Comparison of denture microwave disinfection and conventional antifungal therapy in the treatment of denture stomatitis: a randomized clinical study

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Objective. The aim of this study was to compare the effectiveness of denture microwave disinfection and antifungal therapy on treatment of denture stomatitis.

Study Design. Sixty denture wearers with denture stomatitis (3 groups; n = 20 each), were treated with nystatin or denture microwave disinfection (1 or 3 times/wk) for 14 days. Mycologic samples from palates and dentures were quantified and identified with the use of Chromagar, and clinical photographs of palates were taken. Microbiologic and clinical data were analyzed with the use of a series of statistical tests ($\alpha = .05$).

Results. Both treatments similarly reduced clinical signs of denture stomatitis and growth on palates and dentures at days 14 and 30 ($P > .05$). At sequential appointments, the predominant species ($P < .01$) isolated was *C. albicans* (range 98%-53%), followed by *C. glabrata* (range 22%-12%) and *C. tropicalis* (range 25%-7%).

Conclusions. Microwave disinfection, at once per week for 2 treatments, was as effective as topical antifungal therapy for treating denture stomatitis. (Oral Surg Oral Med Oral Pathol Oral Radiol 2012;114:469-479)

Denture stomatitis is the most common oral opportunistic infection on the palate in elderly denture wearers.[1-3] This recurring disease is mainly caused by *Candida* species. *Candida albicans* is the major pathogen in oral candidiasis,[4-6] but non-*albicans* species can also contribute to the development of this infection.[4-8] Apart from many other factors, candidal adherence to oral epithelia[9] and denture surface[5,8,10] is a crucial first step in the initiation and development of denture stomatitis. Candidal adherence is reported to be enhanced by the transition from blastospores to hyphae,[11] a process that contributes to the virulence of this pathogen.[12] In addition, the roughness of acrylic denture surface

might affect plaque retention or inhibit its removal, and therefore dentures act as reservoirs for yeasts, which may be aggravated by poorly fitting and old dentures.[5,8,10]

The treatment of denture stomatitis involves oral and denture hygiene,[2] removing dentures at night,[10] correction of denture faults,[13] and the use of topical and systemic antifungal agents, such as amphotericin B, nystatin, and azoles.[10,14-18] Amphotericin B and nystatin are used topically and sometimes applied to the denture-fitting surface before its insertion,[19] whereas azoles such as fluconazole, itraconazole, and ketaconazole are available for systemic antifungal treatment.[16,17] There are, however, a few issues in relation to the use of these agents. Poor response to topical antifungal drugs is not uncommon,[10] owing to the dilution and rapid elimination of topically applied drugs by the flushing action of saliva, which can reduce antifungal agents to subtherapeutic concentrations.[14] In addition, these drugs require multiple doses, which can

Statement of Clinical Relevance

This clinical study showed that weekly denture microwave disinfection was as effective as nystatin antifungal therapy in reducing both the clinical and microbiological signs of denture stomatitis. Patients appear to benefit from such protocol for several months.

reduce the patient's compliance.[16] Systemic antifungal drugs are expensive[13] and may present some limitations in terms of toxicity[20] and drug interactions. Furthermore, the widespread use of systemic medications has resulted in the development of resistant species.[21,22]

Strict denture disinfection procedures are recommended to treat and prevent denture stomatitis.[8,10,14,17] Within this context, microwave irradiation has proved to be a safe, simple, effective, and low-cost disinfection method that can be used not only to disinfect dentures,[23–27] but also for the treatment of denture stomatitis.[8,10,14,18] Some studies have shown that irradiation of complete dentures for 1,[14] 6,[10] or 10[8] minutes, associated or not with the use of topical antifungal therapy (nystatin[14] and miconazole[10]), significantly reduced the number of microorganisms found in the dentures, as well as the clinical symptoms affecting the palatal mucosa of patients with denture stomatitis. In addition, denture microwaving was an effective means of reducing the invasive form of *Candida* species (pseudohyphae) from both the dentures and the surface of the patient's palate.[10,14] It is important to point out that this method presents an important advantage in antifungal therapy, in that it is a physical method of disinfection that eliminates the possibility for the survival of microorganisms and selection of resistant yeasts as occurs with systemic and topical antifungals.[8,10,14,17]

Despite the fact that the methods of disinfection through microwaves used in earlier studies[8,10,14,27] are considered to be effective for the sterilization of complete dentures and the treatment of denture stomatitis, it has been demonstrated that the heat generated during microwave irradiation can increase the contraction of the acrylic resins[28,29] and accelerate the water sorption and solubility processes of these materials.[30] There have also been reports of undesirable effects on mechanical properties in denture base resins after microwave disinfection for 6 minutes.[31–34] Therefore, in vitro and in vivo studies were conducted to evaluate both antimicrobial efficacy and effects on acrylic resins of microwave disinfection with the use of reduced irradiation times. A microwave disinfection regimen of 3 minutes at 650 W has proved to have no detrimental effect on several mechanical properties of acrylic resins.[35–40] Moreover, this lower exposure time was effective for sterilizing test samples contaminated with *C. albicans*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus*[23,24] and complete dentures contaminated with different species of *Candida*, including the intrinsically resistant *C. glabrata* and *C. krusei*[26] and methicillin-resistant *S. aureus*.[41] When used in a clinical trial, this disinfection protocol was effective in inactivating denture biofilms in individuals with-

out denture stomatitis.[25] Despite these favorable results, the efficacy of denture microwave disinfection for 3 minutes in the treatment of denture stomatitis has been evaluated by only a single study.[18] Furthermore, the length of treatment and the frequency of denture irradiation would be other factors to consider when using this method of treatment. In the few clinical studies available,[8,10,14,18] the frequency of the disinfection cycles varied from 1 to 7 times per week for periods ranging from 1 to 4 weeks. Considering that the protocol for microwave disinfection (3 minutes at 650 W) can promote complete disinfection of the dentures,[23,25,26] a reduction in the frequency of the irradiation would be possible, making the treatment more convenient by reducing the number of patient visits to the dentist. Therefore, the present clinical study evaluated the effectiveness of 2 frequencies of denture microwave disinfection to treat patients with denture stomatitis and compared them with topical antifungal therapy (nystatin). The hypothesis tested was that there would be no differences among treatments. The effectiveness was assessed microbiologically, through the reduction in *Candida* counts on the palates and denture surfaces, and clinically, by means of resolving the clinical signs of the infection. The prevalence of *Candida* spp. identified in these patients was also evaluated.

MATERIALS AND METHODS

Study design
This study was a 3-group repeated-measures design with random assignment of subjects to treatment groups to compare the effectiveness of denture microwave disinfection and nystatin in the treatment of patients with denture stomatitis. This research was conducted in accordance with the Declaration of Helsinki, and its protocol was approved by the Ethics Committee of the Araraquara Dental School, UNESP-Univ Estadual Paulista. Each of the subjects voluntarily entered the study and signed an informed consent form before enrollment.

Participants
The studied population consisted of patients from the Araraquara Dental School. Only patients wearing a maxillary complete denture, 30-75 years old, and assessed for clinical evidence of denture stomatitis were included. The exclusion criteria for patient selection were as follows: having undergone antibiotic, steroid, or antifungal therapy in the preceding 3 months; with anemia or immunosuppression or undergoing cancer therapy (radio- or chemotherapy); and wearing the same complete denture for >30 years.[18] Full personal, medical, and dental/denture histories were collected from all of the patients. A comprehensive oral examination of each patient was performed by the same

investigator, and the mucosal characteristics were classified according to the criteria proposed by Newton[42]: 0, absence of palatal inflammation; 1 (type I), petechiae dispersed throughout all or any part of palatal mucosa in contact with the denture (localized simple inflammation); 2 (type II), macular erythema without hyperplasia (generalized simple inflammation); and 3 (type III), diffuse or generalized erythema with papillary hyperplasia (inflammatory papillary hyperplasia).

Sample size calculation and randomization

Using data from 2 clinical studies published by the authors' research group,[10,18] sample size calculation resulted in a minimal sample size of 20 patients in each group. To create groups of patients that were similar regarding baseline characteristics that could influence prognosis other than the treatment being considered, namely, risk factors, a stratified randomization was used. The following risk factors were considered in this study: age of the dentures,[1] smoking habits,[43] xerostomia,[1] denture hygiene habits,[2] and nocturnal wear of the dentures.[1] Denture hygiene was classified as good (absence of plaque) or poor (presence of removable and/or nonremovable plaque on the inner and/or outer denture surface).[18]

Interventions

According to the stratified randomization list, the 60 patients were randomly assigned to one of the 3 experimental groups. Patients in the NYS group received topical antifungal medication (nystatin; Cristália, Produtos Químicos Farmacêuticos, Itapira, Brazil). Patients were instructed to remove their dentures from the oral cavity and rinse with 1 mL of the suspension (100,000 UI/mL) for 1 minute, 4 times per day, for 14 days. Patients were informed not to swallow the suspension after rinsing. In the MW1 and MW3 groups, the maxillary complete denture of each patient was individually immersed in a 600-mL beaker containing 200 mL sterile distilled water. Each beaker was placed on the rotational plate in a domestic microwave oven (Sensor Crisp 38; Brastemp, Double Emission System, Manaus, Brazil) and irradiated at 650 W for 3 minutes[23,25,26] 1 time (MW1) or 3 times (MW3) per week for a period of 14 days. After treatment, all patients were followed monthly for 3 months. Over the experimental period, all patients were instructed to scrub their dentures with coconut soap followed by toothpaste before sleeping and after every meal. They were also instructed to remove their dentures and leave them immersed in filtered water (200 mL) while they were asleep.[10,18]

Outcomes

The primary outcomes of interest were the *Candida* colony counts from the palates and dentures surfaces, quantified in cfu/mL, and severity of infection of the palatal mucosal, classified according to the criteria proposed by Newton[42] (0, absence of palatal inflammation; or types I, II, and III), measured before treatments (baseline), at the end of the treatments (day 14), and at follow-ups (days 30, 60, and 90). The secondary outcome was the prevalence of *Candida* spp. identified in the 3 groups of patients at the same intervals.

Microbiologic investigations

Before treatments (day 0), mycologic samples were obtained from the palatal mucosa and the tissue side of the maxillary dentures of each patient in all 3 groups by rubbing with a sterile swab.[5,8,10,17,18,25] Mycologic samples were also taken after treatments started (day 14) and at follow-ups (days 30, 60, and 90). Each swab was put into a test tube containing 10 mL 0.9% sterile saline solution and vortexed for 1 minute to suspend the organisms from the swab. Thereafter, samples from this suspension were cultured on Sabouraud dextrose agar (SDA) containing 0.05% chloramphenicol for yeast growth, and in Chromagar Candida[5,18,25] for isolation and presumptive identification of *Candida* species. The plates were incubated at 30°C for 5 days. *Candida* spp. colonies counts were quantified (cfu/mL) with the use of a digital colony counter (Phoenix CP 600 Plus). All the different colony types observed on Chromagar plates were selected and reinoculated onto SDA for purity. The isolated colonies were identified by chlamydospore production on corn meal agar supplemented with 1% Tween-80 and by the carbohydrate assimilation pattern by using the ID 32C (bioMérieux, Marcy l'Etoile, France).[5,8,18] In addition, a hypertonic Sabouraud broth test was required to distinguish *C. albicans* from *C. dubliniensis*.[44] All microbiologic procedures were carried out by the same operator.

Clinical investigation and blinding

To document the clinical response to the treatments, standardized color photographs were taken of the palatal mucosa of each patient. These photos were taken before treatments (day 0), at the end of treatments (day 14), and at follow-ups (days 30, 60, and 90). All of the photographs were taken with the same digital camera (Cybershot DSC-F717; Sony, Tokyo, Japan), by the same operator and under the same conditions (place, light, angle, and patient position) to facilitate their reproducibility. After standardization of the images, 2 independent observers were engaged to blindly analyze the 5 photographs taken of each patient.[18] In this blind analysis, the observers were instructed to classify the mucosal characteristics of each

Table I. Demographic characteristics and risk factors

	Demographic characteristic		Risk factors				
	Mean age (y)	% female	Mean age of dentures (y)	% of patients with xerostomia	% nonsmokers	% with nocturnal wear of dentures	% with poor hygiene habits
NYS	62.5	90	18.6	65	90	90	80
MW1	59.5	85	17.4	40	90	90	70
MW3	56.8	80	12.8	50	80	90	65
P value	.1420*	.9187†	.3160*	.5020†	.7105†	.9999†	.6752†

*One-way ANOVA (age of patients: Shapiro-Wilks test: $P = .1795$; Bartlett test: $P = .9010$; age of patients' dentures: Shapiro-Wilks test: $P < .0001$; Bartlett test: $P = .9721$).
†Fisher exact test.

patient according to the criteria proposed by Newton[42] (0, absence of palatal inflammation; or types I, II, and III). Observers were blind to risk factors, treatment group, and period of evaluation.

Statistical analysis

Demographic characteristics of the patients and the risk factors were statistically analyzed to ensure homogeneity between the groups using one-way analysis of variance (ANOVA) and Fisher exact tests. In the microbial analyses, the numbers of cfu/mL were $\log_{10}$ transformed ($\log_{10}$ cfu/mL) to achieve a normal distribution, and a 2-way ANOVA using a random effects statistical model was performed. Two factors were considered for analysis: treatment (NYS, MW1, and MW3) and period of time (baseline, day 14, day 30, day 60, and day 90). The values obtained from the palates and dentures were analyzed separately. When differences were found, Tukey post hoc test was implemented and $P < .05$ was taken to be significant. Clinical significance of the 3 treatments on the microbiologic reduction on the palates and dentures was also evaluated by the treatments' effect size[45]: $<0.1 =$ trivial effect; 0.1-0.3 = small effect; 0.3-0.5 = moderate effect; and $>0.5 =$ large difference effect.[10,18] The frequency distributions of the different species of *Candida* isolated from the patients were analyzed by means of a χ^2 test, Bonferroni-corrected confidence interval, and Bonferroni-Holm correction for multiple testing. Also, an odds ratio (OR) was calculated comparing the absence or presence of mixed *Candida* spp. isolated from the palates with that isolated from the dentures.

For the clinical data analysis, the degrees of correlation and concordance of the 2 blind observers were estimated to provide a measure of the reliability and validity of the results. The tests used for this analysis were the kappa measure of agreement, ranging from 0 to 1 (perfect match: $\kappa = 1$; almost perfect: $0.81 \leq \kappa < 1$; substantial: $0.61 \leq \kappa < 0.80$; moderate: $0.41 \leq \kappa < 0.60$; fair: $0.21 \leq \kappa > 0.40$; slight: $0 < \kappa < 0.20$; no concordance: $\kappa = 0$),[46] the Kendall rank correlation coefficient, ranging from -1 to 1 (perfect agreement: $\tau = 1$; no correlation: $\tau = 0$; perfect

disagreement: $\tau = -1$),[47] and the coefficient of concordance (%). The degrees of severity of the mucosal characteristics of patients scored at baseline from the NYS, MW1, and MW3 groups were compared by Fisher exact test to ensure homogeneity between the groups ($\alpha = .05$). To analyze the magnitude of the treatment effect on the evolution of the disease over time, a categoric variable was created.[18] The following general categories were used: decrease in infection scored by 1 point (+, i.e., type III to II, type II to I, or type I to 0), 2 points (++, i.e., type III to I, or type II to 0), or 3 points (+++, i.e., type III to 0); no change in infection status (No); and increase in infection scored by 1 point (−, i.e., type I to II, or type II to III) or 2 points (−−, i.e., type I to III). The percentage of patients in each category was determined for each period, and comparisons between the groups were made by Kruskal-Wallis test ($\alpha = .05$). The percentages of "cured patients" and "patients with recurrence" were also compared among groups by means of the Fisher exact test. In all analyses, differences were considered to be statistically significant at a value of $P < .05$.

RESULTS

Participants, demographic characteristics and risk factors

Table I shows the demographic and clinical characteristics of the subjects. The mean age of the patients ranged from 56.8 to 62.5 years at the time of the initial evaluation, and more women than men were present in the 3 study groups. In all groups, the dentures were made ≥10 years before the trial. In addition, ≥40% of patients complained of xerostomia and few smokers participated. Before the onset of the study, a large percentage of patients wore their dentures at night and most of them showed poor denture hygiene habits. Stratified randomization ensured that the subsamples for the 3 treatments were well balanced for both demographic characteristics and risk factors, because the statistical analysis showed homogeneity among the three groups of study ($P > .05$).

Table II. ANOVAs using a random effects statistical model for mean values of $\log_{10}$ ufc/mL from the palates and dentures

	Numerator df	Denominator df	F value	P value
Palates				
(Intercept)	1	212	56.9892	.0000
Period of time	4	212	8.6790	.0000[a]
Treatment	2	57	2.8242	.0677
Interaction time × treatment	8	212	1.7734	.0837
Dentures				
(Intercept)	1	212	705.9835	.0000
Period of time	4	212	19.8406	.0000[a]
Treatment	2	57	1.0528	.3556
Interaction time × treatment	8	212	0.4669	.8785

[a]Significant difference ($P < .05$).

Table III. Mean values and standard deviation of *Candida* colony counts in $\log_{10}$ cfu/mL from the palates and dentures after treatments and values of treatment effect size

		Period					
Group	Location	Baseline	Day 14	Day 30	Day 60	Day 90	Treatment effect size
NYS	Palate	1.95 (1.53)	0.26 (0.65)[a]	0.81 (1.47)[a]	1.41 (1.56)	1.17 (1.28)	1.78
	Denture	4.24 (0.73)	1.58 (1.79)[a]	2.87 (1.98)[a]	3.46 (1.91)	3.31 (2.18)	2.09
MW1	Palate	0.96 (1.17)	0.52 (0.98)[a]	1.01 (1.21)[a]	1.51 (1.50)	1.05 (1.20)	0.51
	Denture	4.34 (1.05)	1.46 (1.88)[a]	2.63 (2.03)[a]	3.16 (2.04)	3.24 (1.71)	2.95
MW3	Palate	1.72 (1.45)	0.80 (1.43)[a]	1.28 (1.31)[a]	1.32 (1.57)	2.01 (1.53)	1.17
	Denture	4.43 (1.22)	1.65 (1.73)[a]	3.04 (2.14)[a]	3.84 (1.58)	4.24 (1.37)	3.08

[a]Significant difference from baseline by Tukey post hoc test ($P < .05$).

During the follow-up period, a total of 9 patients dropped out or were excluded from the trial (4 from the group NYS, 3 from the group MW1, and 2 from the group MW3) because of the need for antibiotic treatment (5 patients) or withdrawal of consent to participate (4 patients).

Microbiologic results

Two-way ANOVA (Table II) showed that, regardless of the period of time, there were no significant differences in the mean values of $\log_{10}$ cfu/mL when the factor treatment group (NYS, MW1, or MW3) was analyzed for the dentures ($P = .3556$) and for the palates ($P = .0677$). χ^2 test and Bonferroni confidence intervals showed significantly more yeast-negative samples ($P < .00001$) collected from the palates (47.2%, 95% Bonferroni-corrected confidence interval [CI] 0.40-0.53) compared with samples from the dentures (22.5%, 95% Bonferroni-corrected CI 0.16-0.28). When the factor period of time was evaluated, significant differences ($P < .0001$) were found for both palates and dentures (Table II). There was no interaction between the factors of treatment group and period of time for the dentures ($P = .8785$) and for the palates ($P = .0837$).

The mean values of the quantitative mycologic cultures ($\log_{10}$ cfu/mL) of *Candida* spp. with reference to the palatal mucosa and internal surface of the complete dentures are presented in Table III. Tukey post hoc test

showed that all treatments reduced significantly the mean values of $\log_{10}$ cfu/mL from the palates ($P < .001$) and dentures ($P < .001$) at day 14 and day 30 (P values .007 and .050, respectively). The reduction was ~3-fold for the dentures and 2.7-fold for the palates. At days 60 and 90, an increase in the mean values of $\log_{10}$ cfu/mL from the palates and dentures was observed compared with baseline ($P > .05$). Table III shows that the effect size of treatments was large for the palates and very large for the dentures after all treatments.

Table IV demonstrates that regardless of the group and in all trial periods *C. albicans* was the predominant yeast (χ^2 test: $P < .00001$). *C. glabrata* and *C. tropicalis* were the non-*albicans* species most frequently isolated, with no statistical differences between them ($P > .05$). Although a significant decrease ($P < .01$) in the prevalence of *C. albicans* was observed at day 14 and follow-up periods, no significant change was observed for *C. tropicalis* and *C. glabrata*. Other *Candida* species (*lusitanae, colliculosa, famata, parapsilosis, krusei, rugosa, zeylanoides, intermedia, sphaerica, kefyr,* and *guilliermondii*) and additional yeasts from the genus *Cryptococcus* (*laurentii, humicola,* and *albidus*), *Saccharomyces* (*cerevisiae*), *Kloeckera apiculata,* and *Rhodotorula* were detected at low rates (from 1.6% to 13.3% of patients).

Twenty-nine participants (48.3%) had >1 species of yeast isolated from the samples: 11 from the NYS

Table IV. Frequency distributions (%) of *Candida* spp. identified at baseline and at follow-ups

Species	Baseline	Day 14	Day 30	Day 60	Day 90
			Period		
C. albicans	98[Aa]	53[Ba]	69[Ba]	72[Ba]	71[Ba]
C. glabrata	22[Ab]	12[Ab]	14[Ab]	17[Ab]	18[Ab]
C. tropicalis	25[Ab]	7[Ab]	7[Ab]	15[Ab]	20[Ab]

χ^2: baseline: $P = .7149$; day 14: $P = .5207$; day 30: $P = .4214$; day 60: $P = .8397$; day 90: $P = .8365$. 95% Bonferroni-corrected confidence interval: baseline: *C. albicans* 0.94-1.0, *C. tropicalis* 0.11-0.38, and *C. glabrata* 0.08-0.34; day 14: *C. albicans* 0.37-0.68, *C. tropicalis* 0.0-0.14, and *C. glabrata* 0.01-0.21; day 30: *C. albicans* 0.56-0.82, *C. tropicalis* 0.0-0.13, and *C. glabrata* 0.04-0.23; day 60: *C. albicans* 0.59-0.85, *C. tropicalis* 0.04-0.25, and *C. glabrata* 0.05-0.27; day 90: *C. albicans* 0.57-0.84, *C. tropicalis* 0.07-0.31, and *C. glabrata* 0.06-0.29). Horizontally, values designated with the same uppercase superscript letter were not statistically different (Bonferroni-Holm test: $P > .05$). Vertically, values designated with the same lowercase superscript letter were not statistically different.

Table V. Values of kappa measure of agreement (κ), Kendall rank correlation coefficient (τ), and coefficient of concordance (%) for each period of time for the 3 groups of study

Group	Period of time	κ	τ	Coefficient of concordance
NYS	Day 0	0.827	0.874	94.12
	Day 14	0.915	0.960	94.12
	Day 30	1	1	100
	Day 60	0.901	0.838	93.75
	Day 90	0.889	0.948	92.31
MW1	Day 0	1	1	100
	Day 14	1	1	100
	Day 30	1	1	100
	Day 60	0.913	0.954	94.74
	Day 90	1	1	100
MW3	Day 0	0.699	0.756	90
	Day 14	1	1	100
	Day 30	1	1	100
	Day 60	0.839	0.873	89.48
	Day 90	0.923	0.957	94.45

group and 9 from each of the microwave groups (MW1 and MW3). The yeast mixtures isolated were *C. albicans* + *C. glabrata* (38%), *C. albicans* + *C. tropicalis* (27%), and *C. albicans* + *C. glabrata* + *C. tropicalis* (12%). In addition, the frequency distributions of mixed samples isolated from the dentures (52.2%) were significantly higher (χ^2 test: $P = .0115$) than those obtained from the palates (34.8%). Calculation of the OR indicated that the dentures were both 3 times more likely to have *Candida* spp. colonization (OR 3.06; $\alpha = .05$) and 2 times more likely to have mixed populations of *Candida* spp. than the palates (OR 2.03; $\alpha = .05$).

Clinical results

The examination scores assigned by the blind observers were in high degree of correlation and concordance (Table V). Illustrative images of the photos scored by the observers are shown in Figure 1, and a comprehensive description of the frequency distribution of the Newton classification for denture stomatitis over the time is given in Figure 2. Fisher exact test showed homogeneity from the groups of study ($P = .7961$) for the mucosal characteristics of patients at baseline. Kruskal-Wallis test showed no significant differences ($P > .05$) among the magnitude of the clinical efficacy of NYS, MW1, and MW3 on the evolution of the disease over the time (Table VI). All treatments were considered to be successful in treating denture stomatitis, with ≥65% of patients showing improvement in clinical signs of denture stomatitis at the end of the treatment period (day 14). This feature was maintained up to day 60, when ≥50% of all treated patients showed a decrease in the degree of denture stomatitis. Although none of the treated patients showed any increase in the rate of infection at the end of the follow-up period (day 90), 45%-60% of them showed a decrease in the rate of infection (Table VI). Fisher exact test also showed that there were not significant differences in the percentage of "cured patients" or "patients with recurrence" among the 3 groups of treatment (Table VII).

DISCUSSION

The mean age of the patients assessed in the present study (59.6 years) is in agreement with those of earlier studies,[5,10,18] which suggests a higher prevalence of denture stomatitis after the sixth decade of life due to the high number of complete and partial dentures wearers in this age group. Also in agreement with earlier investigations,[1,3,5,10,18] there was a predominance of female patients with denture stomatitis compared with male patients. This difference has been attributed to hormonal changes, especially in the period after menopause[48] as well as women seeking dental treatment more frequently than men.[49]

The age of the dentures is another factor that favors the development and recurrence of denture stomatitis.[3,5,10] In the present study, the mean age of dentures was >10 years. Recently, it was reported that only 25% of individuals using dentures for <1 year were diagnosed with denture stomatitis, whereas >84% of those using dentures for >5 years had the disease.[3] In the present investigation, a large percentage of patients complained of xerostomia and were nonsmokers, and most of them did not remove the dentures for sleeping and showed poor denture hygiene habits. Although not sufficient to treat denture stomatitis,[10] denture and oral hygiene, including the removal of dentures for sleeping,
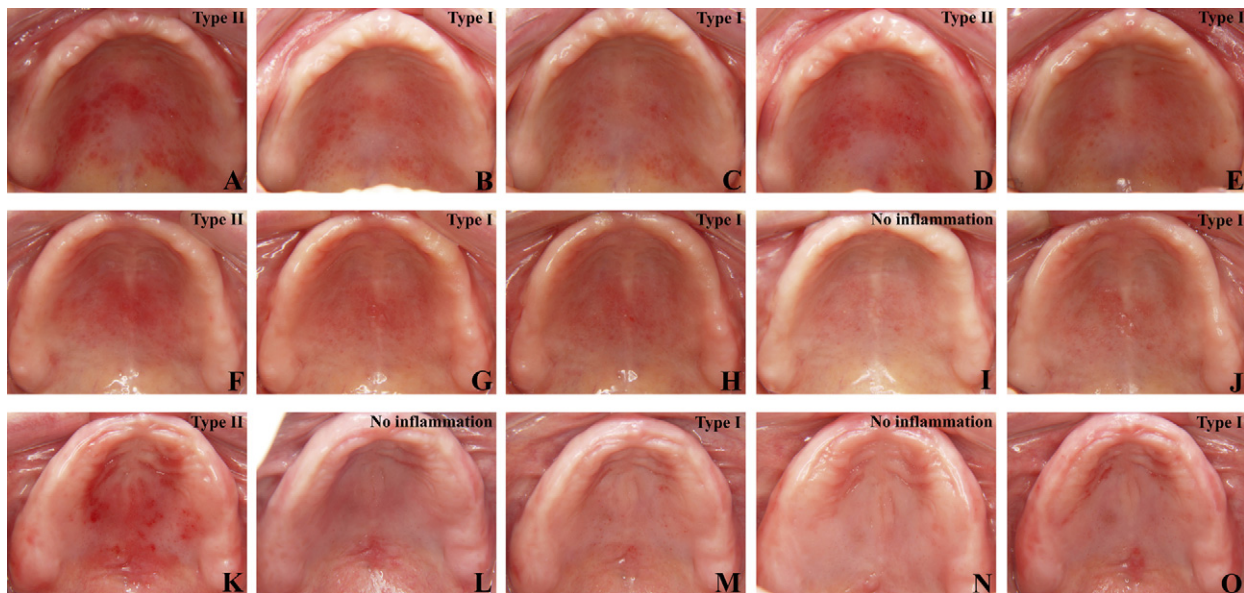
Fig. 1. Palatal mucosa of a patient from the **(A-E)** NYS, **(F-J)** MW1, and **(K-O)** MW3 groups at **(A, F, K)** baseline, **(B, G, L)** day 14, **(C, H, M)** day 30, **(D, I, N)** day 60, and **(E, J, O)** day 90, respectively.
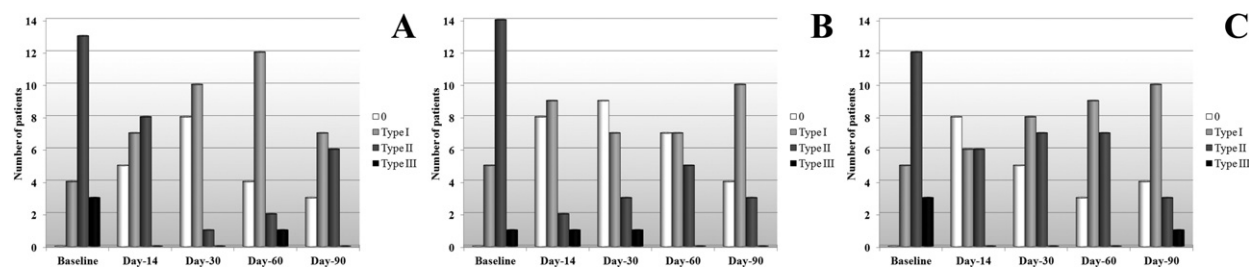

Fig. 2. Number of patients from **(A)** NYS, **(B)** MW1, and **(C)** MW3 groups scored according to the Newton criteria[42] for denture stomatitis.

are essential to maintain a low level of microorganisms on dentures, as well as oral health. Therefore, these instructions were continuously given to patients during this study. Because the statistical analysis showed homogeneity among the 3 groups evaluated, the potential contribution of these risk factors in the results was probably eliminated.

From the microbiologic and clinical assessments at the end of treatments (14 days) and follow-ups (30, 60, and 90 days), the present in vivo study found that microwave irradiation for disinfecting complete dentures, which were immersed in water during the irradiation (3 minutes at 650 W), was as effective as topical antifungal therapy with nystatin in the treatment of denture stomatitis. The results of quantitative mycologic cultures revealed a significant reduction in the cfu/mL values at the end of the treatments (day 14) in all of the groups evaluated. Thus, the use of denture microwave disinfection should be sufficient for many patients with denture stomatitis, without the need of

drugs, which should be reserved for more complicated situations. Nystatin is a polyene antifungal agent that binds to sterol components of the cell membrane to alter membrane permeability and allow outward leakage of vital intracellular components.[50] This alteration reduces the growth and multiplication of fungal cells and the adherence capacity of *Candida* to the epithelial cells and denture surfaces.[9,19] In terms of clinical application, however, the action of topical antifungal agents may be reduced due to the diluent effects of saliva and tongue movement.[14] The multiple daily dosing regimen of this topical agent and its unpleasant taste may have implications for compliance.[16] Furthermore, it is mainly designed to treat the oral mucosa. Despite these shortcomings, nystatin was effective in reducing *Candida* oral colonization, and no significant differences were noted among the treatment groups.

The results from the denture microwave disinfection groups are similar to those from other clinical studies in which denture microwave irradiation was used in the

Table VI. Percentage of patients allocated to each category in each period for the 3 groups

Period of time*	Category	NYS	MW1	MW3	P value†
			Group		
Day 14		(n = 20)	(n = 20)	(n = 20)	.9954
	+	40	40	50	
	+ +	25	30	20	
	+ + +	0	0	5	
	No	25	30	20	
	−	10	0	5	
	− −	0	0	0	
Day 30		(n = 16)	(n = 17)	(n = 18)	.9393
	+	56.25	35.29	50	
	+ +	31.25	41.18	11.11	
	+ + +	0	0	5.56	
	No	12.5	23.53	33.33	
	−	0	0	0	
	− −	0	0	0	
Day 60		(n = 16)	(n = 17)	(n = 18)	.9862
	+	37.5	11.76	44.44	
	+ +	18.75	47.06	16.67	
	+ + +	6.25	0	0	
	No	37.5	35.29	33.33	
	−	0	5.88	5.56	
	− −	0	0	0	
Day 90		(n = 16)	(n = 17)	(n = 18)	.9745
	+	31.25	41.18	38.89	
	+ +	6.25	23.53	22.22	
	+ + +	6.25	0	0	
	No	56.25	35.29	38.89	
	−	0	0	0	
	− −	0	0	0	

*The degree of severity of the infection scored on each period (days 14, 30, 60, and 90) was compared with that scored at baseline.
†Kruskal-Wallis test (α = .05).

Table VII. Percentage of patients cured and with recurrence, for all groups

Group	Day 14	Day 30	Day 60	Day 90	Recurrence Day 90–day 60/30/14*
		Cure			
NYS	25	43.75	25	18.75	50
MW1	41.18	47.06	41.18	23.53	52.94
MW3	38.89	27.78	16.67	22.22	38.89
P value	.5786	.4549	.3029	>.999	.7755

*The degree of severity of the infection scored at day 90 in relation to day 14, 30, or 60.

treatment of denture stomatitis.[8,10,14,18] Banting and Hill[14] associated microwave irradiation (1 minute at 850 W) of complete dentures with the use of topical nystatin and observed that the method was effective in reducing the quantity of invasive forms of *C. albicans* (pseudohyphae) found on the palatal mucosa of patients with denture stomatitis. During treatment (14 days), the complete dentures were submitted to 3 disinfection cycles and the patients used nystatin 3 times a day. Webb et al.[8] observed that, without the use of nystatin, daily denture microwave disinfection (10 minutes at 350 W) for a week significantly reduced the number of *Candida* spp. in complete dentures and in the palate of patients diagnosed with denture stomatitis. Likewise, in the present study, 6 disinfection cycles (MW3 group)

significantly reduced the cfu/mL values in both denture and palate after 14 days. In fact, only 2 disinfection cycles during 14 days (MW1 group) were sufficient to decrease the fungal load from dentures and palate. The advantage of such a protocol is a reduced number of patient visits compared with MW3. In another clinical study,[10] in which the dentures were irradiated at 650 W for 6 minutes, 3 times a week for 30 days, no mycelial forms and viable colonies of *Candida* spp. were detected after 2 weeks from the beginning of the treatment, regardless of the use of a topical antifungal (miconazole). These results supported the choice of 14 days of treatment as adopted in the present study.

According to the results of the biochemical identifications of *Candida* species, *C. albicans* was the most

frequent species identified in the oral cavity of individuals with clinical diagnosis of denture stomatitis. There are several reports in the literature showing that *C. albicans* is much higher than non-*albicans* species in individuals with denture stomatitis.[1,2,4,6−8,12] The high prevalence of *C. albicans* may be explained by the great capacity of this species to adhere to the oral mucosa[5,8−10,18,25] and particularly to the surface of acrylic denture base resins.[4−6,8,10,13,14,18,19,25] Over the past decades, an increase in non-*albicans Candida* species in individuals diagnosed with denture stomatitis has been observed.[1,2,4,6,8,12] In the present study, in addition to *C. albicans* species, several others were identified. Among them, *C. tropicalis* and *C. glabrata* were present in 25% and 22% of the individuals assessed, respectively. The high prevalence of these 2 non-*albicans* species should not be neglected, because they are associated with systemic infection (candidemia) as well as with a higher mortality rate than *C. albicans*.[51,52] Moreover, the presence of mixed populations of *Candida* spp. was observed in 48.3% of the individuals in the present study. The most frequent associations involved the species *C. albicans*, *C. glabrata*, and *C. tropicalis*. These findings demonstrated that these microorganisms were not only growing as single-species biofilms, but also as structured biofilm communities, in both palates and dentures. This points to the importance of biofilm development by different species of *Candida* in the pathogenesis of denture stomatitis. Biofilms may result in a higher pathogenic potential and resistance to antifungal agents and immune defense,[53] facilitating the onset of the infection.

Another important finding of the present study was the higher microbiologic colonization of complete dentures compared with palatal mucosa. Thus, removable dentures can be considered to be the main reservoirs of microorganisms, particularly of *Candida* spp., and eliminating these microorganisms from the surface of the dentures is imperative in the prevention and treatment of denture stomatitis. In addition, besides the quantitative colonization, a relevant difference in the qualitative colonization was found between dentures and palates. The presence of >1 species of *Candida* spp. was observed in 52.7% of the dentures of the individuals assessed, compared with 34.8% obtained from their palates. This could be due to the high ability of *Candida* spp. to adhere to and colonize acrylic surfaces.[5,6,10] Irregularities of acrylic resin, such as roughness and porosities, as well as hydrophobicity of fungal cells, may facilitate the retention of microorganisms, and thus dentures may act as a source of infection. This information combined with the clinical and microbiologic results of the present study reinforces the need that treatment of denture stomatitis should be primarily directed to the dentures.

When assessed by clinical scoring, all treatments were considered to be successful in treating patients with denture stomatitis. The improvement of the clinical signs of denture stomatitis was observed in 65% of individuals, and 25% cured, at the end of the nystatin treatment. Similarly, Bergendal and Isacsson[15] observed a significant reduction in erythematous areas of the palatal mucosa after treating denture stomatitis with nystatin. However, another study demonstrated that, when miconazole was used, no changes in clinical signs were observed.[10] It was also found that the reduction in clinical signs of denture stomatitis was more perceptible in the palatal mucosa of individuals who had their dentures submitted to the microwave disinfection procedure.[10] This trend was seen in the present study, where improvement in clinical signs occurred in 70% and 75% of patients from the MW1 and MW3 groups, respectively, and ~40% of were cured. During the follow-up period, improvement in clinical signs was maintained until day 90 for ≥43% of the patients from the NYS group and 58% and 60% of the patients from the MW1 and MW3 groups, respectively. This finding may be attributed to the reduction of fungal load from dentures and palates after the treatments associated with the patient compliance with the recommendations about oral and denture hygiene, including removing dentures for sleeping. Despite the relevant scores of improvement and cure of the infection, at the end of the follow-up period (day 90), recurrence of denture stomatitis was observed in almost 53%, 39%, and 50% of patients from the MW1, MW3, and NYS groups, respectively. This finding is in agreement with that reported by Banting and Hill,[14] who reported a recurrence rate of 56%. On the other hand, in the study of Neppelenbroek et al.,[10] the recurrence of denture stomatitis was not clinically observed in any of the participants; at the end of treatment (30 days), intraoral photographs obtained from patients who had their dentures irradiated were not different from those observed in other evaluation periods (60 and 90 days). It is important to emphasize that in that study the dentures were submitted to 12 disinfection cycles, whereas in the present study, they were submitted to a maximum of 6 cycles. Recurrence of denture stomatitis may be related to failure in eradicating the colonizing strain or reinfection by an exogenous strain. In addition, denture wearers are also *Candida* carriers even in the absence of clinical signs of infection,[2,4,6] which may lead to recontamination of the dentures and reinfection of the palatal mucosa.

The results of this clinical study can be of great interest to dentistry, because they indicate that micro-

wave disinfection of complete dentures can be used as an effective method for inactivating biofilm from the denture surfaces. Because microwave irradiation is a physical disinfection method, there may be no risk of inducing resistance of microorganisms. Its use prevents the undesirable effects of using antifungal agents, such as nausea, vomiting, and hepatotoxic and nephrotoxic effects,[20] in addition to being a simple, safe, and low-cost method. Moreover, considering that the microwave irradiation of dentures inactivates all microorganisms present in the biofilm,[25] the method can be widely used to prevent not only the denture stomatitis but systemic diseases caused by pathogenic microorganisms present in the denture biofilm, such as fungemia and aspiration pneumonia.[51,52,54]

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