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Effect of Different Exposure Times on Microwave Irradiation on the Disinfection of a Hard Chairside Reline Resin

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Abstract

Purpose: This study evaluated the effectiveness of different exposure times of microwave irradiation on the disinfection of a hard chairside reline resin.
Materials and Methods: Sterile specimens were individually inoculated with one of the tested microorganisms (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, and *Bacillus subtilis*) and incubated for 24 hours at 37°C. For each microorganism, 10 specimens were not microwaved (control), and 50 specimens were microwaved. Control specimens were individually immersed in sterile saline, and replicate aliquots of serial dilutions were plated on selective media appropriate for each organism. Irradiated specimens were immersed in water and microwaved at 650 W for 1, 2, 3, 4, or 5 minutes before serial dilutions and platings. After 48 hours of incubation, colonies on plates were counted. Irradiated specimens were also incubated for 7 days. Some specimens were prepared for scanning electron microscopic (SEM) analysis.
Results: Specimens irradiated for 3, 4, and 5 minutes showed sterilization. After 2 minutes of irradiation, specimens inoculated with *C. albicans* were sterilized, whereas those inoculated with bacteria were disinfected. One minute of irradiation resulted in growth of all microorganisms. SEM examination indicated alteration in cell morphology of sterilized specimens. The effectiveness of microwave irradiation was improved as the exposure time increased.
Conclusion: This study suggests that 3 minutes of microwave irradiation can be used for acrylic resin sterilization, thus preventing cross-contamination.

Dental prostheses brought into a dental office for relining or adjustments are contaminated with pathogenic microorganisms.[1] Concern about transmission of these organisms between the office and laboratory has led to renewed interest in sterilization and disinfection.[2] 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 highly resistant microbial spores.[3]

Effective infection control strategies prevent disease transmission by interrupting one or more links in the chain of infection. To reduce the chances of cross-contamination, prostheses should be completely disinfected before being sent to the laboratory and before insertion.[4] The use of disinfectants has been considered time-consuming and inappropriate.[5,6] Some chemicals may become absorbed into the material porosities and may not be completely eliminated by rinsing. Thus, chemicals may be unintentionally introduced into the oral cavity. Therefore, an alternative method for prosthesis disinfection is necessary. Microwave irradiation is claimed to be a simple, effective, and inexpensive method for prosthesis disinfection.[7–11] A preliminary study evaluated the effectiveness of microwave disinfection on three hard chairside reline resins with specimens immersed in water during irradiation. The authors observed that all immersed specimens showed consistent sterilization of *Staphylococcus aureus, Pseudomonas aeruginosa*, *Bacillus subtilis,* and *Candida albicans* after 6 minutes of microwave exposure at 650 W.[12]

When selecting a disinfection procedure, its effect on the physical and mechanical properties of the denture materials must be carefully considered.[13] Although microwave irradiation for 6 minutes at 650 W has proven to be an effective method for acrylic resin sterilization,[12] it has been observed that this procedure decreased the flexural strength of a hard chairside reline resin and the surface hardness of five brands of acrylic resin denture teeth.[14,15] Pavan et al verified that microwave irradiation for 10 minutes at 604 W produced significant discrepancies in the adaptation of maxillary acrylic resin denture bases to the stone casts.[16] Therefore, reduced microwave exposure times should be chosen in order to produce consistent disinfection without any detrimental effect on acrylic resins. Webb et al[10] observed that the minimal lethal microwave exposure time for *C. albicans* and *Streptococcus gordonii* on dentures at dry state was 2 minutes at 650 W; however, it has been suggested that placing specimens in water during microwave exposure provides uniform heating of the specimens.[8] This procedure is considered suitable to inactivate the microorganisms even within the pores of the materials.[8] Other studies also demonstrated that the wetting of contaminated sponges[17] and underwear[18] before microwave irradiation was necessary to obtain a more effective microbial inactivation. In addition, it is recognized that moist heat is more efficient at killing organisms than dry heat is. Water has a marked effect on the coagulation and denaturation of proteins, while dry heat promotes oxidation of the organic components of the cells (i.e., it makes the cells "burn" gradually).[19] Despite this important effect, the literature does not address the minimum exposure time required to inactivate pathogenic microorganisms on acrylic resins with the specimens immersed in water during irradiation. Using this procedure, this study was conducted to indicate the minimal lethal microwave exposure for pathogenic microorganisms on a hard chairside reline resin. The hypothesis that the effectiveness of microwave disinfection could be influenced by exposure time was tested.

Materials and methods

Specimen preparation and sterilization

The hard chairside reline resin selected for this study was Tokuso Rebase Fast Set (Tokuyama Dental Corp., Tokyo, Japan). Two hundred forty-two specimens were prepared. A stainless steel mold with a breakaway compartment (10 × 10 × 1 mm) was made to fabricate specimens from the resin. The material was mixed according to the manufacturer's instructions and applied into the mold placed on an acetate sheet and a glass slab. A second acetate sheet and a glass slab were placed over the material, and light pressure was applied to expel excess material from the mold. The specimens were then separated from the molds, and the edges were carefully smoothed.

All specimens were sterilized with ethylene oxide (ACECIL, Comércio e Esterilização a Óxido de Etileno Ltda, Campinas, Brazil). To confirm the effectiveness of this procedure, two additional specimens were tested as negative controls. After sterilization, specimens were added individually to 10 ml of Tryptic Soy Broth (TSB, Acumedia Manufacturers, Inc., Baltimore, MD) in sterile test tubes, which were then incubated aerobically at 37°C for 7 days. At 48 hours and 7 days, the broths were evaluated for microbial growth (turbidity). No turbidity in the broth tubes was observed at 48 hours and 7 days.

Contamination and microwave disinfection procedures

The *Handbook of Disinfectants and Antiseptics*[20] recommends 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 in this study were American Type Culture Collection vegetative strains (ATCC, Rockville, MD) of *S. aureus* (25923), *P. aeruginosa* (27853), *C. albicans* (60193), and *B. subtilis* (6633).

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 aerobically for 24 hours at 37°C. The following day, 50 μl of each test microorganism suspension was inoculated into test tubes containing 10 ml of sterile TSB. Immediately after inoculation, each sterile specimen was placed into the inoculated test tubes, vortexed, and incubated aerobically for 24 hours at 37°C. For each microorganism, 10 specimens were not microwaved (positive controls), and 50 specimens were selected for microwaving. For positive controls, the contents of each tube were poured into a sterile beaker. Each specimen was removed with sterile forceps and placed into a test tube filled with 4.5 ml of sterile saline. These tubes were vortexed vigorously 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 aliquots (25 μl) of the suspension were transferred to plates of four selective media (Acumedia Manufactures, Inc., Baltimore, MD): Manitol Salt Agar for *S. aureus*, Miller Hinton Agar for *P. aeruginosa*, Sabouraud Dextrose Agar containing 5 μg/ml gentamicin for *C. albicans*, and Tryptic Soy Agar for *B. subtilis*. The plates were incubated aerobically at 37°C for 48 hours.

Before irradiation, an unmodified domestic microwave oven (Model Sensor Crisp 38, Brastemp, Double Emission System, Manaus, Brazil) was calibrated as described by Thomas and Webb[21] by means of a standard test, in which 1 L of water (room temperature) was heated at one specific setting for 60 seconds. The difference between the starting and final temperatures was multiplied by 70, which established the wattage output.[21] The microwaved specimens were individually immersed in a 200-ml beaker of sterile distilled water and irradiated at 650 W in the microwave oven for 1, 2, 3, 4, or 5 minutes. The microwave timer was used for measuring the exposure time. The microwave oven cavity was allowed to cool to room temperature between exposures. The 200-ml volume of water standard has been calculated by measuring the volume that would completely cover an upper full denture in a standard denture pot.[22] Specimens were then placed in sterile glass tubes containing 4.5 ml of sterile saline and treated identically to positive control specimens.

After aerobic incubation for 48 hours, bacterial and yeast colony counts of each plated specimen were quantified using a Phoenix CP 600 Plus digital colony counter (Phoenix Indústria e Comércio de Equipamentos Científicos Ltda, Araraquara, Brazil). The counts were repeated by the same examiner for accuracy and were made without reference to the specimen, treatment regimen, or previous count. The intraexaminer reliability was evaluated using Pearson's correlation coefficient, which showed agreement of 97%. The logarithm of colony-forming units per milliliter (log cfu/ml) was then calculated. To verify the long-term effectiveness of microwaved sterilization, the TSB tubes with the microwaved specimens were incubated aerobically at 37°C for a further 7 days. Cultures were interpreted by a single microbiologist (DMPS) as positive or negative growth.

SEM procedures

Eight specimens of each experimental group and four specimens of the positive control group were submitted to scanning electron microscopic (SEM) analysis. Two samples of each experimental group and one sample of positive control group were inoculated (10^7 organisms/ml) individually with 10 ml of TSB containing one of the four tested microorganisms. The tubes were incubated aerobically at 37°C for 24 hours. After incubation, specimens of the positive control group were immediately prepared for SEM. The specimens of experimental groups were irradiated. Then, one specimen of each experimental group was prepared for SEM and the other was added to TSB at 37°C for 7 days prior to SEM preparation.

Specimens were fixed for 24 hours in 2.5% glutaraldehyde and then dehydrated for 15 minutes using a graded series of ethanol. The dried specimens were mounted on stubs and sputter coated with gold in a planar magnetron sputter coater (MED 010, OC Oerlikon Balzers AG, Balzers, Liechtenstein). Samples were observed with DSM 940 A SEM (Carl Zeiss, Jena, Germany) at 1000×, 2500×, and 5000×.

Because the log cfu/ml values among the groups had an inhomogeneous distribution, a Kruskal-Wallis one-way analysis of variance on ranks at a 95% confidence level 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

Positive control specimens of Tokuso Rebase Fast Set inoculated with the tested individual suspensions showed substantial microbial growth on plates after 48 hours of aerobic incubation at 37°C. All pairwise multiple comparisons showed that the mean numbers of log cfu/ml for *P. aeruginosa* and *S. aureus* were significantly ($p < 0.05$) higher than those observed for *C. albicans* and *B. subtilis* (Fig 1).

Specimens previously contaminated with individual suspensions of the four microorganisms showed consistent sterilization of all organisms after 3, 4, and 5 minutes of irradiation at 650 W. No evidence of growth was observed at 48 hours for *S. aureus*, *P. aeruginosa*, *C. albicans*, and *B. subtilis* on plates.

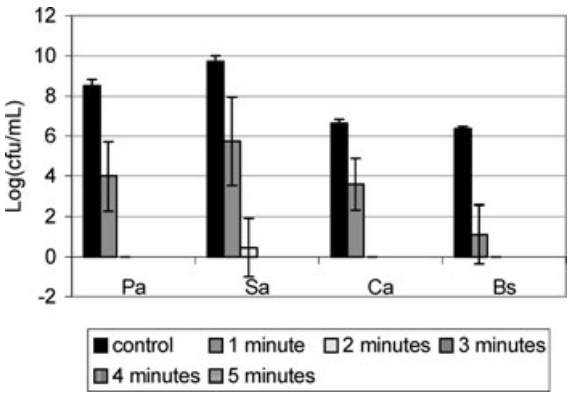

Figure 1 Mean and standard deviation of growth of microorganisms on specimens of the hard chairside reline resin. Pa = *Pseudomonas aeruginosa*, Sa = *Staphylococcus aureus*, Ca = *Candida albicans*, and Bs = *Bacillus subtilis*.

Furthermore, no growth was visible in the TSB tubes of these specimens after 7-day aerobic incubation at 37°C (Table 1).

After 2 minutes of irradiation at 650 W, all specimens contaminated with *C. albicans* showed consistent sterilization after 48 hours and 7 days (Table 1). In addition, after 48 hours at 37°C, no growth was observed on plates of *S. aureus*, *P. aeruginosa*, and *B. subtilis*, except for one specimen previously inoculated with *S. aureus*. Some of these specimens were only disinfected by microwave irradiation for 2 minutes because they exhibited bacterial growth (turbidity) on TSB tubes after 7 days of aerobic incubation (Table 1).

Microwave irradiation for 1 minute at 650 W resulted in survival of the four organisms after 48 hours and 7 days of aerobic incubation (Fig 1). Compared to the positive control, specimens irradiated for 1 minute showed significantly ($p < 0.05$) lower numbers of viable organisms (log cfu/ml) (Fig 1).

SEMs of positive control specimens clearly demonstrated the presence of high numbers of microorganisms on the material (Figs 2A and 3A). SEM analysis of irradiated specimens demonstrated that the alterations in the cells due to microwave exposure were proportional to the exposure time. While the cell morphology of the disinfected specimens was intact (Figs 2B and 3B), the sterilized specimens revealed damage to microorganisms soon after irradiation and after 7 days of incubation (Figs 2C and 3C). SEMs of disinfected specimens at 7 days were comparable to those from positive controls.

Table 1 Numbers of specimens* with microbial growth after 7-day aerobic incubation at 37°C

	Microwave exposure time (min)				
Microorganism	5	4	3	2	1
P. aeruginosa	0	0	0	1	10
S. aureus	0	0	0	2	10
C. albicans	0	0	0	0	10
B. subtilis	0	0	0	4	10

*Ten specimens per microorganism were tested at each group.

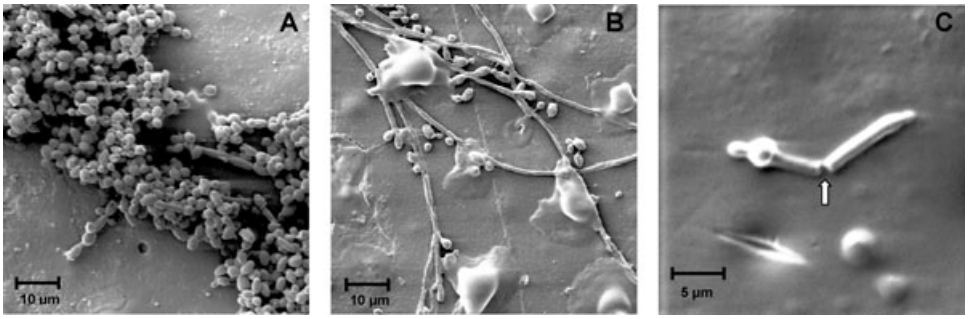

Figure 2 SEM of specimens contaminated with *Candida albicans*: (A) positive control, magnification ×1000; (B) disinfected specimen (1 minute), magnification ×1000; (C) sterilized specimen (2 minutes), showing alteration in cell morphology (arrow), magnification ×2500.

Discussion

The results showed that the sterilization of specimens inoculated with individual suspensions of the four microorganisms was achieved at 3-, 4-, and 5-minute exposure times at 650 W. These results suggest that the experimental protocol advocated by Neppelenbroek et al[12] (6 minutes of microwave exposure at 650 W) could be used with lower exposure times. In another study,[8] 5 minutes of microwave irradiation at full power resulted in sterilization of soft denture liners and a heat-polymerized denture base resin contaminated with *C. albicans*, when the specimens were immersed in water;[8] however, when nonimmersed specimens were submitted to this procedure (dry irradiation), sterilization was not achieved.[8] The favorable results of the present investigation and others[8,12,23,24] have been attributed to water immersion during microwave irradiation. The molecules of water play a major role in absorbing the energy from microwave radiation. The friction of water molecules produces heat, and the increase in temperature could inactivate microorganisms.[23] Moreover, it is recognized that the cells, placed in a hypotonic solution, such as sterile distilled water, may burst from the influx of water.[25]

Studies of denture sterilization by microwave irradiation have been reported. Rohrer and Bulard[9] first reported that 8 minutes of microwave irradiation at 720 W was sufficient to sterilize dentures contaminated with aerobic bacteria and *C. albicans*. These authors emphasized that the sterilization could only be accomplished if the dentures were attached to a 3D rotating device. While the 3D turntable most certainly provides a more even exposure to the irradiation,[9] it is not commercially available or practical for use. More recently, using an unmodified domestic microwave oven with a rotating table, Silva et al[24] demonstrated that the microwave irradiation for 6 minutes at 650 W resulted in sterilization of complete dentures contaminated with *S. aureus* and *C. albicans*. Webb et al[10] also observed in vitro sterilization of acrylic dentures contaminated with *C. albicans* after 2 minutes at 650 W. These previous observations are in agreement with the findings of the present investigation, in which all specimens contaminated with *C. albicans* and submitted to microwave irradiation for 2 minutes showed sterilization after 48 hours and 7 days of aerobic incubation. By using higher power (850 W) and reduced exposure time (1 minute), Banting and Hill[7] found that the microwave irradiation of complete maxillary dentures was more effective than soaking the dentures in 0.2% chlorhexidine solution for eradicating the invasive form of *C. albicans* from denture stomatitis patients. These authors observed that reinfestation of the denture surface and infection of the palatal oral mucosa were delayed in patients whose dentures were microwaved compared with those whose dentures were soaked in chlorhexidine solution. Webb et al[11] verified that microwaving the dentures (350 W for 10 minutes) on a nightly basis for 1 week significantly reduced the number of *Candida* spp. and aerobic bacteria on the dentures and decreased *Candida* spp. on the palate of subjects with denture stomatitis. The results from the present investigation together with those discussed above suggest that

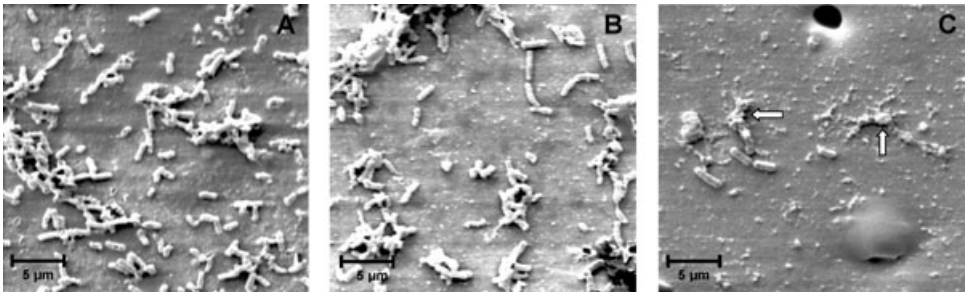

Figure 3 SEM of specimens contaminated with *Bacillus subtilis*: (A) positive control, magnification ×2500; (B) disinfected specimen (1 minute) after 7 days of incubation, magnification ×2500; (C) sterilized specimen (4 minutes) after 7 days of incubation, showing alteration in cell morphology (arrows), magnification ×2500.

the microwave irradiation for 2 minutes at 650 W could be used to prevent or treat denture stomatitis and its recurrence.

For the specimens contaminated with *S. aureus*, *P. aeruginosa*, and *B. subtilis* and irradiated for 2 minutes, no growth was observed after 48 hours at 37°C, except for one specimen previously inoculated with *S. aureus*. Nevertheless, some of these specimens showed bacterial growth (turbidity) on TSB tubes after 7 days of aerobic incubation. It is recognized that the cells of most bacteria are killed in higher temperature than cells of yeasts at the same exposure time of disinfection by moist heat.[19] All specimens of this study were immersed in water during microwave irradiation. Hence, it is likely that the water heating at 2 minutes of microwave irradiation contributed to the killing of the yeast cells (*C. albicans*). Moreover, yeasts such as *C. albicans* are larger cells (5 to 10 μm) compared with bacterial forms such as *S. aureus*, *P. aeruginosa*, and *B. subtilis* (0.5 to 3.0 μm).[12,26] Thus, *C. albicans* cells probably contain more water than the other tested microorganisms and, therefore, have more susceptibility to microwave irradiation. There are some explanations for those cultures with no growth on the plates but growth in the broth. Even a small number of organisms in the interior of the acrylic resin are able to grow rapidly in fresh broth. Furthermore, if only a few viable organisms remain in the 4.5 ml volume tube, the 25 μl aliquot removed for culturing on agar plates may not contain any viable cells. Nevertheless, the broth would exhibit growth after 7 days of aerobic incubation. This emphasizes the importance of the specimen incubation for 7 days.

Microwave exposure for 1 minute resulted in survival of the four organisms after 48 hours and 7 days of aerobic incubation; however, 1 minute of microwave irradiation resulted in reduction in all microorganisms, thus indicating effective disinfection of the specimens. Accordingly, Webb et al[10] observed that microwaving the dentures for 1 minute at 650 W resulted in 11% survival of *C. albicans* and 16.7% survival of *Streptococcus gordonii*. Similar results were observed by Ikawa and Rossen,[17] who found that heating wetted sponges in a microwave oven for 1 minute gave a reduction of bacteria load. These results indicate that, when sterilization is required microwave irradiation exposure time above 1 minute should be used.

Although the lethal action of microwave irradiation on various microorganisms has been documented, the mechanism of destruction is not fully understood. While some studies sustain that the effect of microwave irradiation on microorganisms is directly of thermal character,[27–29] others claim that the killing of the organisms probably also results from the nonthermal effects of microwaves.[30–33] It has been reported that microwave irradiation affects the metabolic activity of *S. aureus* in a manner that could not be explained by thermal effect alone.[34] 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.[30,31,33] This process would detrimentally influence the vital activities of microorganisms. It has also been demonstrated that the volume[23] and composition[29] of the liquid present in the surrounding medium may modify the intensity of the effect of microwave irradiation on microorganisms. Moreover, as most microbial cells bear an electrical charge, usually negative, there exists the possibility of mechanically disrupting the cell by causing it to oscillate rapidly in the high-frequency field.[30] The exact nature of lethal effects of microwave irradiation observed in the present investigation has yet to be elucidated by further research.

The present study showed that the specimens of the control group produced substantial microbial growth on the plates at 48 hours of aerobic incubation. The mean colony counts of *S. aureus* and *P. aeruginosa* were greater than those of *C. albicans* and *B. subtilis*. It has been reported that the cell size and surface roughness of dental materials significantly affected the retention of microorganisms.[26,35] Larger yeast cells required larger surface defects to enhance their retention compared with smaller bacteria. Zissis et al[36] observed that the surface roughness of nine hard relining materials processed against glass slabs ranged from 0.7 to 4.4 μm. As the reline specimens of the present investigation were also processed against glass slabs, it can be supposed that the surface topography of the specimens tested in this study probably restricted the retention of the yeast (*C. albicans*) and aided the retention of the small bacteria (*S. aureus* and *P. aeruginosa*). Despite its small size, the lower mean numbers of log cfu/ml for *B. subtilis* may be related to the spore forming mechanism presented by this microorganism. The *B. subtilis* sporulation is initiated after a period of relatively rapid growth (1 hour).[37] Thus, this microorganism probably produced a high number of spores after the 48 hours of 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.[12]

SEM photomicrographs indicated that microwaving of resin specimens did not remove nonviable bacterial cells but resulted in removal of some nonviable *C. albicans* cells from the resin surface. Verran and Maryan[26] reported that the larger yeasts cells are more easily dislodged from acrylic resin surfaces compared with smaller bacteria. Furthermore, SEM analysis indicated that the microwave irradiation promoted alteration in cell morphology for sterilized specimens but not for disinfected specimens. This is consistent with the findings of Rosaspina et al,[38] who observed that the microwave irradiation produced a progressive series of alterations, which were proportional to exposure time. Photographs of the sterilized specimens 7 days after irradiation appeared similar to those presented in specimens observed immediately after microwave irradiation. In contrast, SEM images of disinfected specimens were comparable to those from the respective controls, illustrating the microbial growth during the 7-day incubation period.

The results of this study indicated that cell viability was reduced as microwave exposure time increased. It was also observed that 3 minutes of microwave irradiation was the minimum exposure time necessary to inactivate all microorganisms evaluated. The interpretation of the results from this report must be made with caution, because only one hard chairside reline resin was evaluated, and in vitro rather than clinical tests were performed. The effect of the microwave exposure times tested in this study on the physical and mechanical properties of the denture base and reline materials also needs further investigation.

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References

1. Powell GL, Runnells RD, Saxon BA, et al: The presence and identification of organisms transmitted to dental laboratories. J Prosthet Dent 1990;64:235-237
2. Kohn WG, Collins AS, Cleveland JL, et al: Centers for Disease Control and Prevention (CDC). Guidelines for infection control in dental health-care settings—2003. MMWR Recomm Rep 2003;19:1-61
3. Molinari JA, Runnells RR: Role of disinfectants in infection control. Dent Clin North Am 1991;35:323-337
4. Henderson CW, Schwartz RS, Herdold ET, et al: Evaluation of the barrier system, an infection control system for the dental laboratory. J Prosthet Dent 1987;58:517-521
5. Chau VB, Saunders TR, Pimsler M, et al: In-depth disinfection of acrylic resins. J Prosthet Dent 1995;74:309-313
6. Pavarina AC, Pizzolitto AC, Machado AL, et al: An infection control protocol: effectiveness of immersion solutions to reduce the microbial growth on dental prostheses. J Oral Rehabil 2003;30:532-536
7. Banting DW, Hill SA: Microwave disinfection of dentures for the treatment of oral candidiasis. Spec Care Dent 2001;21:4-8
8. Dixon DL, Breeding LC, Faler TA: Microwave disinfection of denture base materials colonized with *Candida albicans*. J Prosthet Dent 1999;81:207-214
9. Rohrer MD, Bulard RA: Microwave sterilization. J Am Dent Assoc 1985;110:194-198
10. Webb BC, Thomas CJ, Harty DW, et al: Effectiveness of two methods of denture sterilization. J Oral Rehabil 1998;25: 416-423
11. Webb BC, Thomas CJ, Whittle T: A 2-year study of Candida-associated denture stomatitis treatment in aged care subjects. Gerodontology 2005;22:168-176
12. Neppelenbroek KH, Pavarina AC, Spolidorio DM, et al: Effectiveness of microwave sterilization on three hard chairside reline resins. Int J Prosthodont 2003;16:616-620
13. Smith DC: The cleansing of dentures. Dent Pract Dent Rec 1966;17:39-43
14. Campanha NH, Pavarina AC, Vergani CE, et al: Effect of microwave sterilization and water storage on the Vickers hardness of acrylic resin denture teeth. J Prosthet Dent 2005;93:483-487
15. Pavarina AC, Neppelenbroek KH, Guinesi AS, et al: Effect of microwave disinfection on the flexural strength of hard chairside reline resins. J Dent 2005;33:741-748
16. Pavan S, Arioli Filho JN, Dos Santos PH, et al: Effect of microwave treatments on dimensional accuracy of maxillary acrylic resin denture base. Braz Dent J 2005;16:119-123
17. Ikawa JY, Rossen JS: Reducing bacteria in household sponges. J Environ Health 1999;62:1-5
18. Friedrich EG, Phillips LE: Microwave sterilization of *Candida* on underwear fabric. A preliminary report. J Reproduct Med 1988;33:421-422
19. Pelczar Jr MJ, Chan EC, Krieg NR: Control of microorganisms: principles and physical agents, in Pelczar Jr MJ, Chan EC, Krieg NR (eds): Microbiology: Concepts and Applications, vol 1 (ed 2). New York, NY, McGraw-Hill, 1993, pp. 200-220
20. Cole EC, Robison R: Test methodology for evaluation of germicides, in Ascenzi JM (ed): Handbook of Disinfectants and Antiseptics. New York, NY, Marcel Dekker, 1996, pp. 1-13
21. Thomas CJ, Webb BC: Microwaving of acrylic resin dentures. Eur J Prosthodont Restor Dent 1995;3:179-182
22. Harrison Z, Johnson A, Douglas CW: An in vitro study into the effect of a limited range of denture cleaners on surface roughness and removal of *Candida albicans* from conventional heat-cured acrylic resin denture base material. J Oral Rehabil 2004;31:460-467
23. Najdovski L, Dragas AZ, Kotnik V: The killing activity of microwaves on some non-sporogenic and sporogenic medically important bacterial strains. J Hosp Infect 1991;19:239-247
24. Silva MM, Vergani CE, Giampaolo ET, et al: Effectiveness of microwave irradiation on the disinfection of complete dentures. Int J Prosthodont 2006;19:288-293
25. Pelczar Jr MJ, Chan EC, Krieg NR: Cultivation and growth of microorganisms, in Pelczar Jr MJ, Chan EC, Krieg NR (eds): Microbiology: Concepts and Applications, vol 1 (ed 2). New York, NY, McGraw-Hill, 1993, pp 175-198
26. Verran J, Maryan CJ: Retention of *Candida albicans* on acrylic resin and silicone of different surface topography. J Prosthet Dent 1997;77:535-539
27. Fitzpatrick JA, Kwao-Paul J, Massey J: Sterilization of bacteria by means of microwave heating. J Clin Eng 1978;3:44-47
28. Jeng DK, Kaczmarek KA, Woodworth AG, et al: Mechanism of microwave sterilization in the dry state. Appl Environ Microbiol 1987;53:2133-2137
29. Yeo CB, Watson IA, Stewart-Tull DE, et al: Heat transfer analysis of *Staphylococcus aureus* on stainless steel with microwave radiation. J Appl Microbiol 1999;87:396-401
30. Carrol DE, Lopez A: Lethality of radio-frequency energy upon microorganisms in liquid, buffered, and alcoholic food systems. J Food Sci 1969;34:320-324
31. Culkin KA, Fung DYC: Destruction of *Escherichia coli* and *Salmonella typhimurium* in microwave-cooked soups. J Milk Food Technol 1975;38:8-11
32. Olsen CM: Microwaves inhibit bread mold. Food Eng 1965;37:51-53
33. Rohrer MD, Terry MA, Bulard RA, et al: Microwave sterilization of hydrophilic contact lenses. Am J Ophthalmol 1986;101:49-57
34. Dreyfuss MS, Chipley JR: Comparison of effects of sublethal microwave radiation and conventional heating on the metabolic activity of *Staphylococcus aureus*. Appl Environ Microbiol 1980;39:13-16
35. Taylor R, Maryan C, Verran J: Retention of oral microorganism on cobalt-chromium alloy and dental acrylic resin with different surface finishes. J Prosthet Dent 1998;80:592-597
36. Zissis AJ, Polyzois GL, Yannikakis SA, et al: Roughness of denture materials: a comparative study. Int J Prosthodont 2000;13:136-140
37. Hauser PM, Errington J: Characterization of cell cycle events during the onset of sporulation in *Bacillus subtilis*. J Bacteriol 1995;177:3923-3931
38. Rosaspina S, Salvatorelli G, Anzanel D, et al: Effect of microwave radiation on *Candida albicans*. Microbios 1994;48:55-59

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