

Phase 1 Clinical Trial on the Effect of Palatal Brushing on Denture Stomatitis

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Purpose: This study aimed to assess the efficacy of palatal brushing in the treatment of denture stomatitis. **Materials and Methods:** After screening 143 individuals with a potential diagnosis of denture stomatitis, 48 patients (mean age: 66.0 ± 11.2 years) were enrolled in a two-center phase 1 clinical trial with a one-group pretest/posttest design. The intervention of interest was manual palatal brushing after each meal and before bedtime. Clinical and microbiologic examinations were performed at baseline and 1 and 3 months after treatment. Additional data were obtained using a validated questionnaire. The primary and secondary outcomes were the remission of denture stomatitis and diminution of *Candida* colony-forming units (CFUs), respectively. Descriptive and nonparametric statistical tests were conducted to analyze the data.

Results: At the 3-month follow-up, denture stomatitis was completely cured in 10.4% of the participants, and 70.8% of denture wearers showed improvement in the clinical signs of denture stomatitis. There was a significant reduction in the area and severity of the palatal inflammation ($P < .0001$). The effect size ranged from medium to large (0.34 to 0.54) depending on the classification used for the diagnosis of denture stomatitis. A significant reduction in the number of *Candida* CFUs isolated from the palatal mucosa and dentures ($P \leq .05$) was observed. **Conclusions:**

The results of this study suggest that palatal brushing is an effective treatment of denture stomatitis. *Int J Prosthodont* 2014;27:311–319. doi: 10.11607/ijp.3844

Denture stomatitis is the chronic inflammation of the oral mucosa covered by a removable denture. It is considered the most common mucosal lesion associated with denture use,^{1,2} affecting one in three complete denture wearers.³ Several risk factors have been reported to be associated with denture

stomatitis, including trauma,⁴ denture biofilm,⁵ and bacterial and fungal infections, particularly by *Candida albicans*.⁶ However, the etiology of this pathologic condition remains multifactorial and controversial.^{7,8} A variety of treatments of denture stomatitis have been used in dental practice. These treatments can be classified into one of two categories: the conservative approach and the use of antifungal medications.

Antifungal medications are routinely prescribed for the treatment of denture stomatitis. This approach is based on the hypothesis that infection by *Candida* is the main etiologic factor of this disease.^{9–11} However, a convincing cause-and-effect relationship between the presence of denture stomatitis and *Candida* has never been demonstrated.^{7,8,12} Further, high recurrence rates of denture stomatitis and recolonization of *Candida* have been reported after the cessation of antifungal treatment.^{9,13,14}

Recent findings suggest that trauma from unstable dentures induces local inflammation and creates an environment favorable to the proliferation of microorganisms.⁴ Consecutively, *Candida* colonization becomes a secondary stage in the pathogenesis of denture stomatitis.^{7,15} This suggests that treatments aiming for the remission of inflammation could be effective in the treatment of this disease.

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Table 1 Assessments of Denture Stomatitis***Modified Newton classification**

- 0: Healthy mucosa
- Type IA: Petechiae in normal palatal tissue, usually found around the orifices of the ducts of the palatal mucous glands
- Type IB: Localized area of inflammation of the denture-bearing area
- Type II: Generalized area of inflammation of the denture-bearing area
- Type III: Hyperplastic palatal surface with inflammation of the denture-bearing area

Inflammation area index

- 0: No inflammation
- 1: Inflammation of the palate extending up to 25% of the palatal denture-bearing tissue
- 2: Inflammation of the palate covering between 25% and 50% of the palatal denture-bearing tissue
- 3: Inflammation covering more than 50% of the palatal denture-bearing tissue

Inflammation severity index

- 0: Normal tissue
- 1: Mild inflammation (slight redness, no swelling or edema)
- 2: Moderate inflammation (redness with some edema)
- 3: Severe inflammation (acutely inflamed redness, edema)

*Total inflammation score = area + intensity (range: 0 to 6).

Palatal brushing is a simple procedure that could reduce the extent of inflammation via mechanisms such as the removal of denture plaque and the stimulation of mucosal circulation and salivary flow. However, no previous clinical trial has evaluated palatal brushing as a treatment for denture stomatitis.

Therefore, the objective of this study was to assess the efficacy of palatal brushing in the treatment of denture stomatitis. The null hypotheses was as follows: In individuals with denture stomatitis, there are no differences in the extent of palatal inflammation or number of *Candida* colony-forming units (CFUs) before and 3 months after palatal brushing.

Materials and Methods

Study Design and Participants

A one-group pretest/posttest research design was used to conduct a two-center, phase 1 clinical trial (clinicaltrials.gov no. NCT01643876) at two faculties of dentistry of the Université de Montréal (Canada) and the University of São Paulo (Ribeirão Preto, Brazil). Participants were recruited from the general population of Metropolitan Montreal and Ribeirão Preto by advertisements in local newspapers, flyers placed within dental clinics of the two dental schools, and clinicians during examination at the diagnostic clinics of the two study centers.

The inclusion criteria were as follows: (1) at least 18 years of age, (2) wearing a complete maxillary denture, and (3) having a clinical diagnosis of denture

stomatitis. Patients were excluded if they (1) had any conditions known to promote *Candida* carriage, such as uncontrolled diabetes, anemia, xerostomia, or immunosuppression; (2) received treatment with an antibiotic, antifungal, or corticosteroid or had undergone chemotherapy or radiotherapy in the 4 weeks prior to enrollment in the study; (3) used palatal brushing as a routine oral hygiene procedure; or (4) changed their existing prosthesis during the trial. The study was approved by the Université de Montréal Research Ethics Board and the Institutional Review Board of the Ribeirão Preto Dental School. Written informed consent was obtained from all patients prior to their participation in the trial.

Experimental Procedures

Data collection was conducted at baseline (T0) and 1 (T1) and 3 months (T2) after the intervention using a self-administered questionnaire, clinical examination, and microbiologic investigation.

Intervention. The intervention consisted of palatal brushing with a soft-bristle manual toothbrush (Oral-B CrossAction Pro-Health, Procter & Gamble) after each meal and before sleeping for a period of 3 months. Participants were instructed to brush the palate using horizontal, vertical, and vibration movements. They were also asked to maintain their usual oral and denture hygiene habits during the trial.

Clinical investigation. Denture stomatitis was assessed according to the modified Newton classification¹⁶ and via the area and severity of inflammation indices¹⁷ (Table 1).

The clinical assessment was conducted by two trained calibrated dentists using a front surface mirror and probe (XP23/QW, Hu-Friedy). Photographs of the palate were taken with a Nikon D90 camera (105 mm, f/2.8D, macro flash SB-21, Nikon). These photographs were used to obtain diagnostic consensus among the research team. A good to excellent interrater agreement was obtained ($\kappa = 0.6$ to 0.84).

Microbiologic investigation. Collection of maxillary denture plaque was carried out with the sonication technique, according to the protocol described by Emami et al.⁸ A sample of palatal plaque was also collected using a sterile swab,¹⁸ placed in a tube with 5 mL of saline, and sonicated for 2 minutes. Both denture and palatal plaque sonicates were subsequently mixed by vortex for 1 minute and diluted 10-fold serially with saline (dilution factors: 10^0 , 10^{-1} , and 10^{-2}). Next, 100 μ L of each dilution was spread-plated in duplicate on Sabouraud dextrose 4% agar (Difco, Becton Dickinson). All cultures were incubated at 37°C for 48 hours. CFUs were counted and expressed as CFU/mL after correction for volume and dilution factor. When

growth was observed, an imprint of colonies was obtained on sterile filter paper, which was transferred on a *Candida* selective growth medium (CHROMagar) and incubated under the same conditions. This chromogenic selective medium allows identification of *Candida* species.¹⁹

Outcome measures and explanatory variables. The remission of denture stomatitis was considered the primary outcome variable and defined as a decrease in the level of the modified Newton classification, area and severity of inflammation, and total inflammation score (area + severity).¹⁷

The Budtz-Jorgensen Index²⁰ was used to evaluate the magnitude of the treatment effect (based on the total inflammation score): large effect = inflammation resolved; moderate effect = inflammation reduced; no effect = no change in inflammation; negative effect = increased inflammation.

The secondary outcome was the reduction of *Candida* (CFU).

Explanatory variables included sociodemographic variables (age, sex, education, medical and dental histories, and medication profiles), years of edentulism and age of the maxillary dentures, hygiene habits (cleaning frequency, nocturnal wear, and mouthwash use), and smoking. This information was obtained from a self-administrated questionnaire.⁸ General satisfaction with oral condition was assessed with a 100-mm visual analog scale.⁴ Other independent variables included denture cleanliness, evaluated using the modified Hoad-Reddick classification,^{8,21} and the stability and retention of the maxillary prostheses.^{22,23} Denture stability was evaluated by determining the movement of the prosthesis over the supporting tissues and its resistance to rotational movement. The maxillary prosthesis was grasped in the premolar region with the thumb and index finger, and a rotational force was applied in the occlusal plane. A displacement of 5 mm or more was considered prosthesis instability. The prosthesis was considered retentive if it showed resistance to downward force when grasped using the thumb and index finger.^{22,23}

The wear of the denture teeth, salivary flow, resorption and resilience of the upper residual ridge, and vertical dimension of occlusion²²⁻²⁷ were also evaluated. Further, symptoms of denture stomatitis and side effects of palatal brushing, if any, were documented.

Statistical Analyses

Assuming that the minimum practically important pretest/posttest difference in the mean change score is 20% and the SD of the distribution of the change in score is 0.8,²⁸ a sample size of 44 participants was required to ensure a power of 90% for rejecting the

null hypothesis (if it is indeed false). Additional participants were considered to account for a potential dropout rate of 10%.

Cohen's kappa coefficient²⁹ was used to evaluate the interrater agreement for the diagnosis of denture stomatitis, with $\kappa > 0.75$ representing excellent agreement, 0.40 to 0.75 representing fair to good agreement, and < 0.40 representing poor agreement.

To obtain frequency counts, percentages, and univariate means as well as to test for normality, the data were first subjected to descriptive statistical tests. Nonparametric tests were applied because of the non-normal distribution of the data.

Between-center differences in regard to baseline characteristics of the participants and treatment effects were analyzed using the Fisher exact test, two-sample *t* test, and Mann-Whitney *U* test.

The Wilcoxon signed rank test was used to compare the level of the modified Newton classification, area and severity of inflammation, total inflammation score, and number of *Candida* colonies between baseline and follow-up. The McNemar test was used to compare nominal data on patient-reported symptoms at baseline and follow-up. The level of significance was set at .05.

The Fisher exact test and two-sample *t* test were used to examine the significance of associations between the explanatory variables and the treatment effect. Logistic regression was not conducted because of a nonsignificant association ($P > .10$) in bivariate analyses.

Statistical analyses were performed using SPSS version 20 (SPSS) and SAS version 9.1 (SAS).

Results

Of the 143 individuals who participated in the screening sessions, 48 patients (16 men, 32 women; mean age: 66.0 ± 11.2 years) were enrolled in this study. Figure 1 shows the flow of participants throughout the study. There were no dropouts. Tables 2 and 3 present the sociodemographic characteristics of the participants and their profiles according to denture stomatitis risk factors at baseline, by study center.

The mean years of edentulism in the maxillary arch were 37.2 ± 14.7 , and the mean age of the current maxillary prostheses was 15.3 ± 13.7 years. Signs of wear facets on denture teeth were observed in 77.1% of the participants. The participants used different methods of denture hygiene. The majority of participants brushed their prostheses with or without toothpaste (85.4%), while 4.2% only rinsed their prostheses and 10.4% did not clean their prostheses. Fifteen participants (31.3%) used a denture-cleaning agent, all of which came from the Canadian center. Twenty

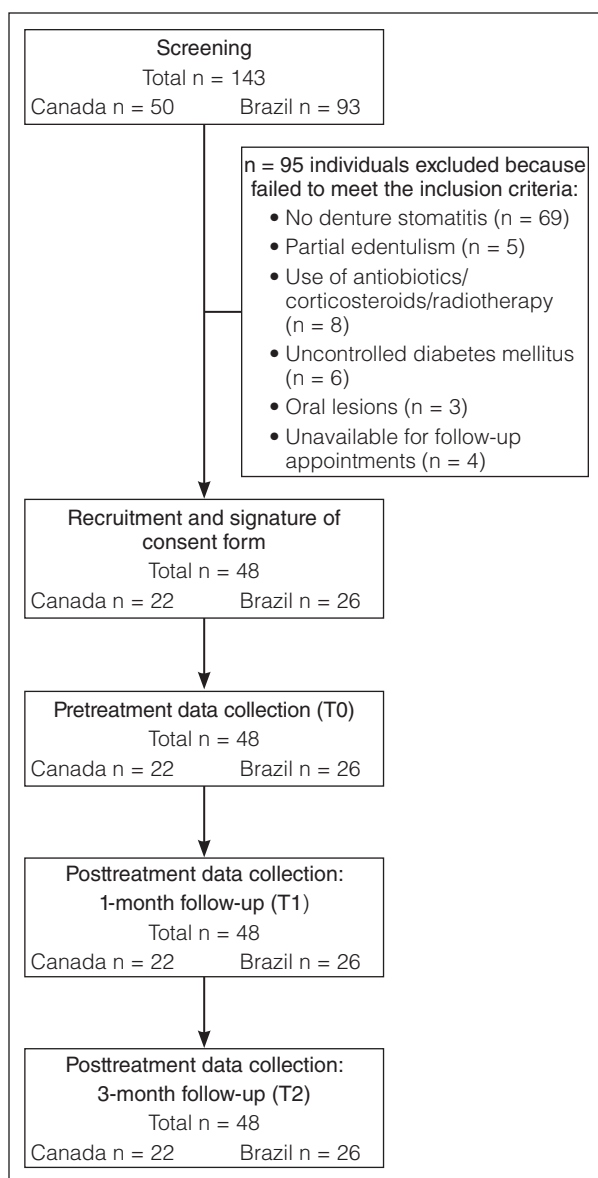


Fig 1 Flowchart of the study.

percent of participants had previously received denture hygiene instructions. Only one participant used denture adhesive on his maxillary dentures. At baseline, the most commonly reported symptoms of denture stomatitis were halitosis (52.1%) and a dry mouth sensation (66.7%). The salivary flow was adequate in 83.3% of the participants, and 91.7% had a well-rounded ridge with sufficient height and width (Class III resorption²⁷). Finally, 39.6% of the patients had resilient tissue covering the residual ridge. There was no statistically significant difference between the two study centers regarding the demographic characteristics or risk factors associated with denture stomatitis, except for sex, income, educational level, denture cleanliness, and the mean age of the current prostheses. The Brazilian center had more women enrolled in

the trial. This center also had participants with a lower income, lower level of education, and older and less clean maxillary prostheses (Tables 2 and 3).

There was no statistically significant association between baseline denture hygiene and intensity of the inflammation.

At baseline, 6.2% of the participants had petechiae (type IA), 16.7% had localized inflammation (type IB), 39.6% had generalized palatal inflammation (type II), and 37.5% had hyperplastic inflammation (type III). In addition, 16.7% of the participants had inflammation extending up to 25% of the palate, 33.3% had inflammation covering between 25% and 50% of the palate, and 50% of the participants had inflammation covering more than 50% of the palatal denture-bearing area (area of inflammation index). Twenty-five percent of the participants had mild inflammation, 43.8% had moderate inflammation, and 31.2% had severe inflammation (severity of inflammation index).

The microbiologic analysis of the denture sonicates at baseline revealed that 39 participants were *Candida* carriers. *C. albicans* was the most frequent species isolated (59% of the cultures from the denture sonicates). Other species included *C. glabrata*, *C. krusei*, *C. tropicalis*, and *C. parapsilosis*. The cultures were negative in 9 patients (18.8%), including those classified as types IA, IB, and II. Cultures from the palatal swab were negative in 77% of the cultures.

There was no statistically significant difference between the two research centers according to the frequency of different types of denture stomatitis or *Candida* carriage at baseline.

There were no statistically significant changes at the 1-month follow-up. At the 3-month follow-up, denture stomatitis was cured in 10.4% of the participants, and 70.8% showed substantial improvement in the clinical signs of denture stomatitis. There was a worsening of the clinical signs of denture stomatitis in only one participant (Fig 2). There was a significant decrease in inflammation according to the modified Newton classification ($P = .001$) as well as in the area ($P < .0001$) and severity ($P < .0001$) of inflammation at the 3-month follow-up. The reduction of the total inflammation was also significant ($P < .0001$; Table 4 and Fig 3). Subgroup analyses showed that there was no change in the inflammation indices for type IA patients. Type II and III patients showed a significant decrease in the total inflammation score ($P < .0001$). However, the hyperplastic tissue remained in all patients affected by type III denture stomatitis.

The effect size ranged from medium to large (0.34 to 0.54) depending on the classification of denture stomatitis used (Table 4). In addition, statistically significant improvements in the perceived oral condition of the participants ($P = .003$) and palatal burning

Table 2 Sociodemographic Characteristics of the Participants at Baseline by Study Center

Variable	Combined		Canada		Brazil		P
Mean age, y (SD)	66.0 (11.2)		64.6 (12.3)		67.3 (10.2)		.407
	n	%	n	%	n	%	
Sex							
Male	16	33.3	12	54.5	4	15.4	.006
Female	32	66.7	10	45.5	22	84.6	
Marital status							
Single/separated/divorced/widowed	23	47.9	9	41	14	53.8	.401
Married/partnered	25	52.1	13	59	12	46.2	
Living arrangements							
Alone	8	16.7	5	22.7	3	11.5	.442
With family or other adults	40	83.3	17	77.3	23	88.5	
Education							
High school or less	39	81.3	13	59	26	100	< .001
College and higher	9	18.7	9	41	0	0	
Yearly income							
Less than \$10,000	14	29.2	0	0	14	53.9	< .001
\$10,000–\$30,000	25	52.1	14	63.6	11	42.3	
> \$30,000	9	18.7	8	36.4	1	3.8	

Table 3 Participants' Profiles by Study Center According to Denture Stomatitis Risk Factors at Baseline

Variable Mean years of edentulism (SD) Mean age of current prosthesis, y (SD)	Combined 37.2 (14.7) 15.3 (13.7)		Canada 40.55 (15.92) 9.93 (9.53)		Brazil 34.3 (13.3) 19.8 (15.1)		<i>P</i> .146 .011
	n	%	n	%	n	%	
Presence of systemic diseases	31	64.6	12	54.5	19	73.1	.232
Medications use	36	75.0	17	77.3	29	73.1	.000
Unacceptable vertical dimension	30	62.5	13	59	17	65.4	.558
Inadequate maxillary retention	17	35.4	9	41	8	30.8	.551
Unstable maxillary denture	22	45.8	12	54.5	10	38.5	.384
Self-reported inadequate denture hygiene	5	10.4	4	18.2	1	3.8	.165
Clinician-reported dirty denture	39	81.3	13	59	26	100	< .0001
No mouthwash use	35	72.9	13	59	22	84.6	.059
Nocturnal wear (maxillary denture)	28	58.3	12	54.5	16	61.5	.770
Smoking	13	27.1	6	27.3	7	26.9	> .999

Fig 2 (right) Treatment effect at the 3-month follow-up (T2).

sensation ($P = .008$) were found at the 3-month follow-up. Overall, 40% of the participants reported minor side effects of palatal brushing, including mild pain and some bleeding during the first days of treatment.

There was no statistically significant difference between the centers regarding the treatment effect. However, there was a statistically significant difference between the two centers regarding the reported side effects of palatal brushing ($P < .0001$), with the majority of reported side effects occurring in the Brazilian center.

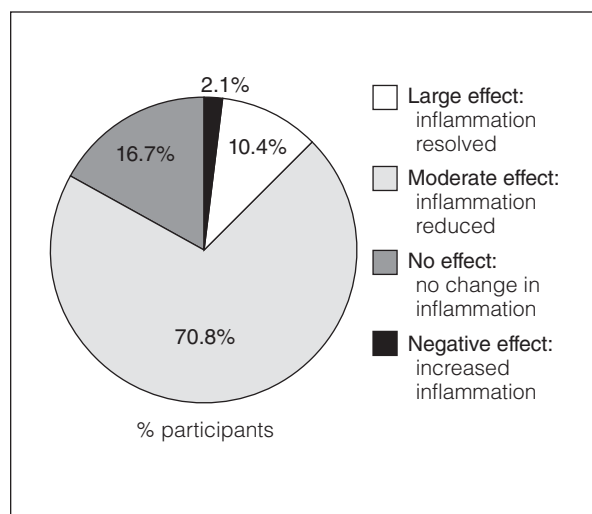


Table 4 Effect of the Intervention at the 3-Month Follow-up

Diagnosis	Worse		Unchanged		Improved/cured		<i>P</i> (T0–T2)	Effect size* (T0–T2)
	n	%	n	%	n	%		
Modified Newton classification	1	2.1	32	66.6	15	31.3	.001	0.34
Inflammation area index	1	2.1	18	37.5	29	60.4	< .0001	0.49
Inflammation intensity index	1	2.1	15	31.2	32	66.7	< .0001	0.52
Total inflammation	1	2.1	8	16.7	39	81.2	< .0001	0.54

T0 = baseline; T2 = 3-month follow-up.

*0.1 = small effect; 0.3 = medium effect; 0.5 = large effect.

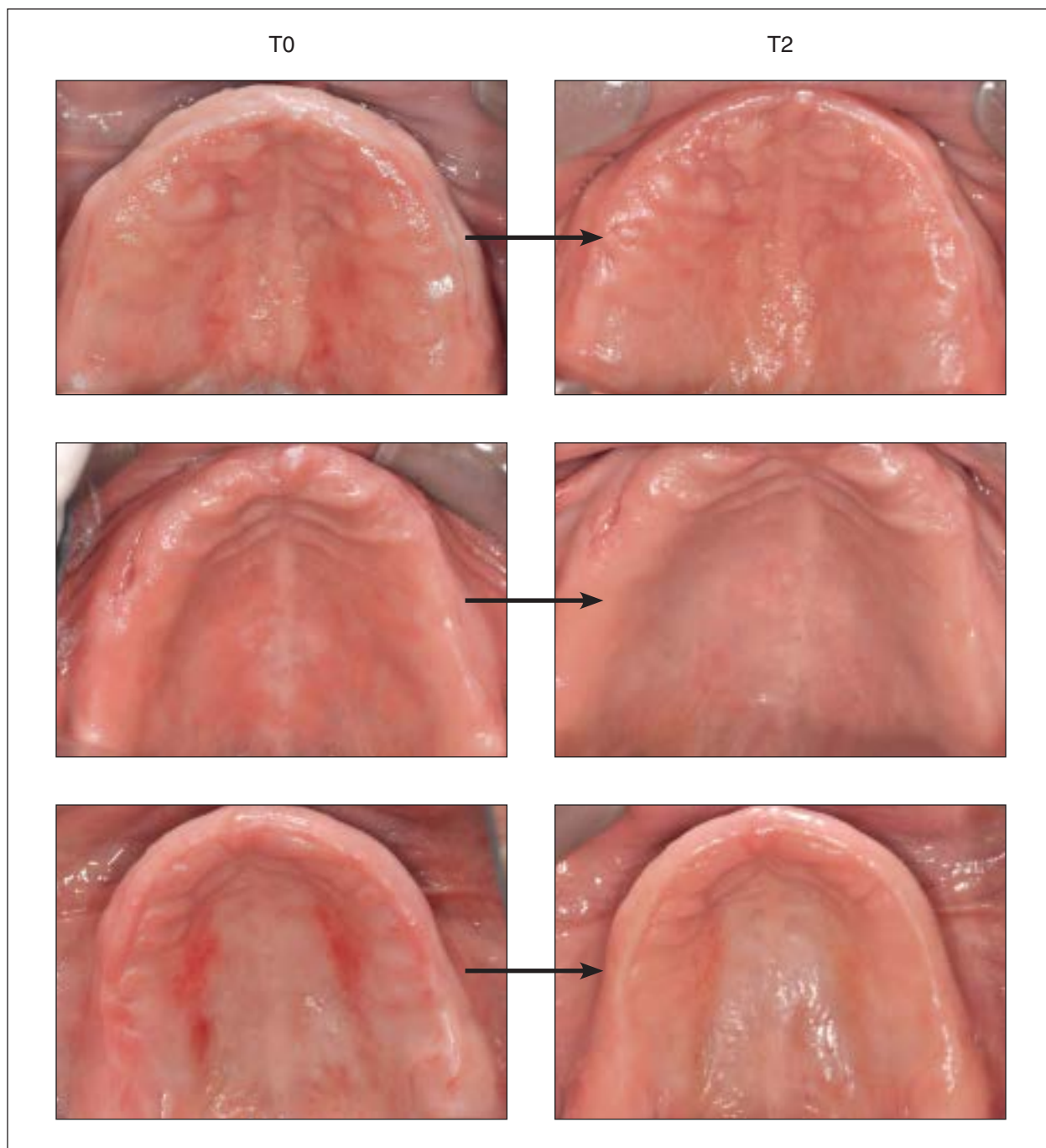
**Fig 3** Palatal mucosa at baseline (T0) and the 1-month follow-up (T2).

Table 5 Effect of the Intervention on the Number of *Candida* Colonies (CFU) at the 3-Month Follow-up

Location	Increased		Unchanged		Decreased		<i>P</i> (T0-T2)	Effect size* (T0-T2)
	n	%	n	%	n	%		
Prosthesis	13	27.1	7	14.6	28	58.3	.050	0.19
Palate	4	8.3	34	70.8	10	20.9	.048	0.20

T0 = baseline; T2 = 3-month follow-up.

*0.1 = small effect; 0.3 = medium effect; 0.5 = large effect.

Microbiologic analyses showed a significant reduction in the *Candida* CFUs isolated from denture plaque sonicates ($P = .05$) and the palatal swabs ($P = .048$) at the 3-month follow-up (Table 5).

Bivariate analyses revealed no statistically significant associations between the explanatory variables and treatment effect.

Discussion

To the authors' knowledge, this is the first clinical trial to provide data on the efficacy of palatal brushing as a treatment for denture stomatitis. The results confirmed previous reports on the positive effect of palatal brushing. In a recent observational study,²⁸ the chance of remission of denture stomatitis was 3.9 times higher in participants who performed palatal brushing. Furthermore, a significant association was found between the lack of palatal brushing and the occurrence of *C albicans*.⁸

Several mechanisms could explain the effect of palatal brushing, including mechanical stimulation and oral biofilm removal.³⁰ Oral biofilm is a protective niche that may harbor a wide array of pathogenic microorganisms encased in extracellular polysaccharide matrix, including aerobic and anaerobic bacteria and yeasts.^{31,32} There is overwhelming evidence that denture and palatal biofilms are important risk factors for denture stomatitis.^{5,33-35} Palatal brushing could eliminate this reservoir of pathogens and thus the source of irritation. This may explain the statistically significant decrease in *Candida* CFUs demonstrated in the present study.

The results of several studies have demonstrated that mechanical stimulation encourages keratinization, reduces the infiltration of inflammatory cells, and enhances the proliferation of fibroblasts and collagen synthesis.^{30,36-38} It has also been shown that, even in the presence of oral biofilm, brushing stimulation can improve tissue microcirculation.³⁹ Consequently, the effects of mechanical stimulation could counteract the effects of the inflammatory process in denture stomatitis, leading to the reestablishment of an undamaged epithelium and basement membrane within the palatal mucosa. This healthy mucosa then

serves as a mechanical barrier against microbiologic colonization.^{40,41}

Impaired salivary flow and xerostomia have been considered predisposing factors in denture stomatitis.^{42,43} Palatal brushing may increase salivary flow by mechanical stimulation of the minor salivary glands of the palate. In turn, the stimulation of the salivary glands could have a mechanical cleansing effect and thus eliminate denture biofilm.^{32,40,44} In addition, saliva acts as a defense mechanism against microorganisms.⁴⁵ In the present study, patient-reported dry mouth was the most commonly reported side effect of palatal brushing. However, in accordance with several studies,^{46,47} the patients' assessment did not correlate with the findings of the clinical examinations. Further research should include a more accurate assessment of unstimulated salivary flow to confirm these results.⁴⁷

There are conflicting hypotheses regarding whether the inflammation in denture stomatitis is associated with trauma from unstable prostheses or with fungal biofilm.^{4,7,41,46,48} However, there is considerable evidence demonstrating the lack of a direct cause-and-effect relationship between the presence of denture stomatitis and *Candida*.^{7,8,12} Further, several studies on the efficacy of antifungal medications in the treatment of denture stomatitis have demonstrated a high recurrence rate of clinical signs of denture stomatitis and recolonization of *Candida* after cessation of antifungal treatment.^{13,14} The present results support the hypothesis that trauma is a primary etiologic factor in denture stomatitis. Thus, the authors encourage oral health-care professionals to use conservative approaches such as oral hygiene instruction, palatal brushing, and prosthesis adjustment rather than antifungal medications in the treatment of denture stomatitis.

This study found significant differences between the Canadian and Brazilian participants regarding the mean age of the maxillary prosthesis, denture hygiene, and use of denture-cleaning agents. However, there was no statistically significant difference between the two research centers in terms of types of denture stomatitis at baseline, *Candida* carriage at baseline, or treatment effect. This may be explained

by the interaction between several risk factors in the development of the disease. The Brazilian participants did not use any denture-cleaning agents; nonetheless, this issue did not influence the baseline diagnosis of denture stomatitis in term of severity and treatment effect. This finding is in agreement with previous studies indicating that mechanical brushing is more efficacious in the control of denture biofilm than the use of denture-cleaning agents.^{49–51} However, these cleaning agents may complement the beneficial effect of mechanical approaches. Further epidemiologic studies (eg, prospective cohort studies) are needed to capture the effect of preventive measures in the initiation of denture stomatitis.

This study used a modified version of the Newton classification. This modification introduced two subtypes for Newton type I denture stomatitis and allowed for differentiation of palatal petechiae (type IA) from localized inflammation (type IB). The findings demonstrated a difference between these two clinical manifestations in terms of treatment effects. The microbiologic analyses of the denture biofilm and palatal swabs of the participants with type IA denture stomatitis were negative for the presence of *Candida*. Further, petechiae remained after palatal brushing. These results suggest that petechiae are merely the widening of the ducts of the minor salivary glands, which could be considered as a variation of the normal anatomy caused by trauma from the denture and not as a pathologic sign of denture stomatitis.^{4,52}

This study also found that the area and severity of inflammation indices^{17,53} permitted a better classification of the clinical signs of denture stomatitis than the Newton classification. The authors recommend the use of these indices in clinical and research training to ensure the standardization of the methods and comparability between trials.

The results of this study should be interpreted with caution since a one-group pretest/posttest design was used; further, this study did not include a control group. No specific approach was used to reinforce compliance; however, the absence of withdrawals or losses to follow-up suggests good patient compliance with palatal brushing. The encouraging results of this study should be confirmed by a phase 2 clinical trial.

Conclusions

The results of this study suggest that palatal brushing is an effective treatment of denture stomatitis. The authors recommend the use of palatal brushing as a crucial adjunct to the routine management of this condition.

Acknowledgments

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