Microwave Denture Disinfection Versus Nystatin in Treating Patients with Well-Controlled Type 2 Diabetes and Denture Stomatitis: A Randomized Clinical Trial

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Purpose: The aim of this randomized clinical trial was to compare the effectiveness of microwave denture disinfection and nystatin in the treatment of well-controlled type 2 diabetic patients with denture stomatitis in terms of microbiologic and clinical outcomes. ***Materials and Methods:*** Diabetic patients wearing maxillary complete dentures with denture stomatitis (n = 40) were divided into two groups: NYS (patients treated with topical nystatin 4 times/day for 14 days) and MW (patients who had their dentures microwaved [650 W for 3 minutes] 3 times/week for 14 days). Mycologic samples were taken from the palates and dentures of the patients for quantification and identification of *Candida,* and standardized photographs of the palates were taken for clinical analysis. Evaluations were repeated at baseline, the end of treatment (day 14), and throughout follow-up (days 30, 60, and 90). Microbiologic data were evaluated by analysis of variance using a random effects statistical model, Tukey post hoc test, and chi-square test (α = .05). Clinical resuts were analyzed using Mann-Whitney and Fisher exact tests (α = .05). ***Results:*** Both treatments were considered successful in reducing the clinical signs of denture stomatitis and significantly reduced the values of colony-forming units/mL from the palates and dentures at days 14 and 30. In addition, 40% of treated patients were cured by the end of treatment. No significant differences in the microbiologic and clinical outcomes were revealed between the two groups ($P > .05$). *C albicans* was the most predominant species isolated ($P < .01$), followed by *C tropicalis* and *C glabrata*. ***Conclusion:*** Denture microwave disinfection was as effective as nystatin for the treatment of diabetic patients with denture stomatitis. *Int J Prosthodont 2012;25:232–244.*

Diabetes mellitus is considered a global public health problem, and the number of adults with diabetes worldwide is expected to increase to 300 million in the next 15 years.[1] This chronic metabolic disorder causes multiple comorbidities and increases the risk of death in those whom it affects. Besides damaging many organs and systems in the body,[2] the consequences of diabetes are strongly associated with several local alterations in the oral mucosa that favor the development of important infections. Oral candidiasis is one of the most common opportunistic infections encountered in diabetic patients,[3] who are more susceptible to fungal infections[4] and show a higher prevalence of *Candida* colonization in the oral cavity compared with nondiabetic individuals.[4–9] A significantly higher incidence of *Candida* infection and increased levels of *Candida spp* were also found in diabetic patients who wore complete dentures,[4,10,11] increasing their vulnerability to *Candida*-induced denture stomatitis. In addition, diabetic patients with dentures had more non-*albicans Candida* species isolated than dentate diabetics.[7,8,11] *Candida spp* have the ability to adhere to the denture tissue surface, which then acts as a reservoir favoring yeast proliferation and enhancing their infective potential.[12,13] Dentures may also induce trauma,[14] thus increasing the permeability of the epithelium to soluble candidal antigens and toxins. As a result, the association of a complete denture and the local and systemic complications of the diabetes can increase the incidence of

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oral *Candida* disorders and the susceptibility of the denture-wearing diabetic patient.

Denture stomatitis is usually treated with topical application of nystatin, chlorhexidine, or miconazole before systemic drugs are used.[12,13,15–17] Nevertheless, failure of topical therapy is not infrequent, especially because of the diluent effect of saliva and movements of the tongue, both of which serve to reduce antifungal agents to subtherapeutic concentrations.[12] In addition, topical agents frequently require multiple doses, and their taste may cause nausea, which can lower patient compliance.[16] If a systemic approach is required, triazole drugs such as fluconazole are frequently used.[16,17] However, these medications must be administered with caution because they may cause hepatotoxic and nephrotoxic side effects.[18] It is also important to emphasize that the widespread use of these medications has promoted selection of resistant species by the development of mutations in yeasts[19] or by shifting colonization to more naturally resistant *Candida spp*, such as *C glabrata*, *C dubliniensis*, and *C krusei,*[20,21] reducing the effectiveness of treatment. The clinical relevance of this epidemiologic shift is the need to establish new strategies for control and management of infections caused by the different *Candida spp*.

While antifungal drugs are aimed at treating the oral mucosa, they do not erradicate the *Candida* that colonizes the denture.[12,13] Thus, the recurrence of infection shortly after treatment has been frequently observed and is attributed to the reemergence of the original infecting strain.[12,13,15–17] For all these reasons and to overcome the limitations and avoid the side effects of these standard medications, strict denture disinfection measures have been recommended for the treatment of denture stomatitis.[12,13,17,22] Microwave irradiation is a simple and inexpensive physical method of denture disinfection whose effectiveness has been demonstrated in vitro.[23,24] Dentures inoculated with different bacteria and *Candida spp*, including the intrinsically resistant *C glabrata*, *C dubliniensis*, and *C krusei*, were sterilized by microwave irradiation for 3 minutes at 650 W.[23,24] A recent in vivo study showed that this microwave regimen inactivated the denture biofilm of 30 individuals who were not diagnosed with denture stomatitis.[25] Moreover, emerging evidence from other clinical studies showed the effectiveness of microwave disinfection of complete dentures in treating patients with denture stomatitis.[12,13,22] The risks of reinfestation of the denture tissue surface and reinfection of the adjacent soft tissue were dramatically reduced in patients whose dentures were microwaved.[12,13] It is important to note that this microwave disinfection regimen (3 minutes at 650 W) had no detrimental effect on the flexural strength,[26] hardness,[27] dimensional stability,[28] or porosity[29] of the denture materials. Therefore, from these studies, an effective, drugless, and safe protocol for the treatment and prevention of denture stomatitis was established.

Although effective in noncompromised patients, microwave denture disinfection for treating denture stomatitis has not been evaluated yet in diabetics. In these patients, the increased number of available receptors for *Candida* as a result of salivary glucose levels[30] and the microvascular degeneration found in oral mucosa of diabetic patients[31] are local factors that facilitate the process of candidal adhesion, colonization, propagation, and mucosal infection. A decrease in salivary flow rate consequent to diabetes may further enhance candidal colonization[7] since saliva possesses several secretory components that inhibit *Candida* cell adhesion to epithelial cells.[32] Some systemic factors, such as a dysfunction in candidacidal activity of neutrophils[33] resulting from decreased phagocytosis, intracellular killing, bactericidal activity, and chemotaxis, may also render the diabetic patient even more prone to candidal infection. For these reasons, the course of infection in diabetic patients is more complicated, and the effectiveness of microwave disinfection in treating denture stomatitis may be more complex and unpredictable. This randomized clinical study tested the hypothesis that microwave disinfection of complete dentures could be as effective as the more conventional topical antifungal medication (nystatin) in the treatment of well-controlled type 2 diabetic patients with denture stomatitis. The effectiveness was assessed microbiologically through the reduction in *Candida* counts on patients' palates and denture surfaces and clinically by means of resolving the clinical signs of the infection. The prevalence of *Candida spp* identified in diabetics was also evaluated.

Materials and Methods

This was a parallel, randomized clinical trial comparing the effectiveness of denture microwave disinfection and nystatin in the treatment of diabetic patients with denture stomatitis. The procedures carried out follow the criteria of Resolution 196/96 of the Brazilian Health Ministry, which regulates research involving human subjects. The project was approved by the ethics committee of the Araraquara Dental School, UNESP–Univ Estadual Paulista, Araraquara, São Paulo, Brazil (no. 06/2006). All participants were made aware of the objectives of the study as well as probable risks and benefits. All subjects entered the study voluntarily and signed an informed consent form before their enrollment.

Table 1 Recommendations for Adults with Diabetes Mellitus

Test	Goal value
Fasting blood glucose level	90–130 mg/dL
Postprandial capillary plasma glucose	< 180 mg/dL
Glycosylated hemoglobin level	< 7%
Serum lipids	
LDL	< 100 mg/dL
Triglycerides	< 150 mg/dL
HDL	> 40 mg/dL
Serum creatinine	0.4–1.3 mg/dL
Urine	
Protein	Absent
Glucose	Absent
Nitrite	Absent
Leukocytes	< 10 units/mm^3

LDL = low-density lipoprotein; HDL = high-density lipoprotein.

Patients were recruited from UNESP–Univ Estadual Paulista and from public health centers in Araraquara, Brazil. Only patients with well-controlled type 2 diabetes ranging between 18 and 75 years of age who wore maxillary complete dentures and were diagnosed with denture stomatitis were included. A comprehensive oral examination of the patients was performed by the same investigator, and their mucosal characteristics were initially classified according to the criteria proposed by Newton: type I (initial stage of localized pin-point hyperemia), type II (diffuse erythema confined to the denture-bearing surface), and type III (inflammatory papillary hyperplasia).[34] All patients were also evaluated in relation to their medical care of diabetes. Based on the recommendations of the Standards of Medical Care in Diabetes 2007,[35] four clinical chemistry tests were performed to assess the degree of diabetic control: fasting blood glucose level, postprandial capillary plasma glucose, glycosylated hemoglobin level, and serum lipids. Serum creatinine and urine tests were also carried out to evaluate the systemic health condition of the diabetic individuals. Only patients fulfilling the recommended goals for the tests (Table 1) and the inclusion criteria were selected. Individuals who had received or were currently receiving treatment with antibiotics, antifungals, or steroids in the past 3 months; patients with anemia, immunosuppression, or cancer therapy (radio- or chemotherapy); and those wearing the same denture for more than 30 years were excluded from the study. Personal, medical, and dental histories of the patients were recorded. Data collection was done at Araraquara Dental School.

Sample Size Calculation and Randomization

A sample size calculation was performed by using data from a pilot study in which 10 patients were recruited (5 in each group) and different tests were performed. The sample size was calculated using two-way analysis of variance (ANOVA) with a random effects statistical model and repeated measures. One factor was group, with two levels (nystatin and microwave irradiation); another factor was time period, with five levels (baseline and days 14, 30, 60, and 90). A minimum sample size of 20 patients in each group enabled detection of an effect size of 0.25 and a within-group correlation of –0.15 at an α value of .05 with a statistical power of 86%. Further, a stochastic simulation by the Monte Carlo method[36] using 12,000 replications was used to determine the power of the Fisher exact and Mann-Whitney tests, which were also used to analyze the pilot results. A sample size of 20 patients in each group would allow detection of a difference between groups at an α of .05 with at least 89% statistical power.

To create groups of patients that were similar with regard to 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,[14] smoking habits,[37] xerostomia,[7,14,32] denture hygiene habits,[38] and nocturnal wear of the dentures.[14] Denture hygiene was classified as good (absence of plaque) or poor (presence of removable and/or nonremovable plaque on the inner or outer denture surface).

Interventions

According to the stratified randomization list, the 40 diabetic patients were assigned randomly to one of the two experimental groups. Patients in group NYS were treated with topical antifungal medication (nystatin; Cristália Produtos Químicos Farmacêuticos). Patients were instructed to remove their dentures from the oral cavity and rinse with 1 mL of the suspension (100,000 IU/mL) for 1 minute four times a day for 14 days. Patients were informed not to swallow the suspension following rinsing. In group MW, each patient had his or her maxillary complete denture individually immersed in a beaker containing 200 mL of sterile distilled water. Each beaker was placed on the rotation plate in a domestic microwave oven (Model Sensor Crisp 38, Brastemp) and irradiated at 650 W for 3 minutes[23–25] three times per week for 14 days. After treatment, patients from both groups were followed up monthly for 3 months. Over the experimental

period, all patients were instructed to scrub their dentures with coconut soap followed by toothpaste after every meal and immerse the dentures in filtered water (200 mL) overnight. Patients received verbal and written instructions describing these routine care procedures and how to use the medication.

To strictly control the diabetes over the 3-month duration of this study, the fasting blood glucose level and postprandial capillary plasma glucose tests were performed every month, while the glycosylated hemoglobin level was measured again at 90 days.

Outcomes

The primary outcomes of interest were the *Candida* colony counts from the palates and denture surfaces, quantified in colony-forming units (cfu)/mL, and severity of infection of the palatal mucosa, classified according to the criteria proposed by Newton[34] (0 = absence of palatal inflammation or types I, II, and III), measured before treatment (baseline), at the end of treatment (day 14), and throughout follow-up (days 30, 60, and 90). The second outcome was the prevalence of *Candida spp* identified in the two groups of diabetics at the same intervals.

Microbiologic Procedures

Oral swab samples were collected from the palates and tissue surfaces of the dentures of all patients.[4,13,17,22,25] Each swab was placed into a test tube containing 5 mL of 0.9% sterile saline and vortexed for 1 minute to suspend the organisms from the swab. For quantification of *Candida* counts, the microbial material of the palates and dentures was diluted using 0.9% sterile saline (10^{-1} to 10^{-3}). Aliquots of the original sample suspension and each dilution (25 µL) were plated in duplicate on Sabouraud dextrose agar (SDA) containing chloramphenicol. The plates were incubated at 30ºC for 48 hours, microbial colony counts of each plate were quantified using a digital colony counter (CP 600 Plus, Phoenix Indústria e Comércio de Equipamentos Científicos), and the cfu/mL was then calculated. Colonies were also submitted to identification procedures for *Candida spp*. Aliquots of 50 µL from the original sample suspension from the palates and dentures were plated on CHROMAgar *Candida*[4,8,25] and incubated at 30ºC for 5 days. Colonies were presumptively identified by colony color and macromorphology. Thereafter, biochemical tests were performed to confirm all identifications. One colony of each color type on CHROMAgar *Candida* was transferred onto fresh SDA for purity. After 48 hours at 30ºC, yeast isolates were identified by the pattern of assimilation of a variety of carbon and nitrogen sources using the ID32C yeast identification system (bioMérieux)[4,22] and by the micromorphologic characteristics produced on corn meal agar with Tween 80 (HiMedia Laboratories).[4,25] In addition, green colonies on CHROMAgar *Candida* were submitted to hypertonic Sabouraud broth testing[39] to discriminate *C albicans* and *C dubliniensis*. All microbiologic procedures were carried out by the same operator.

Clinical Procedures and Blinding

Clinical assessment of the efficacy of the different treatments was carried out by taking standardized color photographs of the palatal mucosa of each patient. These photographs were taken before treatment (baseline), at the end of treatment (day 14), and throughout follow-up (days 30, 60, and 90). All photographs were taken using the same digital camera (Canon EOS Rebel XTI, Canon), by the same operator, and under the same conditions (place, light, angle, and patient position) to facilitate their reproducibility. After standardization of the images, two independent observers were engaged to blindly analyze the five photographs taken of each patient. In this blinded analysis, the observers were instructed to classify the mucosal characteristics of each patient according to the criteria proposed by Newton.[34] Observers were blind to risk factors, treatment group, and period of evaluation.

Statistical Analysis

Demographic characteristics of patients and risk factors were analyzed statistically to ensure homogeneity between the groups by means of the Student *t* test, Fisher exact test, and Wilcoxon signed rank test. Differences were considered statistically significant at a value of $P < .05$.

The numbers of cfu/mL obtained in the microbiologic tests were $\log_{10}$ transformed ($\log_{10}$ cfu/mL) to achieve a normal distribution. A two-way ANOVA using a random effects statistical model was performed to determine whether the treatments differed significantly in their effects on the $\log_{10}$ cfu/mL values over the study period. The values obtained from the palates and dentures were analyzed separately. When differences were found, the Tukey post hoc test was implemented, and $P < .05$ was taken as significant. Clinical significance of both treatments on the microbiologic reduction on the palates and dentures was also evaluated by the treatment's effect size, which was determined by taking the standardized mean

Table 2 Demographic Characteristics and Risk Factors

	Mean age (y)	Sex (% female)	Mean age of dentures (y)	Patients with xerostomia (%)	Nonsmokers (%)	Patients with nocturnal wear of dentures (%)	Patients with poor hygiene habits (%)
NYS	62.6	85	13.1	60	85	80	55
MW	62.2	90	14.3	75	90	75	50
P	.8708*	> .9999†	.3701‡	.5006†	> .999†	> .999†	.7636†

*Student *t* test.
†Fisher exact test.
‡Wilcoxon signed rank test.

difference of $\log_{10}$ cfu/mL between pre- and post-treatment results for each group and dividing by the standard deviation of pretreatment results.[40] To interpret the resulting number, this general guide was used: < 0.1 = trivial effect, 0.1 to 0.3 = small effect, 0.3 to 0.5 = moderate effect, and > 0.5 = large difference effect.[13]

The percentage of different species of *Candida* isolated from the palates and dentures was compared using a nonparametric chi-square test. Differences were considered statistically significant at a value of $P < .05$.

The clinical efficacy of treatment was evaluated by a blinded analysis of the five color photographs taken of the palatal mucosa of each patient. The evaluation of the mucosal characteristics of each photograph by the two blinded observers was scored (0 and types I, II, and III), and their degrees of correlation and concordance were estimated to provide a measure of the reliability and validity of the results. The Kappa measure of agreement (κ) evaluated the interobserver concordance, and its values range between 0 and 1 (perfect match: $\kappa = 1$, almost perfect: $0.81 < \kappa > 1$, substantial: $0.61 < \kappa > 0.80$, moderate: $0.41 < \kappa > 0.60$, fair: $0.21 < \kappa > 0.40$, slight: $0 < \kappa > 0.20$, and no concordance: $\kappa = 0$).[41] The Kendall rank correlation coefficient (τ) evaluated the interobserver correlation, that is, the similarity of scores when ranked by each observer, and its values range between –1 and 1 (perfect agreement: $\tau = 1$, no correlation: $\tau = 0$, and perfect disagreement: $\tau = -1$).[42] The coefficient of concordance (%) was calculated to provide the percentage of same scores among the observers. The analysis of the degree of interobserver correlation and concordance was performed considering the scores recorded for the period of treatment (baseline and day 14) and for the entire trial period (baseline and days 14, 30, 60, and 90).

The degrees of severity of the mucosal characteristics of patients scored at baseline from the NYS and MW groups were compared using the Fisher exact test to ensure homogeneity ($\alpha = .05$). To analyze the magnitude of the effect of treatment on the evolution of the disease over time, a categoric variable was created. This categoric variable was represented by the decrease/increase in infection rates from each period (day 14 to day 90) in relation to baseline. The following general categories were used: +, decrease in infection score by 1 point (ie, type III to II, type II to I, or type I to 0); ++, decrease in infection score by 2 points (ie, type III to I or type II to 0); +++, decrease in infection score by 3 points (ie, type III to 0); No, no change in infection status; –, increase in infection score by 1 point (ie, type I to II or type II to III); and – –, increase in infection score by 2 points (ie, type I to III). The percentage of patients in each category was determined for each period, and comparisons between groups were made using the Mann-Whitney test ($\alpha = .05$). In addition, the percentages of cured patients and patients with recurrence were also compared between the two groups by means of the Fisher exact test ($\alpha = .05$). "Cure" was considered absence of palatal infection and was evaluated over time; "recurrence" was considered increase in infection at day 90 in relation to days 14, 30, or 60. In the present study, the magnitude of the treatment's effect on the disease over time was also measured by percent improvement. It was defined that an improvement of 30% or more corresponded to a clinically significant difference.[13] The outcome measure of success was defined as an increase of 30% in the proportion of patients with a decrease in infection score by 2 and 3 points at the end of treatment and at follow-up.

Results

Demographic Characteristics and Risk Factors

Table 2 shows that at the time of the initial evaluation, the mean age of patients was 62.6 ± 7.45 years (range: 48 to 75 years) in the NYS group and 62.2 ± 6.69 years (range: 46 to 72 years) in the MW group. In both groups, the number of women was higher than that of men, the mean age of dentures was greater than 10 years, most patients complained of xerostomia, and few smokers participated. Before the onset of the

Table 3 Mean (Standard Deviation) *Candida* Colony Counts in $\log_{10}$ cfu/mL from Palates and Dentures Following Treatment and Treatment Effect Size

Group	Location	Baseline	Day 14	Day 30	Day 60	Day 90	Treatment effect size
NYS	Palate	1.21 (1.33)	0.30 (0.75)*	0.37 (0.76)*	0.59 (0.83)	0.85 (1.12)	0.98
	Denture	4.38 (1.04)	1.59 (1.86)*	2.91 (1.08)*	3.27 (1.54)	3.34 (1.81)	2.85
MW	Palate	0.99 (1.17)	0.59 (1.08)*	0.58 (1.09)*	0.98 (1.16)	1.26 (1.14)	0.48
	Denture	3.80 (0.86)	1.45 (1.69)*	2.62 (1.96)*	3.56 (1.85)	3.67 (1.87)	2.12

*Significantly different from baseline (Tukey post hoc test, $P < .05$).

Table 4 ANOVA Using a Random Effects Statistical Model for Mean Values of $\log_{10}$ cfu/mL from Palates and Dentures

	Numerator *df*	Denominator *df*	F	*P*
Palate				
(Intercept)	1	156	56.8789	.0000
Period of time	4	156	3.6765	.0069*
Treatment	1	38	0.7600	.3888
Interaction time × treatment type	4	152	0.5410	.7059
Denture				
(Intercept)	1	156	647.5690	.0000
Period of time	4	156	21.9381	.0000*
Treatment	1	38	1.3351	.2551
Interaction time × treatment type	4	152	0.9910	.4143

*Statistically significant.

Table 5 Frequency Distribution (%) of Different Species Identified in the Palates and Dentures*

	Palatal mucosa	Denture surface
C albicans	43.0[a]	75.5[a]
C tropicalis	2.0[b]	13.5[b,c]
C glabrata	4.5[b,c]	6.0[b]
Other[†]	8.5[c]	11.5[b,c]
Yeast-negative samples	53.0[a]	20.0[c]

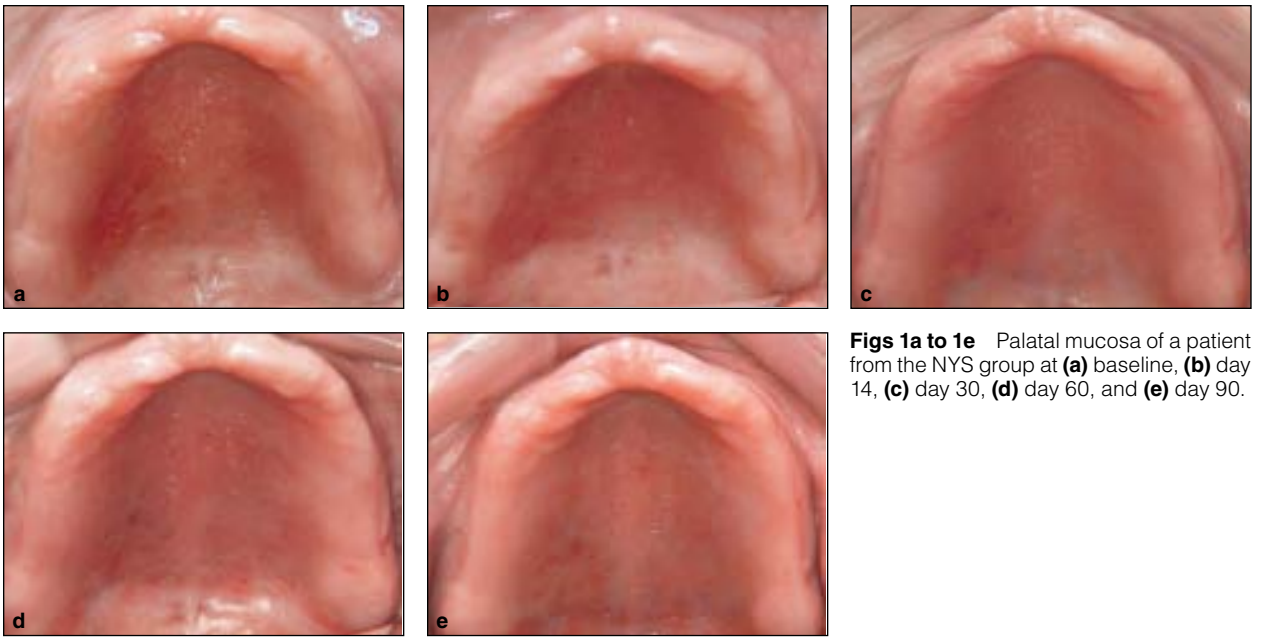
*Horizontal bars connect values that were not significantly different ($P > .05$). Vertically, values designated with the same superscript were not significantly different ($P > .05$).
[†]Other *Candida* species and yeasts from the genus *Cryptococcus*, *Saccharomyces*, *Kloeckera*, and *Rhodotorula*.

study, a large percentage of patients wore their dentures at night, and at least 50% of patients from each group showed poor denture hygiene habits. Stratified randomization ensured that the subsamples for the two treatments were well balanced for both demographic characteristics and risk factors, since the statistical analysis showed homogeneity between the two groups of study ($P > .05$).

Microbiologic Outcomes

The mean values of *Candida* colony counts in $\log_{10}$ cfu/mL obtained from the palates and dentures of both treatment groups are given in Table 3. Two-way ANOVA showed that there were no significant differences in the mean values of $\log_{10}$ cfu/mL when the factor treatment group (NYS or MW) was analyzed for both palates ($P = .3888$) and dentures ($P = .2551$), while statistically significant differences ($P < .01$) were found when the factor period of time was evaluated (Table 4). The Tukey post hoc test (Table 3) showed that both treatments reduced the mean values of $\log_{10}$ cfu/mL from the palates and dentures significantly at days 14 ($P = .0293$ and $P < .001$, respectively) and 30 ($P = .0371$ and $P < .001$, respectively). At days 60 and 90, the mean values of $\log_{10}$ cfu/mL from the palates and dentures were not significantly different from their respective baseline data ($P > .05$). It can also be observed that the effect size of treatments was large for the palates of group NYS (0.98) and moderate for the palates of group MW (0.48) (Table 3). For dentures, the effect size was very large for both groups (NYS: 2.85, MW: 2.12).

Table 5 shows that *C albicans* was the main species encountered ($P < .01$), occurring in 43% and 75.5%

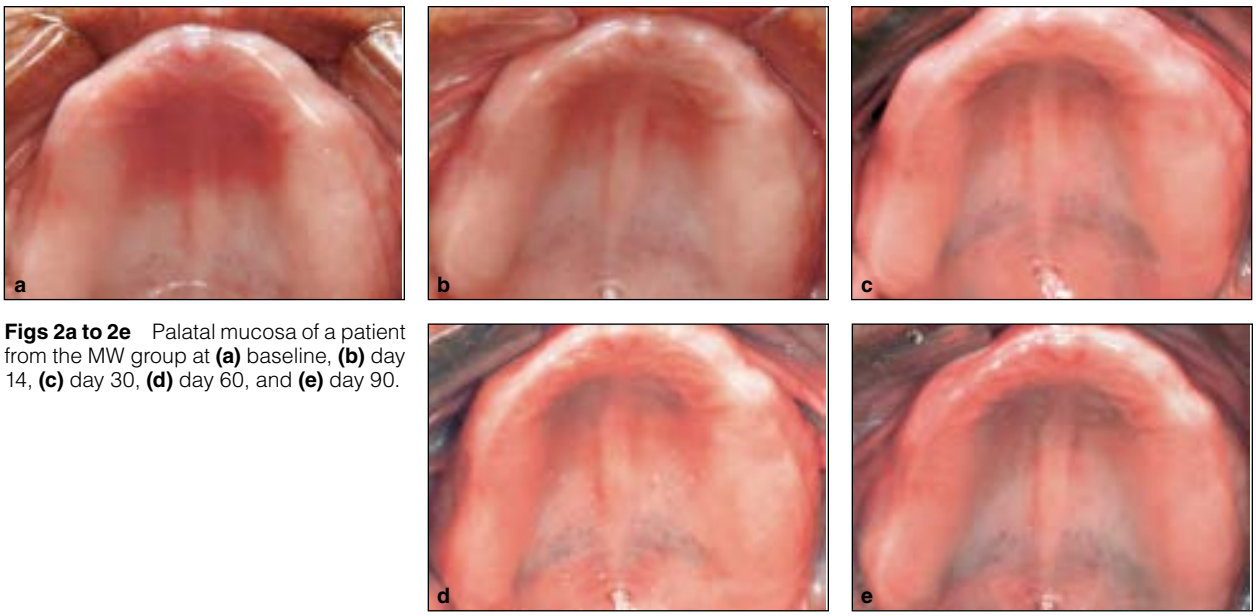


Figs 1a to 1e Palatal mucosa of a patient from the NYS group at **(a)** baseline, **(b)** day 14, **(c)** day 30, **(d)** day 60, and **(e)** day 90.

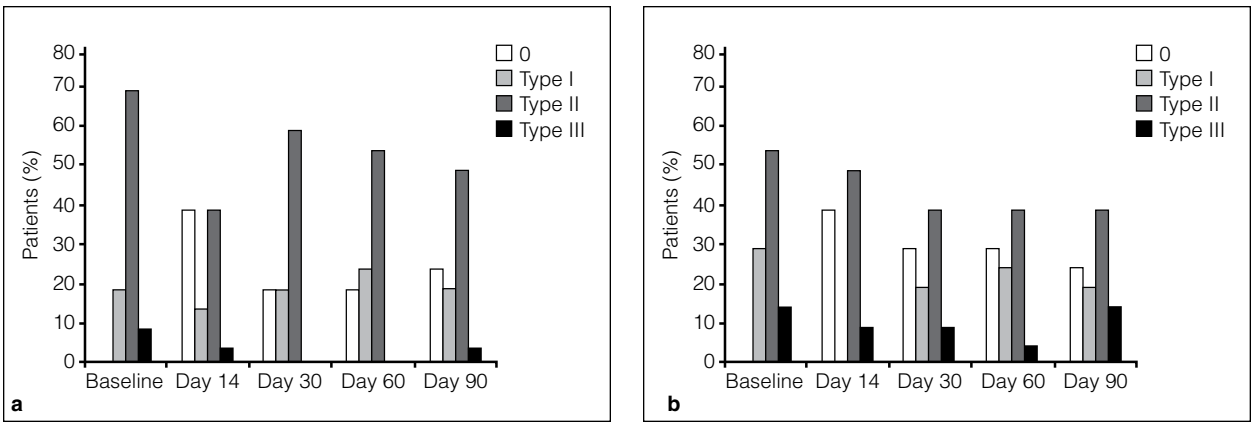
of palates and dentures, respectively. *C tropicalis* and *C glabrata* were the most common non-*albicans* species isolated from the palate and denture samples, with no significant differences between their prevalence ($P > .05$). The frequency distributions of *C albicans* and *C tropicalis* isolated from the dentures were significantly higher than those obtained from the palates ($P < .01$). In addition, significantly more yeast-negative samples were collected from the palates ($P < .01$). Other *Candida* species (*lusitanae*, *colliculosa*, *famata*, *parapsilosis*, *krusei*, *pelliculosa*, *sake*, *rugosa*, *zeylanoides*, *utilis*, and *guilliermondi*) and additional yeasts from the genus *Cryptococcus* (*laurentii*, *humicola*, and *albidus*), *Saccharomyces* (*cerevisiae* and *kluyverii*), *Kloeckera apiculata*, and *Rhodotorula* were detected at a lower rate. Fifteen participants (37.5%) had more than one species of yeast isolated from the palates and dentures. The yeast mixtures isolated were *C albicans* + *C tropicalis* (41%), *C albicans* + *C glabrata* (25%), and *C albicans* + *C glabrata* + *C tropicalis* (14%).

Clinical Outcomes

Considering the scores recorded for the treatment period (baseline and day 14), there was a substantial degree of interobserver concordance ($\kappa = 0.61$)[41] and excellent degree of interobserver correlation ($\tau = 0.85$, coefficient of concordance = 75%).[42] When the entire trial period was evaluated, there was a decrease in the degree of concordance ($\kappa = 0.42$), which was still considered moderate.[41] The degree of interobserver correlation was also satisfactory ($\tau = 0.77$, coefficient of concordance = 60%),[42] indicating a similar consistent trend between the observers' scores. Illustrative images of the photographs scored by the observers are given in Figs 1 and 2. A comprehensive description of the frequency distribution of the Newton classification for denture stomatitis over time is given in Figs 3a and 3b. The Fisher exact test showed homogeneity between the two groups ($P = .6514$) for the mucosal characteristics of patients at baseline. The Mann-Whitney test showed no significant differences ($P > .05$) between the magnitude of the clinical efficacy of NYS and MW on the evolution of the disease over the time (Table 6). Both treatments were considered successful in treating denture stomatitis. At least 50% of all treated patients showed a decrease in infection score from the end of treatment (day 14) to day 60. At the end of follow-up (day 90), approximately 42% of treated patients still showed a decrease in infection score, whereas approximately 60% of patients demonstrated signs of infection. Among them, 46% showed no change and 13% increased their infection score. The Fisher exact test showed that there were no significant differences in the percentage of cured patients among the two groups (Table 7). At the end of treatment, 40% of patients from the NYS and MW groups were cured. Throughout follow-up, the NYS group showed that 20% of patients were cured; in the MW group, 30% of patients were cured at day 30 and day 60 and 25% at day 90. Considering the percentage of patients with recurrence, the Fisher exact test showed that there were no significant differences between the



Figs 2a to 2e Palatal mucosa of a patient from the MW group at **(a)** baseline, **(b)** day 14, **(c)** day 30, **(d)** day 60, and **(e)** day 90.



Figs 3a and 3b Percentage of patients in groups **(a)** NYS and **(b)** MW scored according to the Newton criteria[34] for denture stomatitis.

two groups (Table 7). At day 90, 40% of patients from the NYS group and 55% from the MW group showed an increase in infection score after treatment.

Discussion

In the present sample, the mean age of patients was 62.4 years. This is in agreement with other studies[5,6,13] and also with the observation that older patients had an increased risk of yeast infection resulting from the greater number of denture wearers starting at the age of 50.[4,8] In both groups evaluated, the number of women was more than five times higher than that of men. Other studies also found that denture stomatitis was observed more often in women than in men.[6,8,13,14,43] The hormonal factor and the great incidence of iron deficiency in women[12,14] as well as the fact that women seek dental treatment at a higher rate than men[44] have been suggested as possible reasons for this greater incidence. With regard to the risk factors, the mean age of dentures was greater than 10 years in both groups. The age of dentures has been related to a higher occurrence of denture stomatitis.[14,43] Recently, it was verified that only 25% of individuals using dentures for less than 1 year were diagnosed with denture stomatitis, while more than 84% of those using dentures for more than 5 years had the disease.[43] When considering the other risk factors for denture stomatitis, a large percentage of patients complained of xerostomia and were nonsmokers. Before the onset of the

Table 6 Percentage of Patients Allocated to Each Infection Category in Each Period for Both Groups

Period*	Category	Group NYS	Group MW	$P^{\dagger}$
Day 14–baseline	+	40	25	.8182
	++	20	15	
	+++	0	5	
	No	40	40	
	–	0	15	
	– –	0	0	
Day 30–baseline	+	45	20	> .9999
	++	5	30	
	+++	0	0	
	No	45	25	
	–	5	25	
	– –	0	0	
Day 60–baseline	+	45	35	.4394
	++	5	20	
	+++	0	5	
	No	50	20	
	–	0	15	
	– –	0	5	
Day 90–baseline	+	45	5	.6667
	++	5	20	
	+++	0	5	
	No	40	55	
	–	10	10	
	– –	0	5	

*The degree of severity of the infection scored at each period (days 14, 30, 60, and 90) was compared to that scored at baseline.
†Mann-Whitney test.

Table 7 Percentage of Patients Cured and with Recurrence

	Cured				Recurrence*
	Day 14	Day 30	Day 60	Day 90	Day 90–day 60/30/14
NYS	40	20	20	20	40
MW	40	30	30	25	55
P	.4815	.4079	.6548	.820	.5273

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

study, nocturnal wear of dentures was observed for a large percentage of patients, and at least 50% of them showed poor denture hygiene habits. Since no statistically significant differences between the groups were found for the risk factors, their potential contribution in the development of denture stomatitis[7,14,32,37,38] was similar in both groups and probably had no influence on the outcomes. It is important to mention that over the experimental period, all patients were instructed to scrub their dentures with coconut soap followed by toothpaste after every meal and immerse the dentures in filtered water overnight. Although these recommendations may constitute a form of infection control, evidence from the study of Neppelenbroek et al[13] indicated that scrubbing the dentures after every meal and immersing them in filtered water overnight for 30 days had no effect on proportion of mycelial forms and cfu/mL of *Candida* from the palates and dentures of patients with Newton type I denture stomatitis. Furthermore, it was shown that inflammation of the palatal mucosa was not improved. Thus, maintenance of good denture hygiene and removal of dentures for sleeping alone may not be sufficient to treat denture stomatitis.

No significant differences in the microbiologic and clinical outcomes of treatment were revealed between the two groups. Thus, the tested hypothesis was accepted. In terms of reducing the *Candida* counts at the end of treatment, both nystatin and microwave disinfection significantly reduced the values of cfu/mL from the palates and dentures. In addition, the cfu/mL remained lower than baseline levels at day 30 regardless of treatment. These findings are in agreement with those of Webb et al,[22] who verified that microwave desinfection of dentures on a daily basis for 1 week reduced the numbers of *Candida* on cultures from the palates and dentures of patients with denture stomatitis. Treatment with nystatin for 14 days also decreased the amount of yeast colonies from the palates and dentures of patients.[15] Combining microwave disinfection of complete dentures and topical use of nystatin[12] or miconazole[13] also reduced the cfu/mL values and the invasive form of *Candida* (pseudohyphae) from the palates and dentures.

The mechanisms of action of the treatments used in the present investigation are very different. Nystatin, a polyene antimycotic drug, inhibits the biosynthesis of ergosterol and creates pores in the fungal membrane,[45–47] thus affecting the integrity of the yeast cell wall, causing leakage of intracellular constituents.[47] These changes would not only reduce the ability of candidal adhesion to buccal epithelial cells[46] and denture acrylic resin surfaces[45] but also supress active budding and multiplication.[45,46] Further, nystatin can perturb germ tube formation,[48] modulate the cell surface hydrophobicity,[49] and supress the proteolitic activity of *Candida*.[50] Unlike nystatin, microwave irradiation is a physical method for prosthesis disinfection, and its lethal action is well established in the literature.[13,23–25] One of the most important advantages of microwave irradiation, given that it is a physical method of disinfection, is that the emergence of resistant microorganisms would be avoided. Although the mechanism of destruction is not completely understood, the lethal effects of such radiation have been attributed to a combination of effects. Some investigators stated that the extremely elevated internal

temperatures produced by the vibration of water molecules of the microbial cells when they are exposed to microwaves are responsible for the changes in cell morphology and cell disintegration.[51] In addition, depending on the composition and volume of their surrounding medium, the cells may be selectively heated by microwave irradiation.[52] Others believe that nonthermal mechanisms are also involved,[52,53] and that microwaves may cause a mechanical disruption of the cell wall because of oscillations of the cells in the electromagnetic field.[53] A recent study also verified that microwave irradiation of *Candida* suspensions produced changes in the structural integrity and permeability of the cell membrane and cell metabolism, resulting in cell death.[54] Although both treatments were effective in reducing the colonization of the palates and dentures, microwave disinfection may provide further advantages over nystatin. Nystatin acts on the oral mucosa, which is often less colonized than dentures.[12,13,22] In addition, the multiple daily dosing regimen and unpleasant taste may have implications for compliance.[16] Further, the therapeutic concentration of nystatin can be reduced by the diluent effect of saliva and the cleansing action of the oral musculature, leading to failure of topical therapy.[12] While nystatin is fungicidal, investigations have demonstrated that microwave irradiation produces a broad, nonselective activity against several microorganisms, including several *Candida spp*,[23–25] *Staphylococcus aureus,*[23,25] *Pseudomonas aeruginosa,*[23,25] *Bacillus subtilis,*[23] and *Escherichia coli.*[52] In spite of infection by *Candida* being considered the main etiologic factor of denture stomatitis, the presence of other microorganisms may also be secondarily involved in the pathogenesis of this lesion.[17,55–57] In denture stomatitis, the bacteria possibly favor the adhesion of blastopores (commensal) to the tissue surfaces of dentures by coaggregation.[57] With fungal adhesion, there is an increase in microflora virulence by synergetic interaction, and the blastopores alter their morphology to mycelial, which results in damage to the epithelial cells, and consequently, invasion of the buccal tissues.[57] Therefore, the treatment of denture stomatitis by means of microwave irradiation should simultaneously eliminate the mycelia *Candida* and inhibit bacterial growth in the tissue surfaces of dentures.[22] However, microorganisms other than *Candida* were not evaluated in the present study. This may help explain why the microbiologic outcomes were the same between the nystatin and microwave irradiation groups.

Similar to the findings obtained from noncompromised patients,[4,9,14,22] *C albicans* was by far the predominant yeast isolated from the dentures and palates of the diabetics of this study. *C albicans* is also the most common species isolated in diabetics overall,[4,6–8,10,11] in diabetics with denture stomatitis,[5,9] and in denture wearers with or without denture stomatitis.[14,22] This species is the most virulent and pervasive of all *Candida spp*,[58] which is the reason for its preeminent position in the hierarchy of prevalence. As observed by other investigators,[5,6,8,10,11,14,22] in the current study, non-*albicans* species were also isolated from both palates and dentures, with *C tropicalis* and *C glabrata* being the most prevalent.[5,8,10,14,22] The pathogenicity of *C tropicalis* and *C glabrata* cannot be underestimated, since these two non-*albicans* species have the ability to cause fungemia in humans and are associated with a higher mortality rate than *C albicans*.[59,60] Therefore, more attention must be paid to their appearance. Additional yeasts from the genus *Cryptococcus*, *Saccharomyces*, *Kloeckera*, and *Rhodotorula* were also detected, which is in agreement with other reports.[8,9,14] In the present study, 37.5% of patients had more than one species of yeast on their palates and dentures. The yeast associations noted in the present study were *C albicans* together with *C tropicalis* and/or *C glabrata*, confirming trends found at other institutions.[4,7,14,22] These findings demonstrated that these microorganisms were not only growing as single-species biofilms, but also as structured biofilm communities in both the palates and dentures. This recalls the importance of biofilm development by different species of *Candida* in the pathogenesis of denture stomatitis, since it has the ability to increase resistance of yeasts to antimicrobial agents and immune challenge,[61] facilitating the onset of the infection. It is important to emphasize that both treatments reduced the density of colonization on the palates and dentures significantly, showing that MW and NYS are effective against biofilms of *Candida spp*, including the intrinsically resistant *C glabrata* and *C krusei* identified here.

The frequency distributions of *C albicans* and *C tropicalis* isolated from the dentures were significantly higher than those obtained from the palates, and significantly more yeast-negative samples were collected from the palates. These results are consistent with those of the cfu/mL assay, which showed that the palatal cultures exibited lower cfu/mL than the denture cultures under all conditions. This difference has also been reported by others[12,13,22] and can be partially attributed to the sample technique (swab sampling) employed in the present study.[4,13,17,22,25] A recent study that evaluated the cellular interactions of the yeast-epithelial interphase using a reconstituted human oral epithelium model verified that *C albicans* yeasts can invade the tissue through hyphal penetration into the superficial epithelium together with features of cellular

internalization of yeasts.[62] Thus, it can be assumed that swabbing of the palatal mucosa using a delicate cotton swab may not have removed all *Candida* cells. Hence, as with the oral rinse technique that has been used for sampling of the mouth for *Candida,*[6,8] this technique may be limited in providing precise quantitative information from the palatal mucosa. Virulence factors of *Candida spp* that enhance their adherence potential on acrylic resin surfaces (cell surface hydrophobicity[63] and ability to form biofilm[61]) can also be suggested as a contributing factor for the colony count difference between palates and dentures. Despite this difference, mucosal infection in denture stomatitis has been mainly associated with the proliferation of mycelial forms of *Candida* on the tissue surfaces of removable dentures,[12,13] reinforcing the notion that inactivation of the biofilms attached to the denture surfaces is essential to prevent and treat this disease.

When assessed by clinical scoring, both treatments were considered successful in treating denture stomatitis in diabetic patients. At least 50% of patients showed an improvement in infection score from the end of treatment (day 14) to day 60, and a consistently large percentage of patients (40%) were cured at the end of treatment. These results agree with previous studies that reported that the erythema surface of the palatal mucosa was significantly reduced in noncompromised patients after treatment with nystatin[15] and microwave irradiation.[12,13,22] Despite the high rates of curing and improvement of infection at the end of follow-up (day 90), some patients from both groups NYS (40%) and MW (55%) showed recurrence of the clinical signs of denture stomatitis. One may ask whether the practitioner should use both the microwave and nystatin regimens to enhance the therapeutic efficacy regardless of the risks of using antimycotic medications. However, this seems to be unlikely since a recurrence rate of 56% was reported by Banting and Hill[12] when microwave disinfection of complete dentures and the use of topical nystatin were combined. Likewise, another investigation reported a 40% recurrence rate when microwave denture disinfection was combined with topical miconazole.[13] It is also important to emphasize that the density of colonization does not necessarily correlate with clinical candidiasis.[6,8,11] In fact, although the mean number of yeast colonies cultured from palates and dentures at day 90 was similar to that at admission, at least 20% of all patients had no clinical signs of denture stomatitis. Since the present investigation did not assess the presence of the invasive form (mycelial) of *Candida*, which is an indication of infection, the positive samples in cured patients may be related to noninvasive forms (blastospore) of *Candida* grown on culture medium.[12] There are some explanations for why the clinical and antimycotic effects of nystatin and microwave disinfection were temporary. Reinfection may have occurred via a supply of new organisms from exogenous sources.[17] Also, a significant proportion of patients harbor an abundance of yeasts in the oral cavity, even in the absence of clinical signs of infection,[6,8,11] which may lead to recontamination of the dentures and reinfestation of the adjacent soft tissue.

Because of the increasing resistance,[19–21] the limited power of action,[12,13,16] and the toxicity[18] of some antifungal drugs, new alternatives in the treatment of denture stomatitis are welcome. The present study demonstrated that microwave disinfection of complete dentures by itself was as effective as nystatin—the more conventional topical antifungal medication—in reducing *Candida* counts and the clinical signs of the infection in patients with well-controlled type 2 diabetes. Besides being effective, microwave irradiation is simple, fast, safe, and inexpensive since it requires only a domestic microwave oven and water. The potential relevance of these results to the large population of poorly controlled diabetics remains unkown. The systemic and local predisposing factors found in diabetics without adequate metabolic control might promote an increase in the number of microorganisms and hence the risk of oral candidiasis. Many studies have shown that diabetics are more susceptible to fungal infections[4] and *Candida* colonization[4–9] than nondiabetics and that inadequately controlled diabetes is related to a higher colonization of *Candida*[7] and to a more severe degree of denture stomatitis.[5] Patients with well-controlled type 2 diabetes responded well to both treatments, with outcomes comparable to those expected in noncompromised patients.[12,13,15,16,22] This corroborates with the observations of Richardson et al,[64] that good metabolic control can guide the diabetic individual to a life without or with fewer disease-related complications. Moreover, the results also suggest that when good metabolic control is established, the diabetic patient may be treated using the same methods as for nondiabetic individuals. Therefore, general health care of diabetics should receive increasing recognition for its importance in the treatment of denture stomatitis. This disease is one of the most frequent opportunistic infections found in diabetic patients who wear dentures, and it may extend regionally and result in systemic infection that is associated with high mortality rates.[59,60] Hence, the prevention of colonization of the oropharynx, even in well-controlled diabetics, is critically important in preventing systemic infections resulting from *Candida,* and consequently, the disease-related complications commonly associated

with diabetes mellitus. Therefore, it is imperative that health professionals, including dentists and physicians, aggressively manage the oral health and diabetes of these individuals, thus providing a better quality of life.

Conclusions

- Both microwave disinfection and nystatin treatments significantly reduced the cfu/mL of the palates and dentures at days 14 and 30 and were considered successful in reducing the clinical signs of denture stomatitis.
- Microwave disinfection of maxillary complete dentures was as effective as nystatin in the treatment of well-controlled diabetic patients with denture stomatitis.
- *C albicans* was the most predominant species isolated from patients with well-controlled diabetes and denture stomatitis, followed by *C tropicalis* and *C glabrata*.
- The frequency distributions of *C albicans* and *C tropicalis* isolated from the dentures were significantly higher than those obtained from the palates.

Acknowledgments

This work was supported by the FAPESP–São Paulo Research Foundation (grants 2006/02842-5 and 2007/03895-8) and CNPq–National Council of Scientific and Technological Development (grant 470337/2007-9).

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