

Palatal brushing for the treatment of denture stomatitis: A multicentre randomized controlled trial

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Abstract

Purpose: To determine the effectiveness of palatal brushing in the treatment of denture-related erythematous stomatitis (DES) in complete denture wearers.

Methods: This two-parallel-arm RCT was conducted in three university clinics in Brazil, Canada, and Chile. Participants (n=77) were randomly allocated to receive (i) instructions for palatal brushing and standard oral/denture hygiene ("intervention"); or (ii) standard oral/denture hygiene instructions only ("control"). Data collection was carried out at the baseline and at 3 and 6 months after intervention. Outcomes included the magnitude of oral *Candida* carriage and the degree of inflammation of denture-bearing tissues. Groups were compared using generalized estimating equations and chi-square test ($\alpha=0.05$).

Results: Palatal inflammation levels were reduced significantly in the "intervention" compared to "control" group at 6 months (intervention: 70%, control: 40%; chi-square, $p=0.04$). There was no between-group significant difference in the *Candida* count from denture and palatal biofilms; however, a subgroup analysis restricted to baseline *Candida* carriers showed further reduction with the intervention at 6 months. No adversity was observed by trialist or reported by participants.

Conclusions: Including palatal brushing in oral instructions for denture wearers has positive impact on DES-related mucosal inflammation. Thus, our findings endorse the inclusion of palatal brushing in standard oral hygiene instructions to treat DES.

Keywords: Denture stomatitis, Complete denture, *Candida*, Oral candidosis, Pragmatic trials

Received 6 October 2021, Accepted 3 February 2022, Available online 8 March 2022

1. Introduction

Denture-related erythematous stomatitis (DES) is a chronic inflammatory lesion of the oral mucosa covered by a removable denture, mostly found in the palate[1,2]. This condition is known to affect 15–70% of denture wearers and seems more common in elderly women, although it may also appear in children and adults wearing orthodontic appliances and obturator prosthesis[1–5]. In a large population-based study with 17,235 participants, oral mucosal inflammation conditions such as DES have been associated with an increased risk of cardiovascular diseases, and a significant risk factor for aspiration pneumonia in the frail elderly[2,6,7].

Antifungal medication is the current treatment for DES, although scientific evidence acknowledges the high rate of rapid recurrence after the cessation of antifungals[2,8]. Prescription of antifungal medications relies on the hypothesis that *Candida* infection is the main etiological factor of DES[1,8]. However, previous systematic reviews and meta-analyses have shown only a limited or null effect of antifungals in DES care management[2,8]. Furthermore, resistance to antifungals and severe adverse effects such as gastrointestinal disturbances, liver and kidney toxicity, and hypersensitivity have been reported[1,9].

On the other hand, according to the literature denture hygiene is a critical etiological risk factor for DES[1]. Poor denture cleaning creates an anaerobic environment with low pH, and leads to an increase in palatal and denture biofilm pathogens[1]. The toxins and metabolic wastes produced by these microorganisms foster DES onset and exacerbation of mucosal inflammation[1,9]. The inflammation may also result from the repeated mucosal trauma of ill-fitting prostheses[1,9,10]. The inflammation favours colonization by *Candida*

DOI: https://doi.org/10.2186/jpr.JPR_D_21_00258

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species, meaning that fungal infection is not necessarily a causative factor of DES and it is mainly a moderator[1]. In fact, previous studies have demonstrated the efficacy of various hygienic approaches in preventing DES and decreasing the *Candida* carriage[1,8,9,11–13]. The systematic reviews and meta-analyses on this topic have shown a lower risk of DES recurrence and no outcome difference between hygiene instructions and antifungal treatment in patients suffering from this disease[8,11].

A recent single-arm trial conducted by our team has demonstrated promising results of palatal brushing, a simple and cost-effective procedure, in treating DES[14]. In that study, palatal brushing was used to mechanically remove palatal biofilm and stimulate mucosal circulation and salivary flow[14], and showed a significant reduction of palatal inflammation and *Candida* carriage over a period of 3 months[14]. However, these results should be validated by a randomized clinical trial. Therefore, the primary objective of this trial was to determine if palatal brushing decreases the number of *Candida* colonies on the palatal mucosa and denture of patients with DES. The secondary objective was to demonstrate the efficacy of palatal brushing in reducing the palatal inflammation. We hypothesized that edentulous patients who brush their palate and follow standard hygiene measures will have less denture and palatal *Candida* colonies and less palatal inflammation than those using standard hygiene over a 6-month period.

2. Methods and Materials

2.1. Study design and setting

This pragmatic, multicentre randomized controlled trial was conducted in three university-based clinics in Brazil, Canada, and Chile. The study was designed as a two-arm, parallel-group trial with 1:1 allocation of participants. Institutional review board approval was obtained from each participating institution: A06-M25-16A (McGill University, Canada – coordinating centre), 16-003-CERES-D (Université de Montréal, Canada – clinical site 1), 37033814.60000.54190 (University of São Paulo, Brazil – clinical site 2), and 013/15 (Universidad de la Frontera, Chile – clinical site 3). The protocol trial was registered in ClinicalTrials.gov (#NCT02686632) and published previously[15].

2.2. Study participants

The recruitment of study participants was carried out in 2015–2017 via advertising in local newspapers and distributing informative brochures in dental clinics and long-term care facilities. Research project managers at each study centre called interested individuals, explained the study to them, and assessed them based on primary eligibility criteria. Potential participants were then asked to visit one of the clinical sites, where they were provided with more information related to the study and were carefully re-assessed based on the study's full eligibility criteria.

Potential participants were included if they were (i) older than 18 years; (ii) fully edentulous; (iii) regularly wearing maxillary complete conventional dentures; and (iv) presenting clinical signs compatible with a DES diagnosis. Newton class II and III were not required for inclusion, as in the original protocol[15] (reason for change: to enable adequate study enrollment rate and representability of patients in the Brazilian centre). Exclusion criteria were: (i) having an appointment to replace any existing denture (upper or lower) during the course of the study; (ii) taking antibiotics, steroids, or antifungal

agents within 4 weeks prior to the initiation of the interventions; (iii) systemic conditions predisposing patients to *Candida* infection such as uncontrolled diabetes and history of radiotherapy or chemotherapy; (iv) other oral lesions apart from DES, including patients with other types of oral *Candida* infection (e.g., acute pseudomembranous candidiasis). Those who satisfied the eligibility criteria were asked to provide informed consent prior to initiation of the study and underwent complete clinical examinations, during which the baseline data was collected.

2.3. Intervention

Participants were randomized to either intervention or control group, with both groups receiving standard instructions on oral/denture hygiene measures. These standard instructions were provided verbally by a researcher (with the provision of an explanatory pamphlet) as follows:

- i) Washing all the internal and external surfaces of the denture with tap water to remove all visible debris;
- ii) Brushing all surfaces of the denture using a denture brush (Oral-B Denture Brush; Procter & Gamble) and 2-cm toothpaste for approximately two minutes;
- iii) Rinsing the denture with tap water until visibly clean.

Exclusive instructions for the intervention included brushing the palate without toothpaste. A research trainee (not involved in the provision of standard instructions) provided a soft bristle manual toothbrush (Oral-B CrossAction Pro Health; Procter & Gamble, Iowa, IA, USA) to intervention group participants, and instructed them to perform palatal brushing with vertical, horizontal, and vibration movements, for approximately one minute. Participant in the intervention group also received written instructions of palatal brushing, besides standard instructions.

All participants were asked to follow the given instructions after each meal and before going to sleep, for 6 months. Data collection was conducted at three time points, including baseline (T0), first follow-up at 3 months (T2), and second follow-up (T3) at 6 months post-intervention. Study participants were asked to stop palatal brushing in case of any adverse effects.

2.4. Outcomes

2.4.1. Primary outcome

The primary outcome was the magnitude of oral *Candida* carriage defined by *Candida* spp. colony forming unit (CFU) counts, originating from both denture biofilm and palatal mucosa. Biofilm specimens from dentures were taken using standardized sonication technique[16,17], and palatal biofilm specimens were collected with a sterile swab, which was put on the central part of the palate (1 cm²)[18], then placed in a sterile tube with 5 ml normal saline for subsequent sonication (2 min). All biological samples were collected between 9 a.m. and 12 p.m. to minimize circadian variation in microbial counts[19,20] and were immediately sent to microbiological laboratories at each study centre. Sonicates from palate and the denture were serially diluted in saline (10⁻¹ to 10⁻⁴) and inoculated on Sabouraud dextrose 4% agar (Difco, Becton Dickinson, Sparks, MD, USA) then incubated for 48 hours at 37 °C. CFU/ml in the original sonicate (10⁰) was measured after counting CFUs from the dilutions that yielded 1–300 colonies[21].

2.4.2. Secondary outcomes

The secondary outcomes consisted of the degree of inflammation of denture-bearing tissues, as observed by a clinician. We classified clinical findings according to three classifications, i.e., Modified Newton[14,22], Schwartz[23], and Budtz-Jorgensen[24] classification (**Table S1**). In brief, the modified Newton classification further divides class I into IA (petechiae only) and IB (localized inflamed areas), whereas classes II (generalized area of inflammation) and III (inflammation plus palatal mucosal hyperplasia) remains the same of the original classification.

The Schwartz index was used to grade the area and severity of inflammation, with each aspect based on a 4-point Likert scale (range: 0 to 3 each). Summing the area and severity scores yielded a total score ranging from 0 (healthy mucosa) to 6 (severe and widespread inflammation). Both Newton and Schwartz indices were applied on baseline and during the 3- and 6-month follow-ups. Finally, changes in inflammation over time/treatment effect were classified according to Budtz-Jorgensen, based on the difference between pre- and post-treatment total inflammation scores. Four categories were possible: (i) inflammation resolved/large effect; (ii) inflammation reduced/moderate effect; (iii) no change in inflammation/no effect; and (iv) increased inflammation/negative effect.

The clinicians' diagnostic calibration was conducted with an interobserver agreement of good to excellent ($k=0.6$ to 0.84). Photographs of the palatal mucosa were also taken using a Nikon D90 Camera (105 mm f/2.8 D, macro flash SB-21; Nikon Co., Tokyo, Japan) to ensure diagnostic agreement among clinical investigators. The first author was present at the three clinical sites to train data collectors regarding how to classify palatal inflammation and exploratory variables, before commencing the trial. Those visits included the explanation of trial procedures with representative photos from our previous single-arm study[14], as well as demonstrations with patients that would match the eligibility criteria of this trial. All sites were visited again at least once to ensure good/similar practices, when around 50% of planned recruitment was achieved.

Participants were also asked whether they were experiencing mild pain on each time point, as a potential adverse effect of palatal brushing[14]. Answers were provided on a 4-point Likert scale (0: never; 1: rarely; 2: sometimes; 3: often).

2.4.3. Explanatory variables

At baseline, medical and dental history, sociodemographic, lifestyle, as well as oral health and denture-related data were collected by administering a self-reported questionnaire and performing clinical examinations[15]. In each country, self-reported questionnaires were written in their respective official languages, i.e., French and English for site 1, Portuguese for site 2, and Spanish for site 3. Please refer to Appendix for a copy of data collection forms and detailed information on exploratory variables.

2.5. Randomization, blinding, and allocation

Off-site computer-assisted, centralized block randomization was used for this trial, with stratification done according to each centre. Each participant received a sequentially numbered, sealed, opaque, and tamper-proof envelope from the trial coordinator at the respective centre, containing the concealed allocated intervention.

All clinicians and those in charge of data analysis were blind to group assignment. However, the nature of the intervention did not allow for blinding the participants and the research trainee responsible for palatal brushing instructions. To minimize bias from these sources, participants were scheduled so as to avoid waiting time together, and thus avoid potential contamination. Also, the research trainee opened the envelope for each participant immediately before hygiene instructions, in the absence of other team members.

2.6. Sample size

Based on our pilot study results[14], minimal clinically meaningful inter-group difference in *Candida* CFU/ml mean change score was considered 30.0 with a standard deviation of 31.6. Considering a dropout rate of 10% and power of 85% to reject the null hypothesis, sample size in each group was set to 26 participants. Sample size estimation was calculated by using the Statulator online tool[25].

2.7. Statistical methods

Statistical analyses included descriptive analyses and generalized estimating equations, considering follow-up time (paired factor) and the two-factor interaction. The primary outcome variable (CFU counts) underwent log10 (CFU+1) transformation for better data distribution and interpretation. Furthermore, it was tested based on pre- post-treatment differences, to compensate for mild imbalances at baseline.

An identity link function and generalized score statistics were used in the generalized estimating equations (GEE), and an exchangeable working correlation was assumed. Effect estimates for each factor were shown as mean values and unstandardized regression coefficient (B), with respective 95% confidence intervals. All data analyses were performed on an intention-to-treat basis. The level of statistical significance for all tests was considered as 0.05. SPSS software (v. 22.0, IBM Corp, v. 22.0. Armonk, NY, USA) was used to conduct all analyses.

Given the considerable number of non-carriers of *Candida* spp., we conducted a subgroup analysis including just participants who were *Candida*-positive on baseline. The GEE was redone for the primary outcome as above but considering just participants with ≥ 1 CFU in at least one of the sites (denture and palate).

Missing data was imputed for *Candida* spp. count changes and total inflammation score (Schwartz et al. index)[23]. Five datasets were obtained by multiple imputation based on least squares regression, with GEE redone with pooled imputations.

3. Results

The three clinical sites invited 326 patients to participate in this trial (**Fig. 1** presents the study flowchart). **Tables 1 and 2** present sociodemographic and clinical characteristics of the study participants. The mean age of the study participants (12 men and 57 women) was 69 years (SD: 11). There was no statistically significant baseline data difference between the two study arms. Participants were completely edentulous for a mean time of 30 (SD: 15) years, with an average current upper denture age of 17 (SD: 15) years. All dentures had acrylic resin bases and teeth. Forty-five percent of participants reported that they did not receive any oral and denture hygiene instructions prior to the study. A smaller number of individuals used a denture

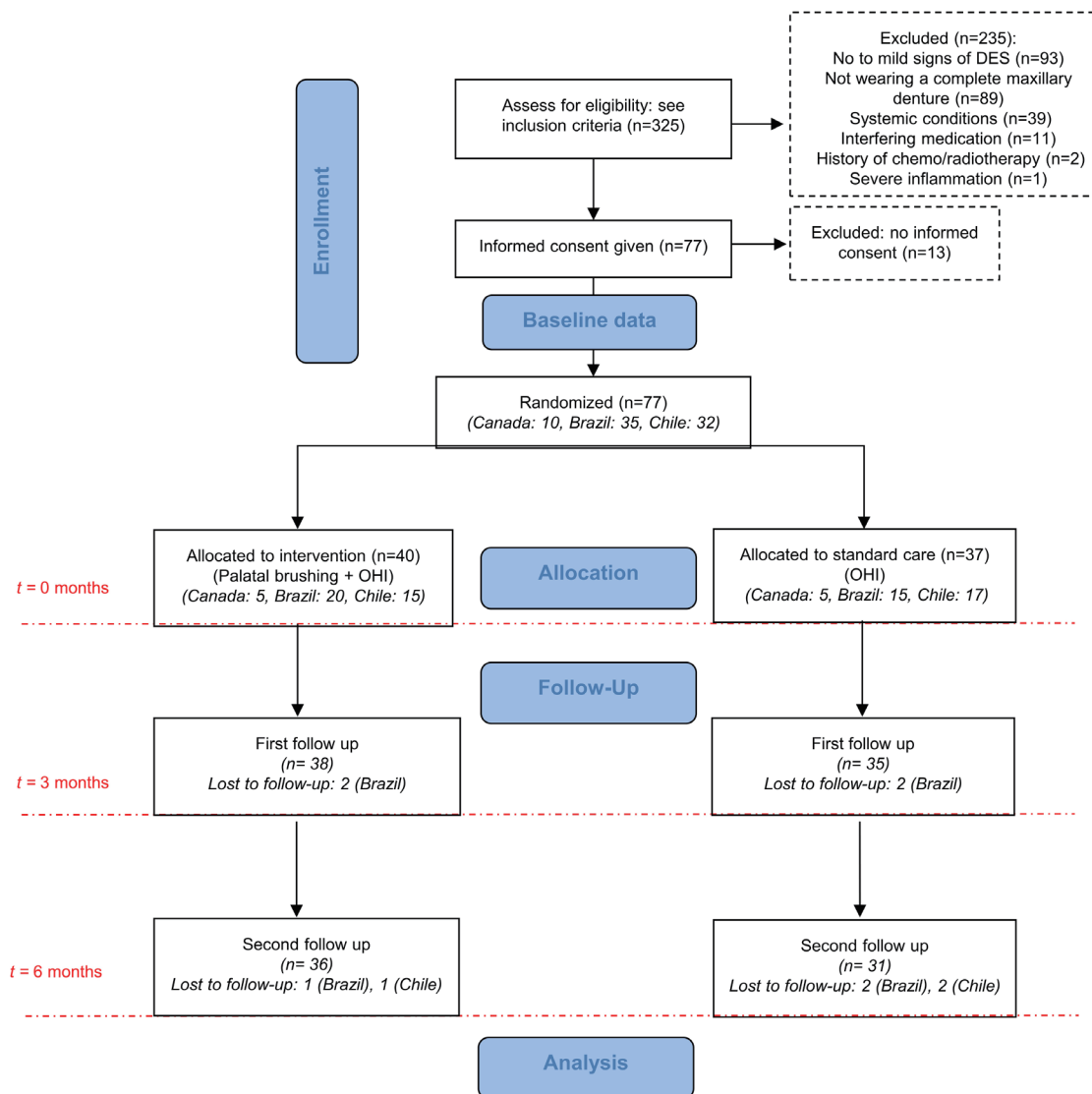


Fig. 1. CONSORT study flowchart, with separate numbers for each clinical site from baseline to 6 months

cleaner (15%) compared to mouthwash (36%), and more than half of the participants (65%) were wearing their dentures overnight. Also, only 6% of participants used any adhesive for their upper dentures. Regarding medical history, most participants (48%) had some systemic disease, the most common being thyroid diseases (16%) and hypertension (14%). Most participants (73%) were using medications, although there was no clear predominant type of medication. Salivary quality was deemed adequate in 83% of participants.

3.1. Primary outcome

Candida spp. were detected in 76% of study participant biological samples, with 71% retrieved from denture biofilm (intervention: 73%; control: 70%) and only 39% from palatal mucosa biofilm (intervention: 30%; control: 49%) (**Fig. 2**). At baseline, there was no significant between-group difference regarding *Candida* spp. colony forming unit (CFU) counts for denture biofilm samples (Mann-Whitney U, $p=0.93$), however, this difference was statistically significant for the palatal mucosa ($p=0.02$).

- Denture Biofilm: Pre-post intervention changes (positive values mean favourable results/reduction after treatment; whereas negative results represent that post-intervention counts were higher than baseline) in *Candida* spp. log10-transformed counts (95% CI) were 0.0 (-0.6 to 0.6) at 3-month follow-up, and 0.5 (-0.2 to 1.3) for the control group. At 6-month follow-up, pre-post intervention difference in transformed counts were 0.6 (95% CI: -0.2 to 1.4) and -0.1 (95% CI: -0.9 to 0.7) for intervention and control, respectively. Although group and time exerted no significant effect on those counts (GEE – complete dataset, $p=0.85$ and 0.10 , respectively), the interaction between the group and the time was significant ($p=0.02$). Parameter estimates were not significant (**Table 3**).

- Palatal mucosa: Pre-post treatment change scores in *Candida* spp. log10-transformed counts were -0.3 (95% CI: -0.7 to 0.2) for intervention group at 3 months, and 0.2 (95% CI: -0.3 to 0.7) for the control group. At 6 months, transformed counts changed to 0.1 (95% CI: -0.2 to 0.4) in the intervention group and -0.1 (95% CI: -0.7 to 0.4) in the control group. Both group and time exerted no significant effect

Table 1. Sociodemographic characteristics of study participants

Variables n (%) or Mean (SD)	Palatal Brushing (40)	Standard Hygiene (37)	Total (77)
Demographic			
Age	70 (SD 9)	67 (SD 13)	69 (SD 11)
Men: Women (%men)	3:33 (8)	9:24 (27)	12:57 (17)
Education level			
• High school or less	32 (89)	31 (94)	63 (91)
• College and higher	4 (11)	2 (6)	6 (9)
Yearly income			
• < \$10,000	9 (26)	14 (44)	23 (34)
• \$10,000-\$29,999	22 (63)	15 (47)	37 (55)
• >\$30,000	4 (11)	3 (9)	7 (11)
Number of past or current smokers	19 (53)	15 (46)	34 (49)
Mean pack/year for past or current smoker	25 (SD 23)	21 (SD 21)	23 (SD 22)
Alcohol consumption			
• Never	12 (33)	12 (39)	24 (36)
• Rarely	9 (25)	6 (19)	15 (22)
• Occasionally	14 (39)	12 (39)	26 (39)
• Frequently	1 (3)	1 (3)	2 (3)

on those counts (GEE – complete dataset, $p=0.69$ and 0.78 , respectively), as well as their interaction ($p=0.09$). Parameter estimates were not significant (**Table 3**).

- Research centres: For denture biofilm and palatal mucosa, centres had no significant effect (GEE, $p=0.24$ and 0.05 , respectively). The interaction between groups and centres was not significant either ($p=0.22$ for denture biofilm and 0.43 for palatal mucosa).

- Subgroup analysis: For the intervention group, the number of *Candida*-positive participants who concluded the 3- and 6-month follow-ups were 27 and 24, respectively; 27 control group participants concluded both 3- and 6-month follow-ups (**Table 3**). For the log-transformed *Candida* count changes in denture biofilm, the group-time interaction was significant (GEE, $p=0.04$), but not for each factor separately (p , group= 0.21 ; time= 0.67). Groups and time yielded more distinct results evident with subgroup analysis, with estimated means being: 3 months, intervention: 0.8 (95% CI: 0.0 to 1.5) and control: 0.7 (95% CI: -0.2 to 1.7); 6 months, intervention: 1.6 (95% CI: 0.7 to 2.6) and control: 0.2 (-0.7 to 1.1). Changes in *Candida* counts from the palatal mucosa were not affected by group (GEE, $p=0.46$), time ($p=0.86$), or their interaction ($p=0.27$). Log-transformed differences at 3 months were: intervention: 0.1 (95% CI: -0.5 to 0.8) and control: 3 months: 0.7 (95% CI: -0.1 to 1.4); at 6 months, intervention: 0.4 (95% CI: 0.1 to 0.8) and control: 0.4 (95% CI: -0.2 to 1.1).

3.2. Secondary outcome

Table 4 presents data on the effect of palatal brushing on inflammation by the Schwartz index. Regardless of the group, almost half of the participants had inflammation affecting 25–50% of the denture-bearing area, and nearly one-quarter had either <25% or >50% in area. Severity of inflammation tended to be moderate in nearly half of the cases, but more participants presented mild rather than severe inflammation. There were no significant between-group differences for total inflammation scores (Mann-Whitney U, $p=0.436$), as well as the area ($p=0.409$) and severity ($p=0.609$) at baseline. As

for the Newton classification, most participants presented stomatitis class II, whereas almost one-fifth had class III, and between 10 to 16% had some of the class I subdivisions.

Figure 3 provides data on palatal inflammation, showing improvement over time and slightly lower scores with palatal brushing. Although time (GEE, $p<0.001$) and the group-time interaction ($p=0.01$) were significant, groups had no significant effect ($p=0.50$). Estimated mean scores after 3 months were 3.8 (95% CI: 3.4 to 4.2) for the intervention group and 3.5 (95% CI: 3.1 to 3.9) for the control group; at 6 months, scores dropped to 2.6 (95% CI: 2.1 to 3.0) for the intervention and 3.2 (95% CI: 2.7 to 3.7) for the control. Post hoc comparisons showed lower scores for the intervention at 6 months compared to both groups at 3 months, whereas intermediate scores were found for the control at 6 months. Transforming 6-month scores into treatment effect (Budtz-Jorgensen Index) showed better improvement with palatal brushing compared to solely hygiene instructions (**Fig. 3**). It showed less inflammation for 70% and 45% of the intervention and control participants, respectively (significant, chi-square, $p=0.04$). Those proportions represented a number needed to treat (NNT) of 3.9 (95% CI: 2.1 to 44.4).

3.3. Multiple imputation

Compared to the primary data, pooled imputed data reached close results for all tested outcome variables, i.e., *Candida* counts in denture and palatal biofilm, and total inflammation scores. Both mean differences between groups and their respective 95% CI were similar for both pooled data and complete cases (**Table 5**).

3.4. Adverse effects

Most participants were experiencing no pain at each time point, and between-group differences were not significant. At baseline, mild pain was never experienced by 25 (63%) intervention and 26 (70%) control group participants (Mann-Whitney, $p=0.674$). After oral hygiene instructions, participants without mild pain corresponded

Table 2. Medical and dental history of study participants

Variables n (%) or Mean (SD:)	Palatal Brushing (40)	Standard Hygiene (37)	Total (77)
Medical history			
Presence of systemic disease	20 (56)	17 (46)	37 (54)
Medication use	24 (60)	26 (70)	50 (73)
Immunosuppressant/Corticosteroid	2 (6)	2 (6)	4 (6)
Dental history and habits			
Consultation of oral health professional			
• Never	6 (17)	3 (9)	9 (13)
• Emergency	16 (44)	22 (67)	38 (55)
• Once a year	8 (22)	3 (9)	11 (16)
• Once or more a year	6 (17)	5 (15)	11 (16)
Received instruction for denture hygiene	15 (43)	15 (47)	30 (45)
Denture cleaner	6 (17)	4 (13)	10 (15)
Mouthwash	15 (43)	9 (28)	24 (36)
Adhesive for upper denture	1 (3)	3 (9)	4 (6)
Difficulty cleaning the denture	3 (9)	6 (19)	9 (13)
Food under the denture			
• Rarely	5 (14)	6 (19)	11 (17)
• Occasionally	14 (40)	7 (22)	21 (31)
• Frequently	9 (26)	9 (28)	18 (27)
• Very often	7 (20)	10 (31)	17 (25)
Nocturnal wear (upper denture)	19 (56)	13 (41)	32 (49)
Oral clinical exam			
Mean years of edentulism	32 (SD 15)	27 (SD 15)	30 (SD 15)
Mean age of current upper prosthesis (year)	19 (SD 16)	14 (SD 13)	17 (SD 15)
Number of upper prostheses	2 (SD 3)	2 (SD 1)	2 (SD 2)
Number of lower prostheses	2 (SD 1)	1 (SD 1)	1 (SD 1)
Upper denture satisfaction (0-100)	50 (SD 32)	50 (SD 37)	50 (SD 34)
Cheilitis	2 (5)	2 (5)	4 (5)
Saliva quality			
• Normal	31 (80)	32 (87)	63 (83)
• Excessive, much mucus	0	1 (3)	1 (1)
• Insufficient, mucinous	8 (21)	4 (11)	12 (16)
Denture plaque			
• Clean	13 (34)	7 (19)	20 (27)
• Mild debris	13 (34)	20 (56)	33 (45)
• Severe debris	12 (32)	9 (25)	21 (28)
Upper denture retention	19 (63)	19 (61)	38 (62)
Upper lateral displacement	13 (43)	19 (61)	32 (53)
Upper denture resilience			
• Firm	25 (64)	25 (68)	50 (66)
• Resilient	9 (23)	5 (13)	14 (18)
• Flabby	5 (13)	7 (19)	12 (16)

Missing data: 0-20% per variable

to: 30 (79%) intervention and 26 (74%) control at 3 months ($p=0.796$); 28 (78%) intervention and 23 (74%) control at 6 months ($p=0.687$). No participant reported any adverse effect that led them to stop palatal brushing or any of the standard oral hygiene methods, including moderate to severe pain or persistent mucosal bleeding.

4. Discussion

To our best knowledge, this is the first randomized trial that has examined the role of palatal brushing in the treatment of DES. This trial provides evidence in favour of adding palatal brushing to the hygiene instructions of denture wearing patients. No statistically significant change in *Candida* carriage on the palatal mucosa was found; however, it is well known that denture biofilm, and not palatal

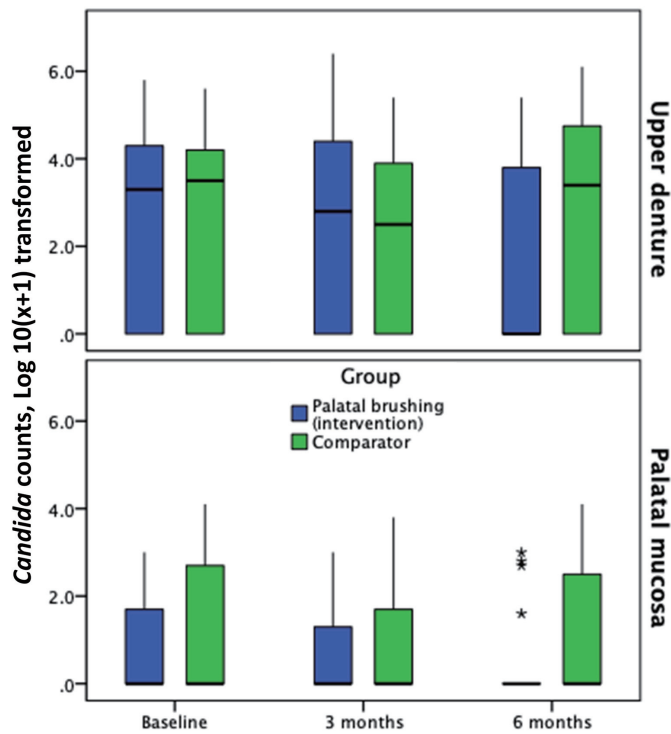


Fig. 2. Box-plot graph with *Candida* spp. counts before and after treatment according to group allocation and site

biofilm, is the primary harbouring site for *Candida*. As for inflammation, a significant decrease was found in both groups in the denture-bearing mucosa, with larger effect in the palatal brushing group. These results are in line with the current literature on the efficacy of palatal brushing and oral hygiene methods in controlling DES[14].

The decrease in *Candida* carriage on upper dentures shows how palatal brushing can treat DES. This finding discloses that mucosal hygiene affects the composition of denture biofilm, most likely

due to the dissipation of by-products of tissue injury/inflammation. Denture biofilm can harbour pathogenic bacteria and yeasts, which can produce toxins and metabolic wastes and thus inflammation of the mucosa[1,14]. A vicious circle takes place, since the inflamed mucosa produces proteins that stimulate candidal growth and virulence[1,26]. Thus, palatal brushing complements standard hygiene methods in breaking that circle, with the first reducing inflammation by-products while the latter removes denture biofilm mechanically.

The relevance of salivary composition and quantity in the results of palatal brushing deserves further comments. Part of the effect of palatal brushing may be explained by the mechanical stimulation of minor palatal salivary glands. An increased salivary flow from those glands may be behind at least part of the overall positive effect of palatal brushing[15]. Indeed, xerostomia is a well-known predisposing factor in the development of DES and *Candida* infection[27–29]. Both proper salivary flow and saliva composition can reduce the risk of DES and *Candida* carriage by offering mechanical cleansing of the denture biofilm and transporting immune factors specific to fungal microorganisms such as lactoferrin, histatin-5, and many others[14,30–33]. Therefore, palatal brushing most likely acts by stimulating the self-cleansing of the oral cavity, besides the mechanical/direct elimination of inflammation by-products.

When *Candida* was counted directly from the palatal mucosa instead of denture biofilm, post-treatment results were not different for both trial arms. This is not surprising since denture biofilm tends to harbour a larger and more diverse yeast population[33–36]. More precisely, unlike living mucosa, the abiotic surface of a denture does not have a self-renewal mechanism that ensures the elimination of biofilms[35–37]. Furthermore, all dentures evaluated were made of acrylic resin, which can be deteriorated over the years by enzymes and metabolites from biofilm[38]. Thus, the ageing of denture base materials has certainly led to increased roughness and susceptibility to *Candida* adhesion[34,36,37]. Therefore, it is not surprising that *Candida* carriage was significantly reduced in the denture and not the palate, but still induced an improvement in clinical signs of DES and inflammation.

Table 3. Estimates from GEE, pre-post differences in *Candida* spp. counts (log10 transformed)

Source				B	95% wald CI
Complete dataset	Denture biofilm	Intercept		0.2 (SD 0.6)	(-1.0 to 1.5)
		Groups	Intervention-Control*	1.3 (SD 0.8)	(-0.2 to 2.8)
		Time	3 months-6 months*	-0.2 (SD 0.3)	(-0.8 to 0.4)
		Interaction	Intervention group at 3 months - Other combinations*	0.2 (SD 0.6)	(-0.9 to 1.3)
	Palatal mucosa	Intercept		0.7 (SD 0.5)	(-0.2 to 1.7)
		Groups	Intervention-Control*	-0.3 (SD 0.5)	(-1.4 to 0.8)
		Time	3 mo-6 mo*	-0.2 (SD 0.2)	(-0.7 to 0.3)
		Interaction	Intervention group at 3 months - Other combinations*	0.3 (SD 0.4)	(-0.5 to 1.1)
<i>Candida</i> -positive on baseline	Denture biofilm	Intercept		0.2 (SD -0.7)	(1.1 to 0.2)
		Groups	Intervention- Control*	1.4 (SD 0.1)	(2.8 to 1.4)
		Time	3 months-6 months*	0.5 (SD -0.2)	(1.3 to 0.5)
		Interaction	Intervention group at 3 months- Other combinations*	-1.4 (SD-2.7)	(-0.1 to -1.4)
	Palatal mucosa	Intercept		0.4 (SD 0.3)	(-0.2 to 1.1)
		Groups	Intervention-Control*	0.0 (SD 0.3)	(-0.6 to 0.6)
		Time	3 months- 6 months*	0.2 (SD 0.3)	(-0.4 to 0.8)
		Interaction	Intervention group at 3 months - Other combinations*	-0.5 (SD 0.4)	(-1.3 to 0.3)

* Reference value

Note: reference values were generated in multiple imputations

Table 4. Effect of brushing on palatal inflammation according to Schwartz index and Newton classification

Variables N (%)	Palatal Brushing n(%)				Total	Standard Hygiene n(%)				Total	p-value*
Inflammation area	0	1	2	3		0	1	2	3		
Baseline	0 (0)	11 (28)	19(48)	10(25)	40	0(0)	13(35)	17(46)	7(19)	37	0.40
3-month follow-up	4(11)	21(55)	8(21)	5(13)	38	2(6)	13(38)	11(32)	8(24)	34	0.07
6-month follow-up	10(29)	13(37)	4(11)	8(23)	35	5(16)	10(32)	8(26)	8(26)	31	0.20
Inflammation severity	0	1	2	3		0	1	2	3		
Baseline	0(0)	15(38)	18(45)	7(18)	40	0(0)	14(38)	20(54)	3(8)	37	0.60
3-month follow-up	4(11)	26(68)	5(13)	3(8)	38	2(6)	18(53)	10(29)	4(12)	34	0.08
6-month follow-up	10(29)	14(41)	8(24)	2(6)	35	5(16)	12(39)	11(36)	3(10)	31	0.13
Newton classification	0	Ia	Ib	II	III	0	Ia	Ib	II	III	
Baseline	0(0)	6(15)	4(10)	23(58)	7(18)	0(0)	6(16)	6(16)	18(49)	7(19)	0.72
3-month follow-up	4(11)	6(16)	15(39)	10(26)	3(8)	2(6)	6(18)	8(24)	15(44)	3(9)	0.24
6-month follow-up	9(26)	6(17)	7(20)	11(31)	2(6)	5(16)	5(16)	6(19)	13(42)	2(6)	0.30

*p-values refer to between-group comparisons within each time period (Mann-Whitney test)

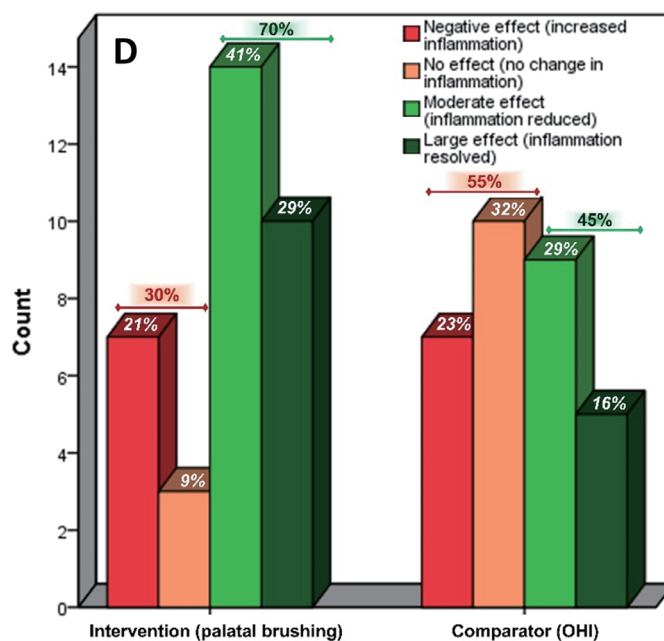
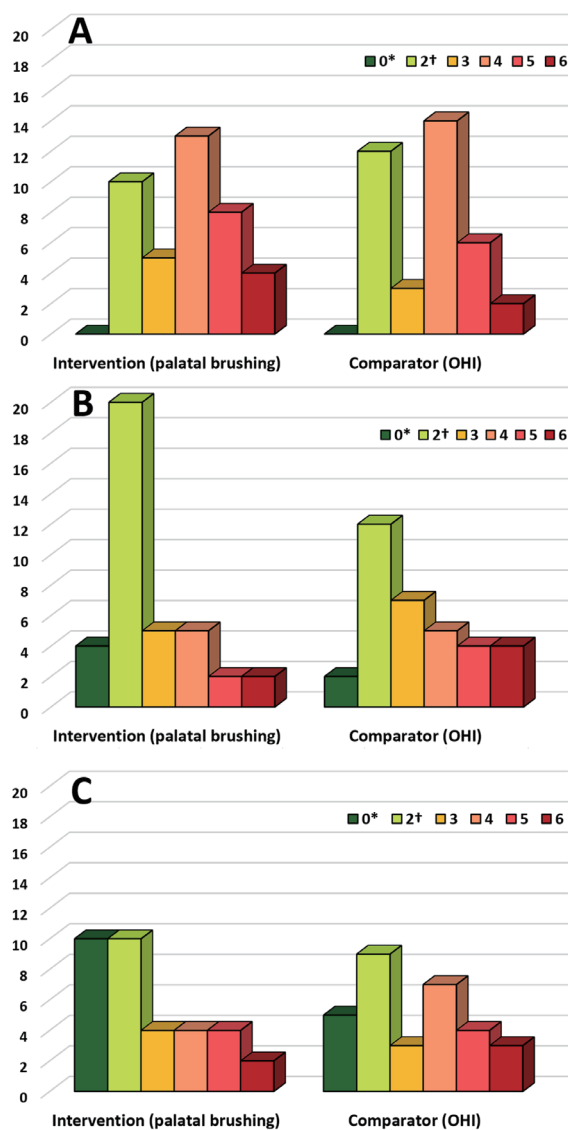


Fig. 3. Clinical aspect of DES, according to the Schwartz et al. index, total inflammation scores (area + severity) at: (A) baseline; (B) 3-month follow-up; and (C) 6-month follow-up. **Figure 1D** shows treatment effect based on pre- and post-treatment differences (Budtz-Jorgensen index). Percent values over horizontal bars represent participants with some benefit for each group. Notes: * not applicable on baseline; † minimum score with DES, i.e., inflammation of mild severity and extending over <25% of the denture-bearing palatal mucosa.

Table 5. Between-group mean differences for each follow-up (and 95% CI)

Variable	Dataset	3 months	6 months
<i>Candida</i> , denture biofilm	Pooled, MI*	0.3 (-0.9 to 1.4)	0.5 (-0.9 to 1.8)
	Complete cases	0.3 (-0.7 to 1.4)	0.7 (-0.5 to 1.8)
<i>Candida</i> , palatal mucosa	Pooled, MI*	-0.3 (-1.1 to 0.5)	0.0 (-0.6 to 0.7)
	Complete cases	-0.4 (-1.2 to 0.4)	0.2 (-0.4 to 0.9)
Inflammation	Pooled, MI*	-0.7 (-1.4 to 0.1)	-0.6 (-1.5 to 0.3)
	Complete cases	-0.7 (-1.4 to 0.1)	-0.6 (-1.6 to 0.3)

*MI: multiple imputation.

Note: *Candida* counts were based on log-transformed pre-post treatment differences, and inflammation refers to the total inflammation scores (area + severity) observed at each follow-up. Positive mean differences mean higher values for Group 1 (palatal brushing).

It is important to highlight that more than half of the participants wore their dentures overnight during our clinical study, which can have affected our results for oral inflammation and *Candida* count[39]. However, we have not included instructions to avoid overnight denture wearing in this trial to maintain a pragmatic framework, i.e., to observe the effect of palatal brushing over “real-life” conditions. It is important to highlight that the rate of overnight wearing was evenly distributed amongst the two study groups on baseline, thus unlikely able to influence post-intervention between-group differences. The same can be said for all sociodemographic and clinical characteristics of participants, including denture retention, the use of denture cleaners and mouthwash.

The significant reduction in inflammation area and severity found at the 6-month follow-up also supports the efficacy of palatal brushing in the care management of DES. Indeed, mechanical stimulation has been demonstrated to stimulate keratinization, reduce inflammatory infiltrates, increase the proliferation of fibroblast and collagen synthesis, improve microcirculation, and as already mentioned, the salivary flow[26,27,29,31]. Through these mechanisms, palatal brushing can stimulate mucosal repair and eliminate the inflammatory processes underlying the development of DES. Moreover, the decrease in inflammation can also partly explain the decrease in *Candida* carriage that was observed. Indeed, as the inflammatory state decreased, the factors produced by the inflamed mucosa that stimulates the development of *Candida* can also decrease, distinguishing palatal brushing from other hygiene methods that solely address the denture biofilm[26]. Supporting this hypothesis, even if standard hygiene instructions did improve the clinical signs of DES in the comparison group, the effect did not reach the extent of improvement seen in the palatal brushing group.

From a clinical perspective, palatal brushing has demonstrated a net superiority in reducing clinical signs of DES. Not only did this method lead to significant improvement in most participants, it also established a number needed to treat of less than four patients. In other words, a clinician recommending palatal brushing to address DES will need to treat four patients to see an improvement in one of them, compared to standard hygiene instructions. It is worth mentioning that most studies using antifungals have reported rapid recurrence of the conditions, some as soon as 14 days after the discontinuation of treatment[8,40]. In contrast, palatal brushing maintained a linear trajectory of decrease in clinical signs of DES, inflammation, and *Candida* carriage. Further investigations need to be carried out to examine long-term outcome of palatal brushing.

The authors acknowledge that this trial has limitations. The

multicentre design of this study might have resulted in discrepancies among study participants from different centres. However, subjects were compared at baseline to ensure comparability of the study groups, and the statistical analysis did not find any centre influence on the outcomes. In reality, a multicentre design could enable a larger sample and enhance the generalizability of results to different populations. This result is in line with the phase one trial findings demonstrating no effect of recruiting centre on baseline data and intervention outcomes[14]. However, contrary to the phase one trial, some participants were lost to follow-up. To address this limitation, an imputation strategy showed that incomplete data has not led to biased results.

The pragmatic approach of this trial and its intention-to-treat analysis also ensure that its results can still be reproduced in the general population. The results obtained in this study can be hypothesized as being as close as possible to a real-life setting where clinicians cannot ensure their patient’s compliance with treatment.

Another noteworthy aspect refers to the finding of lower *Candida* counts with the intervention group, which was statistically significant only in subgroup analysis. This is not surprising, since less effect could be expected when the data are diluted with *Candida*-negative participants. Even though this kind of subgroup analysis often deserves a conservative interpretation, results are strengthened by their consistency with primary analysis and the study findings on the effect of palatal brushing on mucosal inflammation[41].

In summary, this study supports the result of the previous trial and the effectiveness of palatal brushing in DES treatment. The authors recommend using this simple and cost-effective new therapy, along with standard hygiene instructions, to treat DES.

Acknowledgments

This study was supported by funds from the CIHR Phase II Clinician Scientist Award (award #201110, E. Emami), FRQ-S RSBO Recruitment Aid (grant BRDV-FRQS-201-2016-046, RF de Souza), and internal grant by Universidad de la Frontera (grant DIUFRO-DIE15-0003, E. Borie). RF de Souza is the recipient of a “Clinical Research Scholar” salary award from the FRQ-S (dossier 252635). The authors thank Pierre Rompré for his assistance with analytical planning.

Conflicts of interest

The authors have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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