated for 5 minutes. Thirty non-microwaved dentures (n = 10 for each bacteria) 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. After incubation (37°C for 48 hours), colonies were counted (cfu/mL). TSB beakers with the microwaved dentures were incubated at 37°C for a further 7 days to verify long-term disinfection. The data were statistically analyzed by the Kruskal-Wallis test ($\alpha = 0.05$).
Results: No evidence of growth was observed at 48 hours for *S. aureus* and *P. aeruginosa* on plates, and no turbidity was visible in the TSB beakers of these specimens after 7 days of incubation. Dentures contaminated with *B. subtilis* and irradiated for 3 minutes produced microbial growth on six plates and turbidity on all TSB beakers. Microwaving for 5 minutes resulted in survival of *B. subtilis* in two plates and two beakers.
Conclusion: Microwave irradiation for 3 minutes at 650 W produced sterilization of complete dentures contaminated with *S. aureus* and *P. aeruginosa*. Dentures contaminated with *B. subtilis* were disinfected by microwave irradiation after 3 and 5 minutes at 650 W.

Prostheses contaminated with pathogenic microorganisms serve as a potential source of infection transmission between patients and dental personnel.[1] Concern about dissemination of these organisms has produced renewed interest in denture sterilization and disinfection.[2,3] To prevent cross-contamination, prostheses should be completely disinfected before being sent to the laboratory and before insertion.

Several methods of disinfection have been recommended to ensure infection control in dental practice. Proper disinfection of prostheses can be achieved with chemical solutions such as sodium hypochlorite, glutaraldehyde, and chlorine dioxide.[4,5] Nevertheless, these denture-soaking solutions can cause deleterious effects on acrylic resins. Sodium hypochlorite may stain[6] or whiten the plastic components of prostheses.[4] Glutaraldehyde has shown severe risk of cytotoxicity.[7] Chlorine dioxide has a bleaching action on the denture base resin and corrosive effects on the frameworks.[8]

To overcome the problems associated with chemical solutions, a major research effort has been focused on alternative methods for prosthesis decontamination. Microwave irradiation is claimed to be a simple, effective, and inexpensive method for prosthesis disinfection.[9-12] Webb et al[9] indicated that microwaving may be a more effective method of denture sterilization than denture soaking in sodium hypochlorite. A study by

Rohrer and Bulard[13] showed that dentures contaminated with a mixture of four aerobic bacteria and one fungus were sterilized after 10 minutes of microwave irradiation (720 W).

An earlier study[14] evaluated the effectiveness of microwave disinfection (6 minutes/650 W) of three hard chairside reline resins and showed consistent sterilization of four pathogenic microorganisms. Further evidence of the use of microwave irradiation was provided by Silva et al,[12] who demonstrated that 6 minutes of microwave exposure at 650 W resulted in sterilization of complete dentures contaminated with *S. aureus* and *C. albicans*; however, despite its effectiveness, the effect of this disinfection protocol on the physical and mechanical properties of the denture materials must be carefully considered. It has been observed that this procedure promoted a significant increase in the mean linear dimensional change (shrinkage) of denture base and relining materials.[15] Additionally, Seo et al[16] verified that thermal and mechanical stress exerted deleterious effects on the strength of intact and/or relined denture bases. A decrease in the surface hardness of five brands of acrylic resin denture teeth has also been observed.[17]

Thus, reduced microwave exposure should be evaluated to prevent any detrimental effect on acrylic resins. Recently, sterilization of acrylic resin specimens inoculated with individual suspensions of four microorganisms (*C. albicans*, *P. aeruginosa*, *S. aureus*, *B. subtilis*) was achieved at 3-, 4-, and 5-minute exposure times at 650 W.[18] Furthermore, the mechanical properties of the materials evaluated were not detrimentally affected by these exposure times to microwave irradiation.[19]

Therefore, the hypothesis that simulated complete dentures contaminated with three bacteria (*P. aeruginosa*, *S. aureus*, *B. subtilis*) could be consistently disinfected by reduced exposure times to microwave irradiation was tested in this study.

Materials and methods

Simulated denture production

A standardized procedure was followed to fabricate 70 simulated complete dentures.[10] Initially, a stainless steel master die simulating an edentulous maxilla was duplicated via a high-viscosity silicone mold (RTV 3120, Daltomare, Santo Amaro, Brazil) to produce 70 dental stone casts (Herodent, Vigodent, Bonsucesso, Brazil). On one of the prepared casts, a simulated maxillary complete denture base was waxed, and acrylic resin denture teeth were arranged accordingly. This waxed-up denture was duplicated using a high-viscosity silicone, and 70 identical simulated maxillary dentures were obtained. This was accomplished by first placing the acrylic artificial teeth (Dental Vip Ltd, Pirassununga, SP, Brazil) 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, São Paulo, Brazil) 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, São Paulo, Brazil). Two coats of sodium alginate (Isolak, Clássico Dental Products, São Paulo, Brazil) were used as a mold separator.

Poly (methyl methacrylate) dental base resin (Lucitone 550, Dentsply International Inc., York, PA) was prepared in accordance with the manufacturer's directions by mixing 21 g polymer powder to 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 Ltd). The flasks were placed in an automatic polymerization tank (Termotron P-100, Termotron Equipamentos, Piracicaba, Brazil) at 73°C for 90 minutes followed by 30 minutes in 100°C boiling water. After polymerization, the flasks were bench cooled for 30 minutes and placed under running tap water for 15 minutes. The flasks were opened, and the dentures were carefully recovered. The dentures were trimmed using a metal bur (Maxi-Cut, Dentsply-Malleifer, Ballaigues, Switzerland) and finished with a handheld micromotor (Kavo, Biberach/Riss, Germany) using 360-, 400-, 600-, and 1200-grit abrasive papers (Norton, Saint-Gobain Abrasivos Ltd, Guarulhos, Brazil). 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°C ± 1°C for 48 ± 2 hours.[20]

Sterilization of dentures

After 48 ± 2 hours of storage in water, all dentures were sterilized with ethylene oxide (ACECIL, Comércio e Esterilização a Óxido de Etileno Ltd, Campinas, Brazil). To confirm the effectiveness of this procedure, two additional dentures were tested as negative controls. Fifteen days after sterilization,[5] dentures were individually placed in 200 mL of Tryptic Soy Broth (TSB, Acumedia Manufacturers, Inc., Baltimore, MD) in 600-mL sterile beakers, which were sealed with foil. The beakers were then incubated at 37°C for 7 days. At 48 hours and 7 days, the broths were evaluated for microbial growth (turbidity). No turbidity was observed in the two broth beakers at 48 hours and 7 days.

Contamination and microwave disinfection procedures

The microorganisms selected for denture contamination were American Type Culture Collection (ATCC, Rockville, MD) strains of *S. aureus* (25,923), *P. aeruginosa* (27,853), and *B. subtilis* (6633), similar to a preliminary study.[12,14,18] The choice of the bacterial species for this study was based on peer-reviewed scientific data in the *Handbook of Disinfectants and Antiseptics*,[21] which recommends gram-positive *S. aureus*, gram-negative *P. aeruginosa*, and spore-resistant *B. subtilis* as indicators of surrogate pathogen organisms. A standard cell suspension was prepared by inoculating 10 mL of TSB with one of each bacteria (*S. aureus*, *P. aeruginosa*, *B. subtilis*) isolates, and was grown aerobically overnight at 37°C.[12,18] After incubation, the turbidity of inoculated TSB corresponded to 10^7 organisms/mL (0.5 of the McFarland standard).[12,18]

For denture contamination, 15 μL of the standard cell suspension was transferred to each 600-mL sterile beaker containing 200 mL of sterile TSB. Each sterile denture to be tested was aseptically put into these beakers, which were sealed with

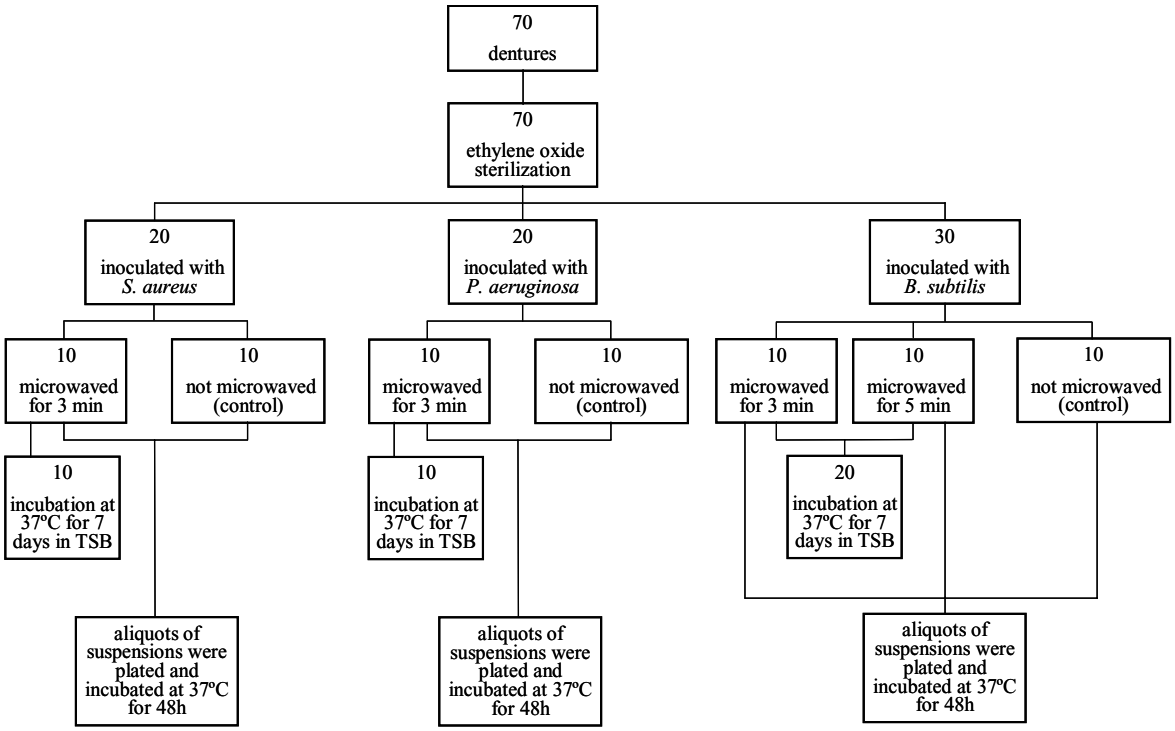

Figure 1 Diagram of experiment.

foil and incubated for 24 hours at 37°C.[12] After incubation, 30 (n = 10 for each bacteria) of the 70 dentures were not microwaved and were used as positive controls. The remaining 40 dentures were submitted to microwave irradiation at 650 W, distributed as follows: 10 dentures contaminated with *S. aureus* were microwaved for 3 minutes; 10 dentures contaminated with *P. aeruginosa* were microwaved for 3 minutes; 10 dentures contaminated with *B. subtilis* were microwaved for 3 minutes; and 10 dentures contaminated with *B. subtilis* were microwaved for 5 minutes (Fig 1).

Positive control dentures were removed from TSB beakers with sterile forceps and placed into a 600-mL beaker filled with 200 mL of sterile saline. The beakers containing positive control dentures were vortexed vigorously in a shaker incubator (Model MA-562, Marconi Equipamentos Laboratoriais Ltd, Piracicaba, Brazil) for 1 minute and allowed to stand for 9 minutes, followed by a short vortex to resuspend any organisms present. The vortex procedure was done to remove adhered cells from the denture surfaces.[5,12,18] To determine the cell survival, aliquots of the contents of each saline beaker were serially diluted 10-fold in sterile saline to give dilutions of 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} times the original concentration. Replicate specimens (25 μL) of the suspensions were transferred to plates of three selective media (Acumedia Manufacturers, Inc.): Mannitol Salt Agar for *S. aureus*, Mueller Hinton for *P. aeruginosa*, and Tryptic Soy Agar for *B. subtilis*. The plates were incubated at 37°C for 48 hours. After incubation, bacterial colony counts of each plated denture were quantified using a digital colony counter (Phoenix CP 600 Plus, Phoenix Ind. e Com. de Equipamentos Científicos Ltd, Araraquara, Brazil). The logarithm of colony-forming units per milliliter (log cfu/mL) was then calculated.

Each denture to be microwaved was aseptically transferred to a 600-mL beaker containing 200 mL of sterile distilled water. Water beakers were placed on the rotational plate in an unmodified domestic microwave oven (Model Sensor Crisp 38, Brastemp, Double Emission System, Manaus, Brazil) and irradiated at 650 W for 3 minutes. Additionally, 10 dentures contaminated with *B. subtilis* were also irradiated for 5 minutes. After microwave irradiation, each denture was removed from distilled water beakers with sterile forceps and placed into a 600-mL beaker filled with 200 mL of sterile saline. Then, the irradiated dentures were submitted to the same procedure as described for positive control dentures. Finally, microwaved dentures were individually placed in sterile beakers containing 200 mL of TSB and incubated at 37°C for 7 days, to verify the long-term effectiveness of microwave irradiation. Cultures were interpreted as positive or negative growth.

Because the log cfu/mL values among the groups had an inhomogeneity distribution, a Kruskal Wallis one-way ANOVA, at 95% confidence level ($\alpha = 0.05$), on ranks was used. If significant differences in the log cfu/mL numbers were found, pairwise multiple comparison procedures (Dunn's method) were performed to analyze the data.

Results

After 3 minutes of exposure to microwaves at 650 W, all dentures previously contaminated with *P. aeruginosa* and *S. aureus* exhibited no evidence of growth on plates incubated at 37°C

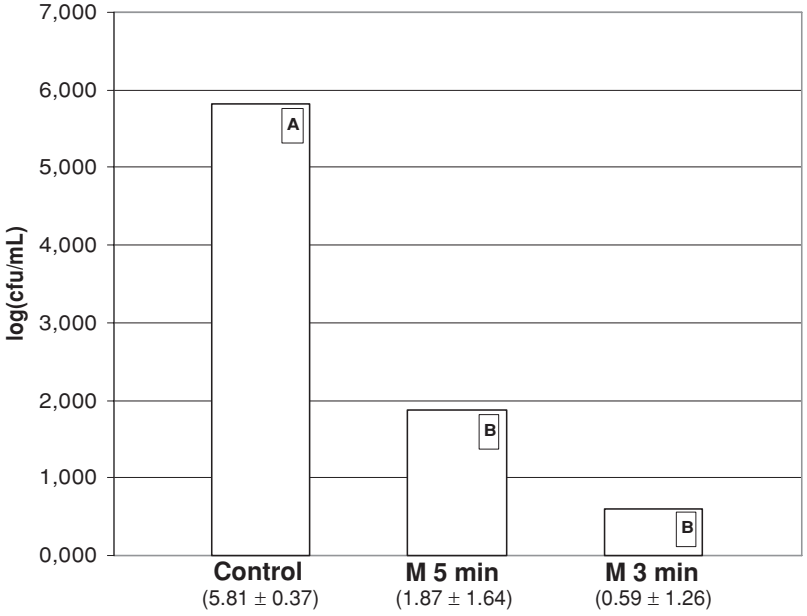

Figure 2 Mean and standard deviation of growth [log(cfu/mL)] of *Bacillus subtilis* on control and experimental dentures. "M 5 min" = microwaved for 5 minutes; "M 3 min" = microwaved for 3 minutes. Columns designated with the same capital letters were not statistically different ($p > 0.05$).

for 48 hours. Moreover, no turbidity was visible in the TSB beakers of these specimens after 7 days of aerobic incubation at 37°C.

Dentures contaminated with *B. subtilis* and irradiated for 3 minutes at 650 W produced microbial growth on six plates and turbidity in all TSB beakers. Microwaving for 5 minutes (650 W) resulted in survival of microorganisms in two plates and two beakers after incubation at 37°C. When compared to the positive control, dentures irradiated for 3 and 5 minutes showed significantly ($p < 0.05$) lower numbers of viable organisms (cfu/mL); however, there was no significant difference ($p > 0.05$) in the numbers of cfu/mL obtained between irradiated dentures for 3 and 5 minutes (Fig 2).

In all non-irradiated or positive control specimens inoculated with the tested individual suspensions, substantial microbial growth on plates was observed after 48 hours of aerobic incubation at 37°C. All pairwise multiple comparisons showed that the values of cfu/mL for *P. aeruginosa* and *S. aureus* were significantly ($p < 0.05$) higher than those observed for *B. subtilis* (Fig 3).

Discussion

The data obtained under the conditions of this study showed that microwaving of dentures contaminated with *S aureus* and *P. aeruginosa* for 3 minutes resulted in sterilization of

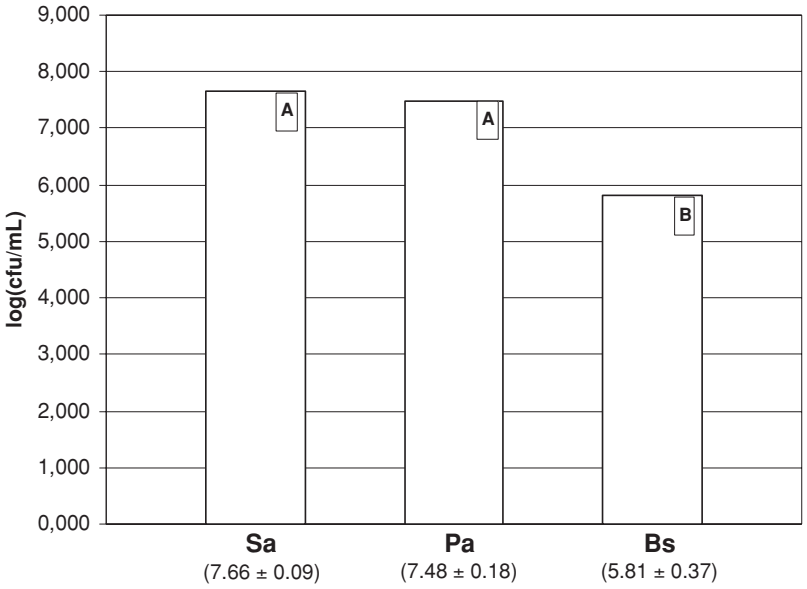

Figure 3 Mean and standard deviation of growth [log(cfu/mL)] of microorganisms on control dentures. Sa = *Staphylococcus aureus*; Pa = *Pseudomonas aeruginosa*; and Bs = *Bacillus subtilis*. Columns designated with the same capital letters were not statistically different ($p > 0.05$).

all specimens. These findings generally agreed with previous reports on denture[9,11,12] and acrylic resin[10,14,18] sterilization. Rohrer and Bulard[13] showed that dentures contaminated with aerobic and anaerobic bacteria, including spore formers, were sterilized after microwave irradiation for 8 minutes at 720 W. Webb et al[9] recommended microwave irradiation for 2 minutes at 650 W as a more effective sterilization method for dentures inoculated with *Streptococcus gordonii* than soaking them in sodium hypochlorite. Nepelenbroek et al[14] demonstrated that acrylic resin specimens contaminated with individual suspensions of three bacteria (*P. aeruginosa*, *S. aureus*, *B. subtilis*) were sterilized by microwave irradiation (6 minutes/650 W). More recently, the same microwave regimen (6 minutes/650 W) was effective for sterilizing complete dentures contaminated with *S. aureus*.[12] These findings support that microwave irradiation may present a suitable alternative for disinfecting complete dentures and will possibly help overcome the limitations of current soak treatments; however, it has been observed that microwaving for 6 minutes at 650 W produced deleterious effects on some physical and mechanical properties of acrylic resin specimens.[16,17,22,23] Thus, reduced microwave exposure times should be chosen to produce consistent disinfection without any adverse effect on acrylic resins.

Mima et al[18] observed that specimens of a hard chairside reline resin showed consistent sterilization of *C. albicans*, *P. aeruginosa*, *S. aureus*, and *B. subtilis* after exposure times shorter than 6 minutes at 650 W. Microwaving for 3 minutes promoted inactivation of all species evaluated.[18] In addition, a recent study showed that microwave disinfection for 3 minutes at 650 W significantly improved denture base adaptation when the traditional flask closure method was used.[24] Thus, in the present study, irradiation for 3 minutes was evaluated against complete dentures contaminated with *P. aeruginosa*, *S. aureus*, and *B. subtilis*. Additional dentures contaminated with *B. subtilis* were irradiated for 5 minutes, based on the evidence provided by Silva et al,[12] who found that microwaving for 6 minutes effectively disinfected dentures contaminated with *B. subtilis*. Thus, it was decided to reduce the irradiation time to 5 minutes and evaluate the effectiveness of this procedure on denture disinfection. The results showed that only *B. subtilis* was not eliminated from dentures after 3 minutes of irradiation. In addition, irradiation for 5 minutes resulted in similar disinfection of the dentures contaminated with *B. subtilis* and those irradiated for 3 minutes.

One possible explanation for complete inactivation of *B. subtilis* obtained by Mima et al[18] is that the microwave exposure was performed on small-dimension specimens (10-mm long, 1-mm thick), which had been processed against an acetate sheet and glass slab. This procedure resulted in the specimens having a mirror-like finish, 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 acrylic resin irregularities are more pronounced.[25,26] Najdovski et al[27] already verified that *B. subtilis* spores were more resistant to microwave irradiation (5 minutes/650 W) than *S. aureus* or *P. aeruginosa*. The authors attributed their results to the sporulation capability of *B. subtilis,* which is associated with less susceptibility to disinfection procedures than *S. aureus* or *P. aeruginosa*.[21] Bacterial spores are metabolically inactive and particularly resistant to a range of stresses, including heat, radiation, desiccation, and toxic chemicals.[28,29] Spores of *B. subtilis* can be formed in response to a variety of adverse environmental conditions, including high levels of temperature.[28] As microwave irradiation promotes heating of the specimens and the surrounding water, there is a possibility of spore formation during this procedure; however, the sporulation capability of *B. subtilis* was not evaluated in this study. Although no sterilization of *B. subtilis* was obtained in the present investigation, a significant reduction in cfu/mL was achieved after 3 and 5 minutes of irradiation in comparison with the control group. Therefore, these procedures were proved to be effective for complete denture disinfection.

The lethal action of microwave irradiation on various microorganisms seems to be well established, even though the mechanisms responsible for the microwave killing are unclear. Some studies maintain that the effect of microwave irradiation on microorganisms is of a directly thermal nature.[30–32] On the other hand, other investigations claim that killing the organisms also probably results from the interaction of the electromagnetic field with the cells and the surrounding liquid medium (nonthermal effects).[33–35] It has been reported that microwave irradiation affects the metabolic activity of *S. aureus* in a manner which could not be explained by the thermal effect alone.[36] A possible explanation of the nonthermal effect of microwave irradiation could lie in the selectivity of absorption of microwaves by certain essential biochemical molecules such as nucleid acids, protein, and the protein-lipopolysaccharide compound of cell membranes.[33,34] This process would detrimentally influence the vital activities of microorganisms. Moreover, since most microbial cells bear an electrical charge, usually negative, the possibility exists of the cell being mechanically disrupted by causing it to oscillate rapidly in the high-frequency field.[33] Despite the nature of the lethality of microwave irradiation, some authors have attributed the favorable results to water immersion during this procedure.[10,37,38] This can probably be explained based on the increase of water temperature during microwave irradiation, which provides uniform heating of the specimens.[10] A study regarding microwave disinfection of acrylic resins contaminated with *C. albicans* demonstrated that all 5-minutes-irradiated acrylic resin specimens immersed in water were effectively sterilized, while the 5-minutes-irradiated specimens in dry state were only disinfected.[10] Whether the nature of the lethality of the microwave irradiation in microorganisms as noted in the present study was molecular, mechanical, or selective heating remains to be investigated.

The present study showed that the control group specimens produced substantial microbial growth on the plates at 48 hours of aerobic incubation. The mean colony counts of *S. aureus* and *P. aeruginosa* were significantly higher than those for *B. subitilis*. Similar results were found by Mima et al[18] and Nepellenbroek et al[14] when specimens with small dimensions (10-mm long, 1-mm thick) were evaluated. The lower mean numbers of log cfu/mL for *B. subtilis* may be related to the spore-forming mechanism presented by this microorganism. *B. subtilis* sporulation is initiated after a period of relatively rapid growth (60 minutes).[39] Thus, this microorganism probably produced a high number of spores after the 48 hours aerobic incubation. It may be that the spore forms are more

easily dislodged from acrylic resin surfaces than bacterial forms, thus resulting in lower colony counts.[14]

The findings of this study suggest that microwave irradiation may be a potential treatment to prevent cross-contamination between the dental office and dental laboratory. Microwaving for 3 minutes was an effective method for disinfection of dentures contaminated with *B. subtilis* and for sterilization of those contaminated with *S. aureus* and *P. aeruginosa*. Additionally, irradiation of dentures contaminated with *B. subtilis* for 5 minutes produced a reduction of viable counts similar to 3 minutes of irradiation. Although properties of denture materials were not evaluated in this study, it has already been demonstrated that mechanical properties of acrylic resins are not detrimentally affected by microwave irradiation for 3 minutes at 650 W.[19] Furthermore, one cycle of microwave disinfection did not negatively affect the denture base adaptation when irradiation was performed for 3 minutes at 650 W.[24] It is important to remember that clinical conditions were not simulated in this investigation, and the interpretation of the results must be made with caution. Further investigations should be conducted to confirm the clinical effectiveness of microwave disinfection and its effects on the integrity of dentures after repeated microwave cycles.

Conclusions

In this study:

1. Microwave irradiation for 3 minutes at 650 W produced sterilization of complete dentures contaminated with *S. aureus* and *P. aeruginosa*.
2. Dentures contaminated with *B. subtilis* were disinfected by microwave irradiation after 3 and 5 minutes at 650 W.

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