The Relationship of Myceliated Colonies of *Candida Albicans* with Denture Stomatitis: An In Vivo/In Vitro Study

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Purpose: Switching from smooth to myceliated colonies, a virulent trait of *Candida albicans*, may be implicated in *Candida*-associated denture stomatitis. The purpose of this study was to verify the relationship between the presence of denture stomatitis and the frequency of myceliated colonies of *C albicans* isolates in denture wearers. Prevalence of denture stomatitis and influence of putative risk factors were also investigated. ***Materials and Methods:*** Demographic and clinical data concerning oral and general health, smoking, denture status, diet, and hygiene habits of 40 complete maxillary denture wearers were collected from an autoevaluation questionnaire and oral examination. Detection of *C albicans* in denture plaque and evaluation of hairy phenotype colonies were carried out on low nutrient media. Eleven subjects were followed-up at 1 month and 3 months after delivery of a new prosthesis. Results were statistically analyzed. ***Results:*** Prevalence of denture stomatitis was 77.5%. No statistically significant relation was found between presence of stomatitis and frequency of myceliated colonies of *C albicans* or presence of yeast. However, the study confirmed a statistically significant difference between Newton types IA and IIB stomatitis in relation to yeast colony-forming units, which were more than 300 times higher in type IIB. A direct relationship was observed between the presence of *C albicans* and nocturnal denture use ($P = .01$) and an inverse relation was observed with brushing of the palate ($P = .03$). ***Conclusion:*** The ability of *C albicans* strains isolated from dentures to produce myceliated colonies may not be directly involved in denture stomatitis. *Int J Prosthodont 2007;20:514–520.*

Denture stomatitis is an inflammatory condition of the palatal mucosa seen in complete denture wearers.[1–5] It is generally recognized that denture stomatitis represents the most frequent form of oral candidosis in elderly patients.[6] The prevalence of denture stomatitis varies between 6.5% and 75% depending on the sample population.[7–13] Classification of denture stomatitis has been generally based on the type, distribution, and extent of the inflammation.[1,12,14] This pathology has a multifactorial etiology. Poor oral/denture hygiene, nocturnal wear of the prosthesis, denture trauma, age of denture, smoking, dietary habits, salivary flow, systemic condition, hypersensivity to denture base material, and bacterial and fungal infection have all been proposed as causal or predisposing factors.[1–5,13–19] Although the correlation between denture stomatitis and *Candida albicans* remains controversial, several studies have demonstrated that denture plaque (denture biofilm) acts as a main reservoir of *C albicans*, which can lead to colonization, invasion of tissues, induction of inflammatory lesions, and denture stomatitis.[1–5,12,15,19–32]

C albicans is an opportunistic pathogen, which is isolated in 30% to 40% of healthy adults and 50% to 60% of people who wear removable dentures.[20,21] One virulence factor of *C albicans* is phenotypic switching, which is the capacity to change colony morphotypes. It is known that on special solid media, *C albicans* can switch from smooth colonies to an array of morphologic variants.[7,21–24] These variants are thought to be the ex-

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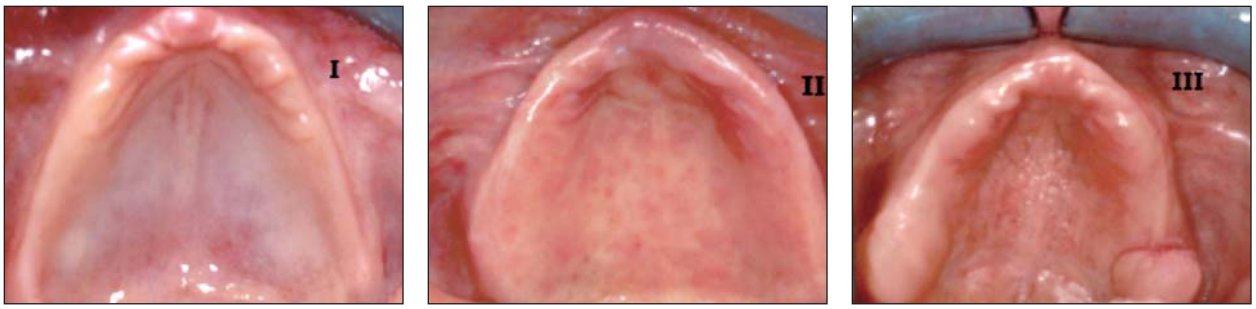


Figs 1a to 1c Palatal mucosa from subjects with denture stomatitis classified according to the modified Newton classification **(a)** Newton type IA; **(b)** Newton type IIB; **(c)** Newton type IIIB.

pression of different blastospore-to-hypha ratios. The hypha is regarded as more virulent because of its greater adherence to epithelial tissue.[7] According to several studies, hyphae may penetrate the host epithelial cell and thus contribute to invasive growth of *C albicans*.[25,26] The phenotypic switch confers to *C albicans* the capacity to express various virulence factors or change their antigenic profile, thus enabling this yeast to colonize different microniches during infection, adapt to different anatomic sites, and escape from the immune system and certain antifungal treatments.[21,24,27] Poor nutrient solid media can allow *C albicans* strains to express characteristic frequency of hairy (myceliated) colony morphotypes. The main goal of this study was to evaluate the relationship between *Candida*-associated denture stomatitis and the frequency of myceliated colonies of *C albicans* strains isolated from healthy subjects and subjects affected by denture stomatitis. Another objective was to determine the prevalence of denture stomatitis and evaluate the risk factors that may influence the condition. The hypothesis was that the high frequency of myceliated colonies of *C albicans* plays a role in *Candida*-associated denture stomatitis.

Materials and Methods

Subjects

Seventy ambulatory subjects (mean age: 64.5 years) from the outpatient prosthodontic clinic of the Université de Montréal consulting for the replacement of complete dentures were invited to take part in the research project. The only inclusion criterion was patients wearing a complete maxillary denture. Subjects with implant-supported prostheses or obturator and metal base prostheses were excluded. After being informed about the project, 40 subjects agreed to participate in the study (29 women and 11 men). The protocol was approved by the ethics committee of the Université de Montréal, and each subject signed a written informed consent form prior to enrollment in the study.

Clinical Investigation

Oral examinations were performed by a single clinician using a front surface mirror and probe (XP23/QW, Hu-Freidy). Diagnosis was confirmed clinically by another clinician and then by a third clinician using photographs taken with a Nikon F70 camera (105 mm f/2.8D; macro flash SB-21; Kodak film ASA 100 Ektachrome). An excellent interobserver reliability was obtained ($\kappa = 0.87$ to 1). Denture stomatitis was classified as Newton type I, II, or III and subclassified as A or B regarding the extent of inflammation according to a modified Newton classification (Fig 1)[12,14]:

- *Newton type I:* Pinpoint hyperemia: localized areas of inflammation in a normal palatal tissue, which are usually found around the orifices of the ducts of the palatal mucous glands.
- *Newton type II:* Diffuse hyperemia: a generalized inflammation of the denture-bearing area.
- *Newton type III:* Granular: hyperplasic palatal surface, which may be generalized or restricted to the central areas.

Each classification is subdivided into an A or B subclass. Subclass A presents inflammation in 1 to 2 quadrants and subclass B indicates inflammation in more than 2 quadrants.

The demographic data (age, sex, medical and dental histories, medication profiles), denture status (years of denture wear, age of denture, stability and retention of denture), hygiene habits (cleaning frequency, palatal brushing, nocturnal wear), sugar consumption, and smoking, were gathered from an autoevaluation questionnaire (Tables 1 to 3).

The level of hygiene was evaluated through questions with categorized answers: How many times per day do you clean your denture? How do you clean your denture? Do you take off your dentures at night (lower/upper denture)? Do you brush your palate? At which frequency do you brush? The answers were binary summarized: daily cleaning of the dentures or

Table 1 Demographic Characteristics of the 40 Subjects

	Healthy subjects (n)	Subjects with denture stomatitis (n)			
		Newton I	Newton II	Newton III	Total
Age group (y)					
41–50	2	2	1	0	5
51–60	1	1	5	1	8
61–70	3	9	8	0	20
71–80	3	3	0	1	7
Total	9	15	14	2	40
Mean age	64.5	66.4	50.4	69.0	64.5
Gender (%) (women/men)	77.8/22.2	–	–	–	71.0/29.0

Table 2 Risk Factors Associated with Denture Stomatitis*

Explanatory variables	Healthy (%) (n = 9)	Denture stomatitis (%) (n = 31)
Years of denture use		
20–30	11.1	22.6
31–40	44.4	29.0
41–50	33.3	38.7
51–60	11.1	6.5
61–70	0	3.2
Age of denture > 5 y	44.4	67.7
Satisfaction with stability	88.9	64.5
Satisfaction with retention	77.8	83.9
Daily denture cleaning	100.0	100.0
No palatal brushing	66.7	64.5
Nocturnal wear	33.3	38.7
Sugar consumption: 2 types or more than once a day	55.6	48.4
Smoking	11.1	16.1%

*All factors: $P > .05$ (Fisher exact test).

Table 3 Risk Factors Associated with *Candida albicans*

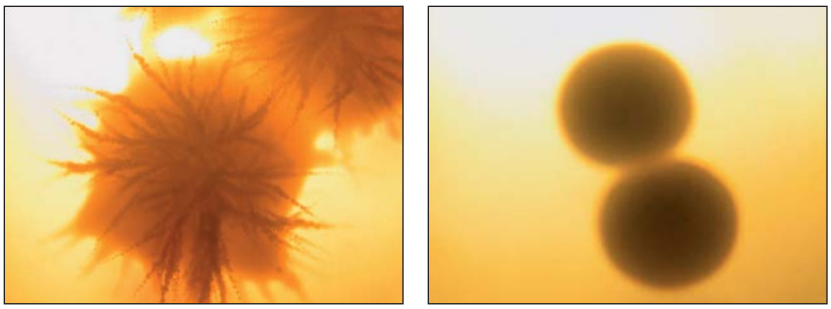
	Subjects with *C albicans* (n = 8)	Subjects without *C albicans* (n = 32)	*P*
Brushing of the palate	0%	43.8%	.03
Nocturnal wear	75%	25%	.014

cleaning less than once a day; brushing the dentures or washing without brushing; brushing the palate at least once a day or brushing less than once a day. Sugar consumption was assessed through questions about the frequency and type of sweet consumption (dessert, sweet drinks, and candies). These nominal data were categorized into 2 main groups: eating once or one type of sugary food in each day or eating twice or two types or more sugar. For smoking, the subjects were divided into 2 groups, nonsmokers and smokers, the latter including regular and occasional smoking.

Microbiologic Investigation

The collection of denture plaque was carried out by treating the prosthesis with an ultrasonic bath. The denture was rinsed under running tap water and inserted in a plastic bag (Ziploc, S.C. Johnson and Son) containing 30 mL of sterile saline (0.85% sodium chloride). This first bag was inserted into a second bag and sonicated for 5 minutes at room temperature in an ultrasonic bath containing distilled water (Cole Parmer 26373, 50/60 Hz, 1.3 Amp). The recovered denture plaque was transferred to a 50-mL sterile tube referred to hereafter as "sonicate" and kept on ice until processing at the laboratory within 24 hours.

All sonicates were mixed by vortex for 1 minute and diluted 10 fold serially with saline (dilution factors: 10^0 [nondiluted], 10^{-1}, and 10^{-2}). A volume of 100 mL was spread-plated in duplicate on different solid culture media: inhibitory mold agar (Becton Dickinson), sabouraud dextrose 4% agar (Difco), and trypticase yeast extract agar (peptone trypticase, yeast extract, sodium chloride, sodium phosphate, agar). Media were prepared at the laboratory. All cultures were incubated at 37°C, 2.5% carbon dioxide, with humidity for 48 hours. The number of colony-forming units (cfu) was recorded visually and expressed as cfu/prosthesis after correction for the volume inoculated and dilution factor. The volume of diluent in which the denture plaque was recovered was standardized. This allowed the evaluation of total numbers of *C albicans* present on the prosthesis. Although the standardization scheme does not account for individual variations of prosthesis surface or accumulated quantity of plaque, these parameters are reflected in the total number of units recovered from the individual dentures. The percentage of colonies with a hairy phenotype, indicating high hyphae-blastospores ratio, was noted and verified using an inverted microscope (Leica, Leitz DM IT) (Fig 2). Identification of *C albicans* was confirmed by microscopic morphology, growth on selective culture medium (CHROMagar *Candida*), serum germination test, and sugar assimilation with API 20C Aux system (bioMerieux-Vitek).



Figs 2a and 2b Phenotypic switching of *C albicans* colonies on trypticase yeast extract agar culture medium. The hairy phenotype **(a)** corresponds to a higher hypha-to-blastospore ratio than the smooth type **(b)**.

Table 4 Prevalence of Denture Stomatitis in the 40 Study Subjects

Diagnosis	No. of subjects (%)
Healthy	9 (22.5)
Stomatitis (all types)	31 (77.5)
Newton type I	15 (37.5)
Subdivision A	15 (37.5)
Subdivision B	0 (0.0)
Newton type II	14 (35.0)
Subdivision A	3 (7.5)
Subdivision B	11 (27.5)
Newton type III	2 (5.0)
Subdivision A	0 (0.0)
Subdivision B	2 (5.0)
Total	40 (100)

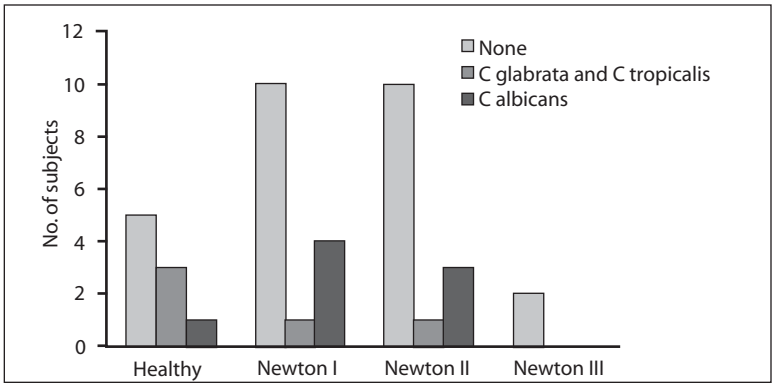


Fig 3 Number of subjects with *Candida* carriage.

Eleven subjects agreed to be followed after wearing the new dentures. The presence of *C albicans* in denture plaque of the new prosthesis was reassessed after 1 month and 3 months.

Statistical Analysis

The data obtained were analyzed by Systat version 10.0 (Systat Inc). For analysis of switch frequencies, Fisher exact test (2-tailed) and Pearson chi-squared analysis were used when appropriate. Odds ratios (95% confident interval) were calculated to measure the strength of association between certain risk factors and denture stomatitis. The influence of cfu and percentage of hairy colonies on denture stomatitis was analyzed using the Mann-Whitney *U* test since these variables did not follow normal distribution. The data were categorized according to the modified Newton classification (Newton type I, II, III and subtypes A and B), and also according to healthy subjects versus subjects with denture stomatitis. All explanatory variables were cross tabulated with diagnosis as well as with the presence of *C albicans*.

Results

The prevalence of denture stomatitis was 77.5% in 40 subjects. The number of subjects with Newton type I (n = 15) and Newton type II (n = 14) denture stomatitis was similar, whereas only 2 subjects were diagnosed with Newton type III and 9 were free of any inflammation (Table 4).

Thirteen subjects (32.5%) carrying *Candida* yeast were identified: 4 healthy and 9 affected by denture stomatitis. Three species of *Candida* were identified: *C glabrata*, *C tropicalis*, and *C albicans*. The subjects with stomatitis showed a higher percentage of *Candida* yeast carriage (69%) than healthy subjects (31%) (Fig 3). However, no statistical difference was found between these groups ($P = .65$, Fisher exact test).

Patients had been completely edentulous and wearing complete denture for 20 to 60 years (mean: 40.3 years), and the mean age of the dentures was 9 years. Ninety-five percent of subjects brushed their dentures and 100% cleaned their dentures at least once a day. Thirty-five percent of subjects reported brushing their palate.

Table 5 Quantitative Evaluation and Phenotypic Expression of *Candida albicans* Strains Isolated from Fresh Denture Sonicates

Subject	Newton classification	Total no. of *C albicans* (cfu)	Hairy phenotype expression (%)
1	Healthy	900	0
2	Type I	3,300	44.4
3	Type I	1,200	75.0
4	Type I	300	0
5	Type I	600	75.0
Median		900	59.7
6	Type II	20,400	12.5
7	Type II	279,000	0
8	Type II	232,800	8.7
Median		232,800	8.7

Statistical analysis showed a significant relationship between nocturnal denture wear and presence of *C albicans* ($P = .01$, OR = 9.0 [1.5 to 53.8]) and an inverse relation between brushing of the palate and the presence of *C albicans* ($P = .03$, OR = 1.8 [1.3 to 2.4]) (Table 3). No relation between presence of yeast and other risk factors was found (Table 2).

The number of *C albicans* colonies and the percentage of hairy colonies isolated from fresh denture plaque are presented in Table 5. There was a statistically significant difference ($P = .03$) between Newton types I and II for the number of *C albicans* colonies, which was higher in subjects with type II stomatitis. The median frequency of hairy colonies was higher for Newton type I. There was no statistically significant relationship between denture stomatitis and frequency of myceliated colonies of *C albicans* ($P > .05$). Among the subjects who agreed to take part in the follow-up, only 2 were carrying *C albicans*. All follow-up subjects showed a reduction in the intensity of inflammation after wearing the new denture for 2 weeks based on a clinical examination; however, based on the Newton classification, the type and extent of the inflammation remained unchanged. Reevaluation of denture plaque on the new prosthesis in subjects with *Candida*-associated denture stomatitis demonstrated that *C albicans* disappeared after 1 month of wearing the new prosthesis but reappeared after 3 months to near the original levels.

Discussion

To the authors' knowledge, this study is the first to examine the relationship between denture stomatitis and the frequency of myceliated colonies of *C albicans* from fresh denture plaque. The results did not show a link between this characteristic of *C albicans* and denture stomatitis. The results are very similar to in vitro studies based on frozen samples of denture plaque, and thus the present study supports the feasibility of studying the phenotypic commutation of *C albicans* in fresh denture plaque using simple culture media like trypticase yeast extract without glucose or R2A, which was originally designed to cultivate waterborne bacteria. It is known that experimental conditions (medium, temperature, moisture, carbon dioxide) can influence the phenomenon of dimorphism of *C albicans*. Lo et al[28] noted that the capacity of *C albicans* to alternate from blastospores to hyphae would be an important factor of virulence.

The recovery of intraoral *C albicans* varies greatly with the sensitivity of the collection method used.[29] In the present study, a sonication technique was used. This technique is described in protocols used for assessing denture-associated candidosis, isolation of biofilm matrix material, and denture plaque.[19,30–33] It has been shown that the mechanical forces caused by cavitation in an ultrasonic bath are not sufficient to kill bacteria and yeast.[30,34] Several factors, such as exposure time, presoak immersion, and synergistic effect of cleaning solutions contribute to the impact of ultrasonic cleaning on microorganisms.[34–36] All of these factors were taken into account during the collection of denture plaque. Furthermore, there is a large body of evidence indicating that dentures act as a reservoir that harbors *Candida* biofilms.[37–40] These biofilms consist of a dense network of blastospores, hyhae, and pseudohyphae, and it is possible that the phenotypic switching may be one of the factors contributing to the cell differentiation in *Candida* biofilms.[41–43] Germination tubes or hyphae with associated surface adhesins may be responsible for enhanced adherence to acrylic material.[44] Hyphae may then penetrate the host epithelial cell and thus contribute to invasive growth of *C albicans*.[21,24,27] Although palatal swabs and smears were used in several studies for isolation of *Candida* and hyphae,[7] the literature, as well as the laboratory experience of the present authors, have shown that the smear method is less sensitive than other methods, depending on the sample's site. Furthermore *C albicans* colonies can be isolated less frequently from the palatal mucosa than from acrylic resin dentures.[19,29,38,45] In addition, a swab can remove surface epithelial layers[29] and does not generally permit quantification of the *Candida*.

The hypothesis that denture stomatitis may not be strictly linked to the presence of *C albicans* can be explained by considering the following facts: *(1) C albicans* does not invade the palatal mucous membrane even in severe stomatitis,[46] *(2)* not all subjects with denture stomatitis harbor *C albicans*,[12,16,19] and *(3)* many subjects without denture stomatitis are *C albicans* carriers.[47] While some virulence factors of *C albicans* could be involved in denture stomatitis, frequency of phenotypic switching as measured on solid media does

not appear to be a predictive factor by itself. This does not exclude the possibility that phenotypic switching may occur in situ. Phenotypic switching is probably the result of the local conditions under the prosthesis: low partial pressure of oxygen, low pH, composition of denture biofilm, and accumulation of desquamative epithelial cells.[43]

In this study, the prevalence of the various types of denture stomatitis was similar to the highest prevalence mentioned in literature.[8,12,16] This may reflect differences in the study population, such as the homogeneity and geographic and socioeconomic situations of the subjects; however, it primarily reflects the divergent diagnostic criteria of denture stomatitis. In this study, the diagnosis of Newton type I denture stomatitis was based on inflammatory reactions localized around the excretory ducts of salivary glands, which could be diagnosed as normal in other reports.[19] Most studies on denture stomatitis are based on diagnosis, but clinically there is a lack of calibration or standardization of the classification. The majority of reports used the Newton classification[8,48,49] or the classification based on the intensity and severity of inflammation.[10,50,51] In this study, a modified Newton classification was used with consideration for the extent of the inflammation as well as its type.[12] A revision of the classification for diagnosing denture stomatitis is necessary for 2 reasons: *(1)* none of the existing classifications are complete in terms of the above-mentioned criteria, and *(2)* the classification used can influence results and thus the clinical application of treatment choices.

This study shows that the subjects in subcategory B had more *C albicans*, which confirms the results of Barbeau et al,[12] who hypothesized that the extent of inflammation influences the colonization of *Candida*. Since inflammation can modify the expression of adhesion molecules and surface structures on a variety of cells and tissues, it can reduce or promote adhesion of pathogens. Therefore, it can be assumed that in Newton type IA, the mechanical trauma or mechanical occlusion of the excretory ducts of the minor palatal salivary gland could be an inducing factor for denture stomatitis. However, the low level of inflammation may not promote high *Candida* colonization.

A factor considered important in the etiology of denture stomatitis is wearing dentures during the night.[48,52–54] Several studies showed the importance of maintaining good oral and prosthetic hygiene in the treatment of denture stomatitis.[48,51] Although no statistically significant association between hygiene habits and the presence of denture stomatitis was shown in this study, a statistically significant association between the negligence of brushing the palate and the presence of *C albicans* was found. However, identifying the details of how brushing of palate (frequency, method) or nocturnal wearing of prostheses can influence mucosal health was beyond the limits of this study. Contrary to certain studies[55–57] and similar to another,[12] no statistically significant relation was found between denture stomatitis and certain putative risk factors such as presence of *C albicans*, smoking, and sugar consumption.

It can be assumed that the disappearance of *C albicans* after patients received the new prosthesis can be related to temporary changes in the oral flora. The reappearance of yeasts suggests that it was still in the oral environment and simply could not be detected or that *C albicans* was in another body location (eg, gastrointestinal tract). Oral carriage may not be dependant on the presence of the prosthesis, but it can be proposed that the denture represents the principal reservoir of the yeast. Longitudinal studies with a larger number of patients may provide important data on factors that promote the association of *C albicans* with inflammation observed in denture stomatitis.

It is important to mention that the small sample size in this study increases the likelihood of type 2 errors and that punctual data can hide long-term reality. However, the authors believe that it is clinically sound to encourage preventive treatment and microbiologic studies before any treatment is carried out.

Conclusion

Within the limitations of this study, nocturnal wear of the prosthesis remains the essential etiologic factor for the presence of *Candida*-associated denture stomatitis. The frequency of myceliated colonies of *C albicans* may not be directly involved in the development of denture stomatitis.

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