



Original article

Adding denture cleanser to microwave disinfection regimen to reduce the irradiation time and the exposure of dentures to high temperatures

Plinio M. Senna¹, Bruno S. Sotto-Maior², Wander J. da Silva¹ and Altair A. Del Bel Cury¹¹Department of Prosthodontics and Periodontology, Piracicaba Dental School, State University of Campinas, Piracicaba, São Paulo, Brazil;²Department of Implantology, Sacred Heart University, Bauru, São Paulo, Brazil

doi: 10.1111/j.1741-2358.2012.00641.x

Adding denture cleanser to microwave disinfection regimen to reduce the irradiation time and the exposure of dentures to high temperatures

Background: The microwave energy is an efficient disinfection method; however, it can generate high temperatures that can result in distortion of the dentures.

objectives: To evaluate whether the addition of an enzymatic cleanser to microwave disinfection regimen would disinfect dentures with shorter irradiation time.

Materials and methods: Seven resin discs colonized with *Candida albicans* biofilm were placed on the palatal surface of sterile dentures to be randomly assigned to the following treatments: immersion in distilled water for 3 min with 0 (DW), 1 (DW + M1), 2 (DW + M2), or 3 min (DW + M3) of microwave irradiation; or immersion in denture cleanser for 3 min with 0 (DC), 1 (DC + M1), 2 (DC + M2) or 3 min (DC + M3) of irradiation. After the treatments, the viable cells were counted by a blinded examiner. The temperature was measured immediately after irradiation. The data were analyzed by ANOVA and Tukey *post hoc* tests ($\alpha = 0.05$).

Results: No viable cells were found after DC + M2, DC + M3, and DW + M3 treatments, of which DC + M2 achieved the lowest temperature. No significant difference was found between the effectiveness of DW, DW + M1 and DC treatments ($p > 0.05$).

Conclusion: Within the limits of this study, the association of a denture cleanser and microwave energy is efficient to disinfect dentures in lower irradiation time and temperature.

Keywords: denture cleanser, denture hygiene, microwave disinfection.

Accepted 25 September 2011

Introduction

Candida spp. biofilm can accumulate on dentures, leading to the occurrence of oral candidosis in up to 65% of denture wearers¹. Although this pathology is usually restricted to the local mucosa, immunocompromised subjects may present an aggravated systemic infection, known as candidemia, which extends the hospitalization time and is associated with a 40% mortality rate². Therefore, the prevention and treatment of oral candidosis must be directed towards controlling the biofilm on the denture³. Although mechanical brushing is effective for preventing fungal adhesion by removing food debris and microorganisms^{4,5}, some patients

do not have the visual acuity or manual dexterity to perform adequate denture hygiene. This scenario can lead to biofilm accumulation, and highlights the need for auxiliary cleaning methods⁶.

The use of denture cleansers is increasing in the healthcare market as the main disinfection method for elderly patients, many of whom may be unable to brush their dentures effectively because of disease or dementia⁷. Denture cleaners, which are classified into different groups according to their main components, are generally effervescent tablets that readily form an alkaline peroxide solution when dissolved in water. This process releases oxygen bubbles and enzymes for mechanical and chemical cleaning⁸. Although denture cleansers are

able to reduce the biofilm mass when they are used alone, viable cells are still present on dentures, which can be a reservoir of microorganisms causing infection^{9,10}.

Microwave energy has been proposed as a clean and low-cost disinfection method that can effectively reduce the recurrence of denture stomatitis¹¹ by irradiating the denture for 3 min at 650 W^{12,13}. However, this procedure causes fast heating of the water solution in which the dentures are immersed, achieving high temperatures with only 3 min of irradiation¹³. When dentures are exposed to temperatures >71°C, some distortion of the poly(methylmethacrylate) (PMMA) polymer matrix may occur, due to the relaxation of the internal stresses acquired during polymerization^{14,15}.

To avoid PMMA distortion, there is a need for a low microwave irradiation regimen (low exposure time and low power) capable of disinfecting at a lower temperature^{16,17}. It was previously proposed the 450 W power, which is an efficient disinfection method and achieves lower final temperatures with 3 min of irradiation; however, the final temperature is still above 71°C¹⁶. Therefore, the aim of this study was to evaluate if the use of a denture cleanser with a low-power microwave disinfection regimen would adequately disinfect dentures with shorter irradiation time and consequently lower temperatures.

Materials and methods

Experimental design

Poly(methylmethacrylate) (resin discs, on which *Candida albicans* biofilm were developed for 72 h, were placed on the palatal surface of sterile dentures. Each denture was randomly assigned to one of the following disinfection regimens ($n = 8$ dentures each): immersion in distilled water for 3 min with 0 (DW), 1 (DW + M1), 2 (DW + M2), or 3 min (DW + M3) of 450 W microwave irradiation; or immersion in denture cleanser for 3 min with 0 (DC), 1 (DC + M1), 2 (DC + M2) or 3 min (DC + M3) of 450 W microwave irradiation.

After the disinfection treatments, the denture was removed. The discs were transferred to and sonicated in a tube containing phosphate buffered saline (PBS) for disaggregation of the biofilm. The sonicated solution was serially diluted in PBS and plated in triplicate on Sabouraud Dextrose Agar (SDA). The viable cells were counted by a blinded examiner with a stereomicroscope. The results were expressed in cells/ml for each denture. After the microwave irradiation procedures, the final temper-

ature of the solution was measured to evaluate the temperatures at which the dentures were exposed.

Denture and disc fabrication

Sixty-four denture replicas were made with heat-polymerizing PMMA resin (Lucitone 550; Dentsply International Inc., Chicago, IL, USA) in accordance with the manufacturer's instructions. These dentures had seven niches (11 × 1.5 mm deep) on the palatal surface to seat the discs during the microwave irradiation assay. After finishing and polishing, the dentures were immersed in distilled water for 48 h (23 ± 1.0°C) for residual monomer release¹⁸. Next, the dentures were ultrasonically cleaned for 20 min in an ultrasonic bath (Thornton T 740; Thornton-Inpec Eletrônica LTDA, Vinhedo, Brazil) and sterilized with ethylene oxide (ACECIL Comércio e Esterilização a Óxido de Etileno Ltda, Campinas, Brazil).

Heat-polymerized PMMA discs (10 × 2 mm) (Lucitone 550) were made in accordance with the manufacturer's instructions. The discs were finished and polished by using progressively smoother aluminium oxide papers (grits 320, 400, and 600) in a horizontal polisher (model APL-4; Arotec, São Paulo, Brazil). The final roughness was 3.3 ± 0.5 Ra, checked with a profilometer (Surfcorder SE 1700; Kozaka Laboratory Ltd., Tokyo, Japan)¹⁶. Then, the discs were immersed in distilled water for residual monomer release and disinfected in an ultrasonic bath for 20 min.

Candida albicans biofilm

Stimulated saliva was collected from a single healthy volunteer. The saliva was sterilized by 0.22-µm membrane filtration (TPP, Trasadingen, Switzerland) after clarification by centrifugation at 10 000 *g* for 10 min at 4°C. Discs were incubated for 30 min in 1 ml of saliva solution, into a sterile 24-well tissue plate to form an acquired pellicle. After this period, the discs were removed, washed twice with sterile PBS, and immediately used in the biofilm development assay.

Candida albicans ATCC 90028 was aerobically cultured at 37°C for 24 h on SDA. A loopful of growth was inoculated into Yeast Nitrogen Base (YNB) broth (Difco Laboratories, Detroit, MI, USA) supplemented with 50 mM glucose for analysis of the disinfection effectiveness. After 18–20 h of incubation, the cells were washed twice with PBS and suspended in YNB supplemented with 100 mM glucose. The cell concentration was ascertained spectrophotometrically ($OD_{520 \text{ nm}} = 0.250$) (Du

530 Spectrophotometer; Beckman Coulter Inc., Brea, CA USA) and was standardized to $1-5 \times 10^7$ cells/ml¹⁹.

Aliquots of 2.0 ml of standard yeast cell suspensions were transferred into each well containing one PMMA disc and incubated for 90 min at 37°C in an orbital shaker (Lab-line Incubator Shaker; Elliott Bay Laboratory Services, Kenmore, WA, USA) at 75 rpm for cell adhesion. Each specimen was washed twice with PBS. Then, 2.0 ml of freshly prepared YNB supplemented with 100 mM glucose were added to each well for biofilm development. The plates were incubated for 72 h at 37°C at 75 rpm, and the medium was renewed every 24 h.

Microwave disinfection assay

After 72 h of biofilm development, seven discs were distributed among the palatal niches of the 64 sterile dentures. Because a small drop in output power was verified after the first use of the microwave oven, before any further use, 1 l of distilled water was irradiated for 2 min at full power to warm up the microwave oven (AW-42 model; Continental, Manaus, Brazil)²⁰.

For the dentures not exposed to denture cleanser, each denture was immersed for 3 min in a glass beaker containing 200 ml of distilled water at room temperature. The beaker was placed in the centre of the microwave oven and was irradiated at 450 W¹⁶ for 1 (DW + M1), 2 (DW + M2), or 3 min (DW + M3), or received no irradiation (DW).

For the dentures exposed to denture cleanser treatment, one tablet of alkaline peroxide containing enzyme (Polident 3-min; GlaxoSmithKline, Philadelphia, PA, USA) was dissolved in 200 ml of distilled water in a glass beaker at room temperature. Each denture was immersed immediately in the solution for 3 min, in accordance with the manufacturer's directions. The beaker was placed in the centre of the microwave oven and was irradiated at 450 W for 1 (DC + M1), 2 (DC + M2), or 3 min (DC + M3), or received no irradiation (DC).

After each disinfection procedure, the denture was immediately removed and washed twice with sterile PBS. The seven discs were transferred to a plastic tube containing 5 ml of sterile PBS. The discs were sonicated at 7 W for 30 s for disaggregation of the biofilm¹⁶. The sonicated solutions were serially diluted in PBS, and 20- μ l specimens were plated in triplicate on SDA. The plates were incubated at 37°C under aerobic conditions for 48 h.

The viable cells were counted by a blinded examiner with a stereomicroscope (Coleman Equipa-

mentos para Laboratórios, Santo André, Brazil), and the results were expressed in cells/ml for each denture. The solution temperature was measured with a digital infrared laser thermometer with 0.1°C of resolution (IP-550; Impac, São Paulo, Brazil) immediately after the irradiation.

Statistical analysis

All analyses were performed by using SAS software (SAS Institute Inc., version 9.0, Cary, NC, USA), with the level of significance fixed at 5%. The normality of the error distribution and the degree of nonconstant variance were checked for the response variable (visible growth of viable cells and final temperature). The cell count values were transformed by logarithm ($\log_{10}(\chi)$). The disinfection treatments were analysed with one-way ANOVA. For post-ANOVA comparison, Tukey's HSD test was used.

Results

Table 1 shows the viable cell counts and temperatures for dentures microwave irradiated, with or without the use of denture cleanser, for 0–3 min. No viable cells were found on dentures irradiated for 2 min immersed in denture cleanser (DC + M2) and on both groups irradiated for 3 min (DC + M3 and DW + M3). Microwave irradiation for 2 min alone (DW + M2) was not effective despite of the reduction of viable cells ($p < 0.05$).

The dentures disinfected by denture cleanser, used alone (DC) or in combination with 1 min microwave irradiation (DC + M1), showed lower reduction of number of viable cells than the den-

Table 1 Number of viable cells after the disinfection treatments and the temperature of solution immediately after microwave irradiation (mean \pm SD; $n = 8$ dentures per group).

Disinfection treatments	Viable cells ($\times 10^5$)	Final temperature (°C)
DW	7.47 \pm 1.78 a	21.25 \pm 0.05
DC	4.82 \pm 1.18 ab	21.24 \pm 0.06
DW + M1	4.49 \pm 2.99 ab	40.70 \pm 0.94
DC + M1	2.64 \pm 1.77 b	41.55 \pm 0.83
DW + M2	0.10 \pm 0.06 c	62.50 \pm 1.10
DC + M2	0.00 \pm 0.00 d	59.65 \pm 1.01
DW + M3	0.00 \pm 0.00 d	76.21 \pm 2.21
DC + M3	0.00 \pm 0.00 d	75.78 \pm 1.79

Different letters indicate significant difference between the disinfection treatments ($p < 0.05$).

tures irradiated for 2 min immersed in distilled water (DW + M2) ($p < 0.05$).

Only 3 min of microwave irradiation was able to expose dentures to temperatures above 71°C. No significant difference was found between distilled water and denture cleanser solution for final temperatures ($p < 0.05$).

The use of denture cleanser combined with microwave irradiation allowed faster disinfection than the use of microwave irradiation alone (Fig. 1).

Discussion

Previously, it was reported that microwave disinfection can affect the biofilm mass present on dentures¹⁶. Therefore, the present study showed that the reduction of biofilm caused by the use of denture cleanser was sufficient to reduce the irradiation time needed during a microwave disinfection procedure. In other words, the synergism between denture cleanser and microwave disinfection enabled an effective disinfection regimen with only 2 min of irradiation that exposes the dentures to lower temperatures, which would avoid distortion of the PMMA resin.

Mechanical cleaning could also be performed to reduce the number of viable cells before 2 min microwave irradiation in distilled water in order to improve its effectiveness⁴. However, patients without adequate visual acuity or manual dexterity to brush their denture^{6,7} need an auxiliary disinfection method to be realized once a day to avoid biofilm accumulation. Among these methods, denture cleanser solutions are not an efficient disinfection method when used alone^{9,10} and 3-min of microwave irradiation at 450 W could be harmful

to dentures due to cumulative distortion on PMMA caused by the heat^{13–15}. Therefore, the association of these two methods can reduce their own limitations, generating an efficient protocol that disinfects dentures at temperatures below 71°C.

The fungus *Candida albicans* is the main etiological factor of oral candidosis, due to its high virulence and ability to adhere to and colonize on PMMA surfaces²¹. Thus, the *C. albicans* biofilm growth model used in the present study simulated *in vivo* conditions of static biofilm growth found on the tissue-contacting surface of a denture²², enabling the effectiveness evaluation of the disinfection treatments.

It has been established that 650 W of microwave irradiation for 3 min is effective for disinfection^{12,23,24}; however, its effectiveness may be related to the fact that water starts to boil after 1.5 min¹³. Although boiling water is desirable for sterilization, it exposes the PMMA resin to temperatures close to its glass transition temperature (Tg 100.4°C), which can distort the denture by releasing internal stress²⁵. Microwave disinfection should expose the denture to temperatures <71°C to avoid any damage to the PMMA^{14,15}. To achieve lower temperatures, lower irradiation powers have been proposed to be harmless to the PMMA resin^{16,17}. At the 3 min exposure time, the 450 and 650 W powers showed the same effectiveness¹⁶; however, even the lower power is still able to expose the denture to temperatures of >71°C, even if for a short period (Table 1). Despite the relatively safety of these regimens, they may cause distortion of the denture^{14,15}.

Two minutes of irradiation at 650¹² or 450 W¹⁶ previously was shown to be ineffective at disinfection when used alone. In the present study, microwave irradiation at 450 W for 2 min associated with the denture cleanser solution produced an effective disinfection. Similarly, studies have shown that 2 min of microwave irradiation improves the effectiveness of a commercial denture cleanser^{26,27}; however, these previous studies did not identify the irradiation power used, which influences the temperature of the solution. A reduction in irradiation time to 2 min^{12,16,23,24} enabled effective disinfection at temperatures <71°C, combining both efficiency and efficacy in the procedure.

Microwave heating is dependent on the properties of the solution. Microwave energy causes rapid and intense heating of polar molecules, due to its selective dielectric heating property. Thus, the molecules are excited by dipolar polarization and ionic conduction, which is reflected in the pro-

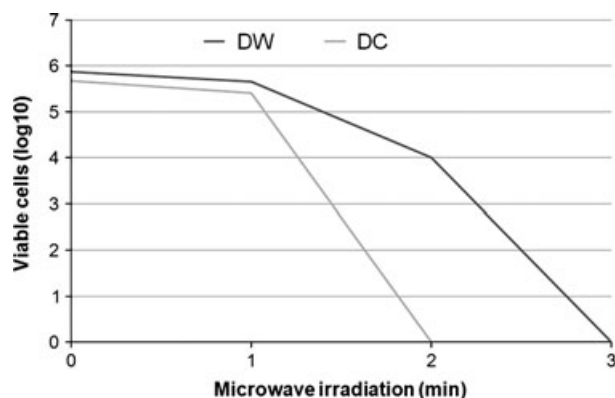


Figure 1 Comparison of the killing rates of microwave disinfection with dentures immersed in distilled water or in denture cleanser.

duction of heat²⁸. Denture cleansers generally are composed of citric acid, sodium carbonate, potassium peroxymonosulfate, and sodium perborate monohydrate²⁹. It is expected that the ions released in the solution, from the acid and salt dissolution, experience more heating than distilled water. However, in the present study, the presence of charged ions in the solution did not appear to influence the final temperature, and no difference from distilled water was observed.

The final temperature also depends on the volume of solution: solutions with high volumes will have lower final temperatures than solutions with low volumes. In this study, 200 ml of distilled water was used because it is a sufficient volume to cover a denture completely³⁰, and it is sufficient for protecting the microwave oven from the excess energy reflected inside the oven³¹.

Because dentures may function as reservoirs of microorganisms to cause infection, the objectives of disinfecting dentures are to remove the biofilm and to decontaminate the surface by eliminating all the microorganisms. Thus, a vital goal of denture cleaning protocols is to avoid recolonization of the oral cavity³². Although the present study does not fully mimic the oral environment (only one *Candida* species was evaluated) and only one denture cleanser was tested, we conclude that the combination of denture cleanser and microwave irradiation is an efficient disinfection protocol in which the denture is exposed to lower temperature.

Conclusion

The combined use of denture cleanser and low-power microwave irradiation is an efficient protocol to disinfect dentures.

References

1. **Marcos-Arias C, Vicente JL, Sahand IH et al.** Isolation of *Candida dubliniensis* in denture stomatitis. *Arch Oral Biol* 2009; **54**: 127–131.
2. **Leleu G, Aegerter P, Guidet B.** Systemic candidiasis in intensive care units: a multicenter, matched-cohort study. *J Crit Care* 2002; **17**: 168–175.
3. **Webb BC, Thomas CJ, Willcox MD, Harty DW, Knox KW.** Candida-associated denture stomatitis. Aetiology and management: a review. Part 3. Treatment of oral candidosis. *Aust Dent J* 1998; **43**: 244–249.
4. **Barnabe W, de Mendonca Neto T, Pimenta FC, Pegoraro LF, Scolaro JM.** Efficacy of sodium hypochlorite and coconut soap used as disinfecting agents in the reduction of denture stomatitis, *Streptococcus mutans* and *Candida albicans*. *J Oral Rehabil* 2004; **31**: 453–459.
5. **Coelho CM, Sousa YT, Dare AM.** Denture-related oral mucosal lesions in a Brazilian school of dentistry. *J Oral Rehabil* 2004; **31**: 135–139.
6. **Shay K.** Denture hygiene: a review and update. *J Contemp Dent Pract* 2000; **1**: 28–41.
7. **Gornitsky M, Paradis I, Landaverde G, Malo AM, Velly AM.** A clinical and microbiological evaluation of denture cleansers for geriatric patients in long-term care institutions. *J Can Dent Assoc* 2002; **68**: 39–45.
8. **Nikawa H, Hamada T, Yamashiro H, Kumagai H.** A review of in vitro and in vivo methods to evaluate the efficacy of denture cleansers. *Int J Prosthodont* 1999; **12**: 153–159.
9. **Vieira AP, Senna PM, Silva WJ, Del Bel Cury AA.** Long-term efficacy of denture cleansers in preventing *Candida* spp. biofilm recolonization on liner surface. *Braz Oral Res* 2010; **24**: 342–348.
10. **de Freitas Fernandes FS, Pereira-Cenci T, da Silva WJ, Filho AP, Straioto FG, Del Bel Cury AA.** Efficacy of denture cleansers on *Candida* spp. biofilm formed on polyamide and polymethyl methacrylate resins. *J Prosthet Dent* 2011; **105**: 51–58.
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. **Ribeiro DG, Pavarina AC, Dovigo LN, Palomari Spolidorio DM, Giampaolo ET, Vergani CE.** Denture disinfection by microwave irradiation: a randomized clinical study. *J Dent* 2009; **37**: 666–672.
13. **Mima EG, Pavarina AC, Neppelenbroek KH, Vergani CE, Spolidorio DM, Machado AL.** Effect of different exposure times on microwave irradiation on the disinfection of a hard chairside reline resin. *J Prosthodont* 2008; **17**: 312–317.
14. **Basso MF, Giampaolo ET, Vergani CE, Machado AL, Pavarina AC, Compagnoni MA.** Influence of microwave disinfection on the linear dimensional stability of complete dentures: a clinical study. *Int J Prosthodont* 2010; **23**: 318–320.
15. **Sartori EA, Schmidt CB, Mota EG, Hirakata LM, Shinkai RS.** Cumulative effect of disinfection procedures on microhardness and tridimensional stability of a poly(methyl methacrylate) denture base resin. *J Biomed Mater Res B Appl Biomater* 2008; **86B**: 360–364.
16. **Senna PM, Da Silva WJ, Del Bel Cury AA.** Denture disinfection by microwave energy: influence of *Candida albicans* biofilm. *Gerodontology* 2010 doi: 10.1111/j.1741-2358.2010.00439.x.
17. **Thomas CJ, Webb BC.** Microwaving of acrylic resin dentures. *Eur J Prosthodont Restor Dent* 1995; **3**: 179–182.
18. **Moura JS, da Silva WJ, Pereira T, Del Bel Cury AA, Rodrigues Garcia RC.** Influence of acrylic resin polymerization methods and saliva on the adherence

- of four *Candida* species. *J Prosthet Dent* 2006; **96**: 205–211.
19. **da Silva WJ, Seneviratne J, Parahitiyawa N, Rosa EA, Samaranyake LP, Del Bel Cury AA.** Improvement of XTT assay performance for studies involving *Candida albicans* biofilms. *Braz Dent J* 2008; **19**: 364–369.
 20. **Reipert S, Kotisch H, Wysoudil B, Wiche G.** Rapid microwave fixation of cell monolayers preserves microtubule-associated cell structures. *J Histochem Cytochem* 2008; **56**: 697–709.
 21. **Figueiral MH, Azul A, Pinto E, Fonseca PA, Branco FM, Scully C.** Denture-related stomatitis: identification of aetiological and predisposing factors - a large cohort. *J Oral Rehabil* 2007; **34**: 448–455.
 22. **Pusateri CR, Monaco EA, Edgerton M.** Sensitivity of *Candida albicans* biofilm cells grown on denture acrylic to antifungal proteins and chlorhexidine. *Arch Oral Biol* 2009; **54**: 588–594.
 23. **Campanha NH, Pavarina AC, Brunetti IL, Vergani CE, Machado AL, Spolidorio DM.** *Candida albicans* inactivation and cell membrane integrity damage by microwave irradiation. *Mycoses* 2007; **50**: 140–147.
 24. **Silva MM, Vergani CE, Giampaolo ET, Neppe-lenbroek KH, Spolidorio DM, Machado AL.** Effectiveness of microwave irradiation on the disinfection of complete dentures. *Int J Prosthodont* 2006; **19**: 288–293.
 25. **Urban VM, Machado AL, Oliveira RV, Vergani CE, Pavarina AC, Cass QB.** Residual monomer of reline acrylic resins. Effect of water-bath and microwave post-polymerization treatments. *Dent Mater* 2007; **23**: 363–368.
 26. **Goodson LB, Glass RT, Bullard JW, Conrad RS.** A statistical comparison of denture sanitation using a commercially available denture cleaner with and without microwaving. *Gen Dent* 2003; **51**: 148–151.
 27. **Glass RT, Goodson LB, Bullard JW, Conrad RS.** Comparison of the effectiveness of several denture sanitizing systems: a clinical study. *Compend Contin Educ Dent* 2001; **22**: 1093–1096 1098, 1100–2 passim; quiz 1108.
 28. **Zielinski M, Krzemieniewski M.** The effect of microwave electromagnetic radiation on organic compounds removal efficiency in a reactor with a biofilm. *Environ Technol* 2007; **28**: 41–47.
 29. **Alam M, Jagger R, Vowles R, Moran J.** Comparative stain removal properties of four commercially available denture cleaning products: an in vitro study. *Int J Dent Hyg* 2011; **9**: 37–42.
 30. **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.
 31. **Baysan A, Whiley R, Wright PS.** Use of microwave energy to disinfect a long-term soft lining material contaminated with *Candida albicans* or *Staphylococcus aureus*. *J Prosthet Dent* 1998; **79**: 454–458.
 32. **Ferreira MA, Pereira-Cenci T, Rodrigues de Vasconcelos LM, Rodrigues-Garcia RC, Del Bel Cury AA.** Efficacy of denture cleansers on denture liners contaminated with *Candida* species. *Clin Oral Investig* 2009; **13**: 237–242.

Correspondence to:

Altair A. Del Bel Cury, Department of Prosthodontics and Periodontology, Piracicaba Dental School, State University of Campinas, PO Box 52, Av. Limeira, 901 – Piracicaba, Sao Paulo 13414-903, Brazil.

Tel.: 55 19 21065294

Fax: 55 19 21065211

E-mail: altcurry@fop.unicamp.br