Can New Dentures Decrease Candida Levels?

Tatiana Pereira-Cenci, PhD^a/Frederico S.F. Fernandes, PhD^b/Jovito A. Skupien, DDS, MSc^c/ Mauro E. Mesko, DDS^c/Fabiana G. Straioto, PhD^b/Atair A. Del Bel Cury, PhD^d

> Purpose: The aim of this study was to evaluate the influence of time-course changes and various types of removable dentures on the oral levels of Candida species. Materials and Methods: In this prospective clinical trial, 72 subjects were divided into three groups according to the type of denture replaced: Kennedy Class I or II removable partial dentures (RPDs), Kennedy Class III or IV RPDs, and complete dentures. Whole saliva and biofilm samples from the palate, tongue, dentures, and inner surface of the cheek were obtained and evaluated for Candida diversity before the delivery of the new prostheses (baseline) and at 1, 6, and 12 months postdelivery. The results were analyzed using a three-way analysis of variance, followed by a post-hoc Student-Newman-Keuls test. **Results:** Candida levels decreased after the insertion of the new dentures; however, after 6 months, Candida levels were similar to baseline, and complete denture wearers presented higher *Candida* counts than RPD wearers. *Conclusion:* The type of denture does not seem to be a decisive factor in Candida levels. After 6 months, Candida colonization was well established in all types of removable prostheses. Denture replacement alone did not guarantee a decrease in Candida levels for more than 6 months. Int J Prosthodont 2013;26:470-477. doi: 10.11607/ijp.3047

Oral candidiasis is the most common fungal oral infection diagnosed in humans,¹ with *Candida* species as the primary etiologic agent.² High levels of *Candida* are often associated with denture stomatitis, a mucosal infection in tissue in contact with the prosthesis.³ Although this microorganism is a normal commensal in the mouth,⁴ several predisposing factors, such as immunosuppression resulting from the treatment of various diseases, drugs including broadspectrum antibiotics, and wearing prosthetic appliances, can lead to an overgrowth of *Candida* species⁵ and, therefore, the development of oral candidiasis. In particular, among elderly subjects, these predisposing factors are associated with systemic conditions such as malignancies, broad-spectrum antibiotics,

xerostomia, dietary factors, diabetes mellitus, and iron and vitamin deficiencies,^{6,7} which often lead to severe candidal infections.

The ability of *Candida* species to adhere to and form biofilm on the surfaces of dentures protects these organisms from detachment by shear forces, such as the flushing action of saliva, and from the antifungal proteins in saliva.^{8,9} Additionally, the space between the denture and the mucous membrane has a relatively low pH value,¹⁰ which can provide a suitable microenvironment for *Candida* proliferation.¹¹ The prosthetic environment created by inadequate oral and denture hygiene and continuously wearing dentures is hospitable for an overgrowth of *Candida* species.¹²

Although *C albicans* is considered to be the main pathogen responsible for the development of denture stomatitis,^{2,13,14} other non-*albicans* species have been isolated on acrylic resin denture surfaces and oral mucosa.^{8,9,15} The discovery of other *Candida* species is significant because they are frequently resistant to commonly used antifungal agents.^{8,16} Therefore, candidiasis associated with high levels of these non-*albicans* species is extremely difficult to treat¹⁷ and associated with bloodstream infections with a high mortality rate.^{18,19}

Although cross-sectional studies have attempted to establish a relationship between the use of removable dentures and the prevalence of *Candida* species in the oral environment,^{6,15,20} the literature contains few

^aResearcher, Graduate Program in Dentistry, Federal University of Pelotas, Pelotas, Rio Grande do Sul, Brazil.

^bStudent, Piracicaba Dental School, Unicamp, Piracicaba, Sao Paulo, Brazil.

^cStudent, Graduate Program in Dentistry, Federal University of Pelotas, Pelotas, Rio Grande do Sul, Brazil.

^dProfessor, Piracicaba Dental School, Unicamp, Piracicaba, Sao Paulo, Brazil.

Correspondence to: Dr Altair A. Del Bel Cury, PO Box 52, 13414-903, Piracicaba, SP, Brazil. Fax +55 19 2106 5302. Email: altcury@fop.unicamp.br

^{©2013} by Quintessence Publishing Co Inc.

^{© 2013} BY QUINTESSENCE PUBLISHING CO, INC. PRINTING OF THIS DOCUMENT IS RESTRICTED TO PERSONAL USE ONLY. NO PART MAY BE REPRODUCED OR TRANSMITTED IN ANY FORM WITHOUT WRITTEN PERMISSION FROM THE PUBLISHER.

longitudinal studies supporting this relationship. The aim of this prospective study was to evaluate timecourse changes in the oral levels of *Candida* species in patients who underwent prosthetic treatment with complete or removable partial dentures (RPDs).

Materials and Methods

Experimental Design

This prospective study involved a single-blind design (biofilm analyses) in which patients had their removable dentures replaced (complete dentures) or inserted for the first time (RPDs). *Candida* levels were evaluated at four timepoints—baseline and 1, 6, and 12 months postdelivery—at various sites (inner surface of the cheek, hard palate, tongue, dentures, and saliva). Ethical approval was obtained from the Research and Ethics Committee of the Federal University of Pelotas (protocol 040/2006). Patients who met the inclusion criteria were selected from dental clinics run by the Piracicaba Dental School in Brazil and were free to withdraw without justification at any stage of the trial.

Seventy-two patients (mean age, 57.8 y) were divided into three groups according to the type of denture worn: Kennedy Class I or II RPDs (n = 32), Kennedy Class III or IV RPDs (n = 17), and complete dentures (n = 23). Two operators collected all biofilm samples. Each patient received a new pair of removable dentures. Samples were evaluated using specific medium and biochemical tests at baseline and 1, 6, and 12 months after the delivery of the new dentures.

Assessment Procedures

Inclusion and exclusion criteria are listed in Table 1. Stimulated saliva (Parafilm M, American National Can) were collected from each subject for 5 minutes in the morning and 2 hours after the last meal to assess normal stimulated salivary flow rate (> 1 mL/min). The classification for good health included individuals without any systemic disease such as diabetes mellitus or a heart or lung condition. Intraoral devices such as obturator prostheses (for cleft palates) and occlusal appliances (for temporomandibular disorder [TMD] patients) were exclusion criteria. All individuals presenting with TMD (n = 2) wore occlusal appliances, which could provide an additional area for biofilm development and, therefore, insert a bias into the study.

All complete denture wearers had their dentures for more than 5 years and received replacement dentures. Patients who had never worn RPDs before received prostheses for the first time. All patients had

Table 1 Criteria for Patient Selection
--

Inclusion criteria	Exclusion criteria
Adults of both sexes	Use of antifungal agents,
Complete denture wearers for 5 y	antiseptic mouthwashes, or any medication or medical condition known to create a predisposition
Need for removable partial denture	to oral candidosis (eg, diabetes mellitus or iron and vitamin deficiencies)
Normal salivary flow rate	Temporomandibular joint
Good health	disorder (use of an occlusal appliance)
Available for follow-up	

similar Gingival Index (GI) scores and socioeconomic status (three times the Brazilian minimum wage, approximately US\$1,050). GI scores were the same at baseline because the patients were being treated at a dental school with a strict protocol giving dentures only to patients who either had good oral hygiene or had undergone any necessary periodontal and restorative treatment. The population studied also had similar socioeconomic status because the dental school program through which they are treated is free and gives priority to low-income people. Therefore, study participants had very similar oral status at the beginning of the study.

At delivery of the removable dentures, baseline saliva and biofilm samples were collected. Each subject received instructions on oral hygiene, which addressed the health maintenance of the remaining teeth (if any) and cleaning of the dentures. Oral hygiene was performed twice a day using dentifrice and a soft toothbrush, and none of the subjects used denture adhesives for retention. In addition, the patients were instructed to wear their prostheses during the day and night (removable partial or complete dentures). At each evaluation, the oral hygiene instructions were repeated, and the patients were asked if they still wore the prostheses at night. No other attempt to control the nocturnal wear of dentures was done.

Two calibrated investigators working independently collected samples at baseline and 1-, 6-, and 12-month evaluations. Each subject was asked to refrain from eating and performing any oral hygiene for 2 hours before the sample collection procedure. Stimulated saliva and biofilm samples (palate, cheek, and tongue) were collected immediately before delivery of the dentures. Volunteers' whole saliva was collected during masticatory stimulation with Parafilm M in an ice-chilled polypropylene tube and then serially diluted in phosphate buffer saline (PBS). For complete denture wearers, biofilm from their older dentures was also collected.

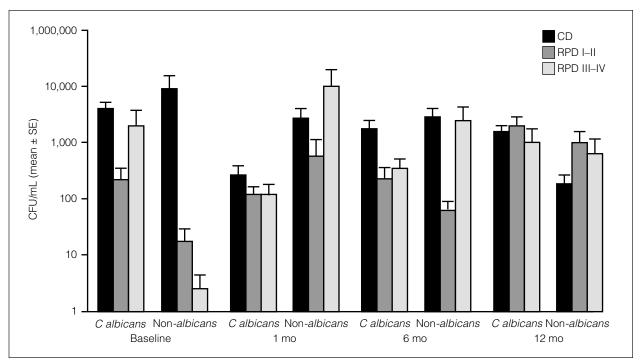


Fig 1 Analysis of type of denture, time of data collection, and *Candida* species for saliva (CD = complete denture; RPD I-II = Kennedy Class I or II removable partial denture; RPD II-IV = Kennedy Class III or IV removable partial denture).

Microbiologic Analysis

Biofilm samples from the tongue, inner surface of the cheek, hard palate, and internal denture surfaces were collected by swabbing the areas for 1 minute. Swabs were inserted in a test tube and sonicated at 30 w with three pulses of 10 seconds in 5 mL PBS. The test tubes containing the saliva samples were individually homogenized in a vortex mixer for 1 minute. All suspensions were serially diluted in PBS, and 20-µl samples were plated in duplicate on CHROMagar Candida. Plates were incubated at 37°C under aerobic conditions for 24 to 96 hours. Colony-forming units (CFUs) were counted using a stereomicroscope, and the results expressed in CFU/mL. Candida species were identified according to the colony color and biochemical tests and classified as Candida albicans or Candida non-albicans.

Statistical Analysis

Statistical analysis was done using SigmaStat (SigmaStat, version 3.5, Systat Software) employing a significance level fixed at 5%. The null hypothesis assumed no differences among the type of prosthesis, *Candida* species, and timepoint. The data were transformed by rank to fit the assumptions of the equality

of variances and normal distribution of errors. The results were analyzed using a three-way analysis of variance followed by a post hoc Student-Newman-Keuls test.

Results

There were no statistically significant differences between the Kennedy Classes I–II and Classes III–IV groups; thus, Tables 2, 3, and 4 show the Kennedy Classes I–II and III–IV groups together. Saliva assessment found no statistically significant differences regardless of the timepoint, type of prosthesis, or *Candida* species (P = .674; P = .112; P = .245, respectively) (Fig 1). On the inner surface of the cheek, complete dentures had the highest counts regardless of timepoint (P = .005), and *C albicans* had higher counts than non-*albicans* species regardless of the timepoint and type of prosthesis (P < .001) (Tables 2 and 3).

Similarly, on complete dentures, higher counts of *Candida* species were found (P < .001), and *C albicans* was more common than non-*albicans* (P = .005). However, there was a statistically significant difference among timepoints (P = .04), with the baseline and 1-month values presenting higher counts than the 12-month value (Tables 2 and 3).

© 2013 BY QUINTESSENCE PUBLISHING CO, INC. PRINTING OF THIS DOCUMENT IS RESTRICTED TO PERSONAL USE ONLY. NO PART MAY BE REPRODUCED OR TRANSMITTED IN ANY FORM WITHOUT WRITTEN PERMISSION FROM THE PUBLISHER.

-	<u> </u>						
Type of	Chee	ek	Ton	igue	Hard palate		
prosthesis	C albicans ^A	Non-albicans ^B	C albicans ^A	Non-albicans ^B	C albicans ^A	Non-albicans ^B	
Baseline							
CD	13.7 (6.5; 1.0–26.4)*	0.2 (0.1; 0.0-0.4) *	180.5 (79.8; 24.1–336.9)*	136.8 (72.0; -4.4-278.0)*	6.3 (2.4; 1.6–11.0)*	95.0 (61.2; -25.0-215.0)*	
RPD I-II	1.2 (0.5; 0.2–2.2)	0.7 (0.4; -0.1-1.5)	4.3 (2.1; 0.2–8.4)	0.5 (0.22; 0.0–1.0)	1.2 (0.9; -0.5-2.9)	0.0 (0; 0–0)	
RPD III-IV	0.8 (0.5; -0.2-1.8)	0.5 (0.3; -0.2-1.2)	8.1 (4.0; 0.3–15.9)	0.4 (0.29; -0.2–1.0)	4.8 (4.1; -3.3-12.9)	0.0 (0; 0–0)	
1 mo							
CD	0.5 (0.2; 0.2–0.8)*	0.6 (0.3; 0.1–1.1)*	5.4 (2.1; 1.3–9.5)*	58.6 (46.4; -32.3-149.5)*	4.6 (2.6; -0.6-9.8)*	101.8 (64; -23.7-227.3)*	
RPD I-II	1.6 (0.7; 0.2–3.0)	1.7 (1.3; -0.9-4.3)	2.2 (0.8; 0.6–3.8)	7.6 (6.55; -5.3-20.5)	0.4 (0.2; 0.1–0.7)	2.0 (1.8; -1.7-5.7)	
RPD III-IV	0.4 (0.2; 0.0–0.8)	3.9 (3.2; -2.4-10.2)	2.9 (0.9; 1.0-4.8)	63.9 (47.5; -29.3-157.1)	0.4 (0.2; 0.0–0.8)	32.5 (26.4; –19.3–84.3)	
6 mo							
CD	69.3 (53.7; -35.9-174.5)*	1.1 (0.6; -0.2-2.4)*	12.1 (3.3; 5.6–18.6)*	138.6 (72.9; -4.4-281.6)*	3.2 (1.2; 0.7–5.7)*	201.9 (86.1; 33.1–370.7)*	
RPD I-II	35.6 (32.8; -28.7-99.9)	0.2 (0.1; 0.0–0.4)	4.9 (1.8; 1.4–8.4)	1.3 (0.6; 0.1–2.5)	1.9 (1.1; -0.2-4.0)	0.5 (0.3; -0.2-1.2)	
RPD III-IV	2.2 (1.5; -0.7-5.1)	1.0 (0.9; –0.7–2.7)	61.6 (56.8; -49.8-173.0)	56.1 (57.14; 55.9–168.1)	56.8 (57.1; -55.1-168.7)	55.9 (57.1; -56.1-167.9)	
12 mo							
CD	19.6 (4.9; 10.0–29.2)*	3.0 (1.3; 0.5–5.5)*	60.0 (17.3; 26.0–94.0)	31.6 (9.7; 12.5–50.7)	6.6 (1.6; 3.5–9.7)*	2.0 (0.9; 0.2–3.8)*	
RPD I-II	17.5 (9.2; -0.6-35.6)	1.0 (0.5; -0.1-2.1)	50.1 (37.5; -23.5-123.7)	11.7 (7.0; -2.1-25.5)	98.0 (51.9; -3.7-199.7)	8.5 (4.6; -0.5-17.5)	
RPD III-IV	10.6 (5.5; -0.3-21.5)	0.6 (0.4; -0.1-1.3)	11.4 (5.5; 0.6–22.2)	6.0 (2.3; 1.5–10.5)	2.4 (1.4; -0.5-5.3)	72.6 (64.7; -54.3-199.5)	

 Table 2
 Microbiologic Analysis for Candida Species in the Biofilm According to the Experimental Conditions

*Indicates differences among types of dentures.

Values are mean (SE; confidence interval) CFU/mL (\times 10²). Within the same site, upper case letters indicate differences between *Candida* species (P < .05). CD = complete denture; RPD I-II = Kennedy Class I or II removable partial denture; RPD II-IV = Kennedy Class III or IV removable partial denture.

Table 3	Percentage of Increased, Decreased	, or Maintained Counts Accordin	g to the Experimental Conditions*

	Cheek		Tongue		Hard palate		Maxillary prosthesis		Mandibular prosthesis						
	Increase I	Decrease	Equal	Increase I	Decrease	Equal	Increase [Decrease	Equal	Increase I	Decrease	Equal	Increase	Decrease	Equal
CD															
1 mo	20/25	45/5	35/70	15/15	50/30	35/55	10/15	40/20	50/65	10/40	40/10	50/50	10/30	65/15	25/55
6 mo	33/20	20/13	47/67	53/49	7/20	40/40	27/13	27/13	46/74	13/13	13/33	74/54	53/7	0/27	47/66
12 mo	80/40	0/0	20/60	80/40	0/40	20/20	60/20	20/20	20/60	40/40	20/20	40/40	40/40	0/0	60/60
RPD I-	41														
1 mo	21/14	24/7	55/79	21/28	31/21	48/51	14/7	14/3	72/90	56/17	0/0	44/83	16/20	0/0	84/80
6 mo	17/3	12/21	71/76	25/21	29/25	46/54	17/12	12/8	71/80	60/20	15/10	25/70	35/22	4/4	61/74
12 mo	33/19	10/5	57/76	48/38	10/5	42/57	29/14	14/10	57/76	25/31	19/19	56/50	35/40	10/15	55/45
RPD II	I-IV														
1 mo	12/12	24/12	64/76	24/18	35/12	41/70	0/12	29/0	71/88	47/27	0/0	53/73	13/19	0/0	87/81
6 mo	17/11	0/6	83/83	39/17	17/6	44/77	28/17	6/6	66/77	56/38	6/6	38/56	33/33	7/13	60/54
12 mo	29/14	21/14	50/72	36/50	21/14	43/36	14/7	29/7	57/86	33/25	17/33	50/42	31/46	8/8	61/46

*Values are albicans/non-albicans species.

CD = complete denture; RPD I-II = Kennedy Class I or II removable partial denture; RPD II-IV = Kennedy Class III or IV removable partial denture.

The hard palate showed statistically significant differences among timepoint, type of prosthesis, and *Candida* species. The hard palate had higher levels of *Candida* species at the delivery of the new dentures, which decreased 1 month after delivery but increased after 6 and 12 months. Complete dentures (P < .001), non-*albicans* species (P = .002), and the 6- to 12-month follow-up evaluations (P = .006) presented the highest values (Tables 2 and 3).

Type of	Mandibular p	prosthesis	Maxillary prosthesis			
prosthesis	C albicans	Non-albicans	C albicans	Non-albicans		
Baseline						
CD	398.1 (102.9; 196.3–599.9) ^{Aa}	89.3 (59.9; -28.2-206.8) ^{Aa}	576.9 (103.1; 374.8-779.0) ^{Aa}	90.4 (59.9; -27.1-207.9) ^{Aa}		
RPD I-II	0 (0; 0–0) ^{ACb}	0 (0; 0–0) ^{ACb}	0 (0; 0–0) ^{Aa}	0 (0; 0-0) ^{Aa}		
RPD III-IV	0 (0; 0–0) ^{Aab}	0 (0; 0–0) ^{Aab}	0 (0; 0–0) ^{Aa}	0 (0; 0–0) ^{Aa}		
1 mo						
CD	51.1 (46.6; -40.2-142.4) ^{Aa}	54.4 (46.5; -36.7-145.5) ^{Aa}	351.9 (101.7; 152.5–551.3) ^{Aa}	304.3 (118.7; 71.7-536.9) ^{Aa}		
RPD I-II	0.6 (0.2; 0.1–1.1) ^{Bb}	18.8 (11.3; -3.3-40.9) ^{Bb}	98.6 (46.3; 7.8–189.4) ^{Ba}	1.1 (0.5; 0.0-2.2) ^{Ba}		
RPD III-IV	24.1 (22.8; -20.6-68.8) ^{Bc}	17.6 (15.3; -12.4-47.6) ^{Bc}	26.5 (13.6; -0.3-53.3) ^{BCa}	5.2 (4.5; -3.7-14.1) ^{BCa}		
6 mo						
CD	467.7 (107.5; 257.1–678.3) ^{ABa}	2.5 (1.9; -1.2-6.2) ^{ABa}	467.9 (107.4; 257.3–678.5) ^{Aa}	203.5 (86.0; 35.0–372.0) ^{Aa}		
RPD I-II	177.1 (68.3; 43.3–310.9) ^{Cb}	18.9 (23.9; -28.0-65.8) ^{Cb}	294.7 (80.9; 136.2-453.2) ^{Ab}	49.2 (39.4; -28.0-126.4) ^{Ab}		
RPD III-IV	137.7 (85.0; -28.8-304.2) ^{Bc}	1 (0.5; 0.1–1.9) ^{Bc}	316.2 (115.5; 89.8-542.6) ^{BCc}	128.9 (85.1; -38.0-295.8) ^{BCc}		
12 mo						
CD	492.4 (103.4; 289.6–695.2) ^{Ba}	201 (93.1; 18.4-383.6) ^{Ca}	327.2 (88.5; 153.6-500.8) ^{Ba}	7.2 (2.2; 2.8–11.6) ^{Ba}		
RPD I-II	258 (77.8; 105.5-410.5) ^{Ba}	112 (54.1; 6.0–218.0) ^{Ca}	564.1 (90.2; 387.2-741.0) ^{Ab}	71.7 (44; -14.5-157.9) ^{Ab}		
RPD III-IV	310.5 (116.1; 83.0–538.0) ^{Ca}	3.9 (1.9; 0.2–7.6) ^{Ca}	588.3 (123.4; 346.3-830.3) ^{ACab}	6.8 (3.5; -0.1-13.7) ^{ACab}		

Table 4	Microbiologic	Analysis for	Candida Species	in the Biofilm
---------	---------------	--------------	-----------------	----------------

Values are mean (SE; confidence interval) CFU/mL (x 10²). Capitalized letters represent differences among timepoints within the same type of prosthesis; lowercase letters represent statistically significant differences among the types of prosthesis at the same timepoint. CD = complete denture; RPD I-II = Kennedy Class I or II removable partial denture; RPD II-IV = Kennedy Class III or IV removable partial denture.

For the mandibular prostheses, only complete denture wearers showed statistically significant differences between Candida species (P < .001). In addition, complete denture wearers had higher levels of *C albicans* than the RPD groups (P < .001). That trend did not occur with non-albicans species (P > .05, Tables 3 and 4). For the maxillary prostheses, statistical analysis revealed the interaction among the three variables tested (P = .024) (Tables 3 and 4). At the C albicans level (P = .003), there was a significant difference associated with the interaction of the timepoint and type of denture; complete dentures and Kennedy Class I and II RPDs had higher counts than Kennedy Class III and IV. Complete denture wearers had a statistically significant difference among timepoints for C albicans (P < .001), with baseline values differing among timepoints. A similar trend occurred in the Kennedy Classes III-IV RPD group; C albicans levels were highest at 6 months and decreased after 12 months (P < .001). For all denture types, baseline values of non-albicans species were higher at 1 month (P = .003), then decreased after 12 months (P = .011).

Discussion

This study showed that the location of sample collection plays a key role in the analysis of biofilm because the six sites assessed had different outcomes for the variables evaluated. However, the results of this study cannot be extrapolated for all types of prostheses in all sites presented. Another limitation of this study is that with a larger sample size (more than 1,000 subjects), a multivariate analysis considering data dependence could have been performed, yielding more complete results. This project could be the subject of future research. For a better understanding of Candida colonization in oral environments, it is important to assess all variables potentially involved in the process. This study also contributes to the growing evidence that more than one Candida species may simultaneously colonize oral habitats.²¹ Generally, in this study, Candida species simultaneously colonized the biofilm collected from the various sites of the oral cavity.

Saliva has a regulating role in inhibiting the adherence of *Candida* species.⁶ Unsurprisingly, especially

474 | The International Journal of Prosthodontics

© 2013 BY QUINTESSENCE PUBLISHING CO, INC. PRINTING OF THIS DOCUMENT IS RESTRICTED TO PERSONAL USE ONLY. NO PART MAY BE REPRODUCED OR TRANSMITTED IN ANY FORM WITHOUT WRITTEN PERMISSION FROM THE PUBLISHER.

considering that the study participants were healthy, the present results showed no difference in saliva counts among the evaluated variables. Possibly, this occurred because anti-Candida salivary components and innate defense mechanisms, such as the flushing effect of saliva, affect Candida physiology and decrease Candida adherence to oral surfaces.²² This result is in accordance with other studies showing that the Candida count decreased when individuals with hyposalivation and high Candida levels received salivary stimulation,²³ indicating that low salivary flow rates are associated with higher oral Candida counts,24 not because of the amount of Candida but because of the cleaning effect. Although complete denture wearers had the highest amount of C albicans species in the inner surface of the cheek, this site also had the lowest Candida counts. This seeming discrepancy could be explained by the fact that the inner surface of the cheek is constantly in a state of attrition, modifying the biofilm and making Candida colonization more difficult.

While systemic host factors such as diabetes mellitus, HIV infections, iron deficiencies, hypoendocrine states, blood disorders, drug therapies, or xerostomia could create a predisposition to denture stomatitis, the participants in this study were comparable because the host factors that potentially could create differences among them were controlled. In addition, giving all participants instructions in the same oral hygiene procedures assessed whether the new denture alone could bring any benefit to patients positive for Candida. Special emphasis was placed on oral hygiene because microbial accumulation on the dentures is known as a potential problem. The surface roughness and concentration of exotoxins and metabolic products caused by fungal growth on the prosthesis destroy the surface quality and can irritate oral tissues. In clinical terms, the surface of an old denture (highly roughened) could facilitate colonization by microorganisms and acid production, mainly by yeasts, which have been identified as major etiologic factors in denture stomatitis. The participants' similarities could be considered a limitation of this study, however, as the findings are applicable only to healthy individuals.

Previous studies showed that a maxillary denture, or hard palate, encloses specific microorganisms in its base, creating a local microenvironment suitable for yeast adhesion and growth, depending more on local factors than individual variations.^{25,26} In addition, the denture's surface roughness directly influences microorganisms' initial adherence to surfaces, biofilm development, and *Candida* species colonization.^{27,28} The high levels of *Candida* at baseline in complete denture wearers could be attributable to the roughness of the

tongue. All patients had worn their older dentures for a long time, so tongue roughness still reflects the old denture's bacterial species. This study demonstrated that after 6 months, complete denture wearers had higher Candida counts than RPD wearers, demonstrating the importance of oral hygiene for denture wearers. All subjects enrolled in the study were asked not to remove the prosthesis at night. Although in many countries denture wearers customarily remove their dentures at night, the individuals in this study did not for several reasons: many patients kept their denture use a secret from their families and, most importantly, the practice had already been part of their routine for many years. Thus, all patients would show compliance with the study simply by maintaining their day-by-day routine, including sleeping with a prosthesis. In addition, it has been shown that instructions and motivations about denture hygiene are the most important issues with which patients are concerned.²⁹ At the study's follow-up evaluations, all patients reported that they used their dentures both day and night, only removing them for cleaning. The question prompted most of the study participants to ask if they should have been removing the dentures, which is a good indication that they were wearing the dentures at night. The findings on nocturnal wear, though, are based solely on the participants' answers.

Asymptomatic oral carriage of Candida has been recognized for many years. As the elderly population, and therefore their need for dental treatment, is rapidly growing, ³⁰ studies on their oral hygiene³¹ are becoming increasingly important. In a recent cross-sectional study, Zaremba et al¹⁵ showed that C albicans is found more often in denture-wearing than nondenture-wearing edentulous individuals. This research corroborates the findings of this study that the dentures of all patients were colonized, especially complete denture wearers who had higher Candida levels than RPD wearers. The type of material used in these prostheses could explain these findings. While complete dentures are fabricated with heat-cured acrylic resin, RPDs also contain metal, which sometimes decreases fungal growth.26 In addition, it is important to point out that complete dentures have a larger area of heat-cured acrylic resin than RPDs, which allows a larger area to be colonized by microorganisms and, consequently, could lead to higher Candida levels.

It is important to emphasize that 1 month after the prosthesis delivery, in general, the level of yeast had decreased, followed by an increase to the initial levels (or even higher) after 6 months. The literature showed an association between oral candidiasis and the duration of denture wear,²¹ and this study demonstrated

Volume 26. Number 5. 2013

475

that the regular replacement of a prosthesis does not guarantee a lack of Candida colonization even for a short period of time. It is important to highlight the limitation imposed on this study by evaluating two distinct populations: complete denture wearers who already used dentures and RPD wearers receiving dentures for the first time. Therefore, it was impossible to know how the latter would respond to the new RPDs since the complete denture wearers had baseline Candida values for the old dentures. Second, although Kennedy Class I dentures might be expected to have a higher volume of acrylic resin than Class II dentures, no statistically significant difference was found between the two groups, which is why they were grouped together in the tables. This finding could have occurred because the volume of acrylic resin is more important when dealing with complete dentures, which cover the palate, preventing the cleansing action of the tongue and saliva. The mere presence of Candida in the oral environment does not mean that an individual necessarily has or will develop Candida-related pathologies, which depend on complex fungi-bacteria-host interaction that modulates the host's response, possibly leading to inflammation. Nevertheless, if a slight inflammation is not controlled and plaque accumulation continues, this neglect could have a detrimental impact on the patient's health. Moreover, as a result of their large size, fungi contribute more mass to the biofilm than bacteria.³² According to the ecologic plaque hypothesis,³³ the proportions of pathogenic microorganisms, not the presence of any particular species, dictates the changes that transform health into disease. This theory highlights the need for effective, physical removal of denture plaque, which can be accomplished by regular chemical cleansing (eg, immersion in a sodium hypochlorite solution).

Conclusion

Within the limitations of this study design, it can be concluded that complete denture wearers have a higher count of *Candida* species than RPD wearers and, therefore, should be treated with more caution. Moreover, the type of RPD does not seem to be a decisive factor in *Candida* levels, and after 6 months, *Candida* colonization is well established in all types of prostheses.

Acknowledgment

The authors thank Renato Azevedo de Azevedo for his valuable contributions. The authors reported no conflicts of interest related to this study.

References

- Muzyka BC. Oral fungal infections. Dent Clin North Am 2005; 49:49–65.
- Figueiral MH, Azul A, Pinto E, Fonseca PA, Branco FM, Scully C. Denture-related stomatitis: Identification of aetiological and predisposing factors—A large cohort. J Oral Rehabil 2007; 34:448–455.
- Espinoza I, Rojas R, Aranda W, Gamonal J. Prevalence of oral mucosal lesions in elderly people in Santiago, Chile. J Oral Pathol Med 2003;32:571–575.
- Ten Cate JM, Klis FM, Pereira-Cenci T, Crielaard W, de Groot PW. Molecular and cellular mechanisms that lead to Candida biofilm formation. J Dent Res 2009;88:105–115.
- Akpan A, Morgan R. Oral candidiasis. Postgrad Med J 2002; 78:455–459.
- Pereira-Cenci T, Del Bel Cury AA, Crielaard W, Ten Cate JM. Development of Candida-associated denture stomatitis: New insights. J Appl Oral Sci 2008;16:86–94.
- Soysa NS, Samaranayake LP, Ellepola AN. Diabetes mellitus as a contributory factor in oral candidosis. Diabet Med 2006; 23:455–459.
- Li L, Redding S, Dongari-Batgtzoglou. Candida glabrata, an emerging oral opportunistic pathogen. J Dent Res 2007;86: 204–215.
- Coco BJ, Bagg J, Cross LJ, Jose A, Cross J, Ramage G. Mixed Candida albicans and Candida glabrata populations associated with the pathogenesis of denture stomatitis. Oral Microbiol Immunol 2008;23:377–383.
- Budtz-Jörgensen E. Etiology, pathogenesis, therapy, and prophylaxis of oral yeast infections. Acta Odontol Scand 1990;48:61–69.
- Sánchez-Vargas LO, Pérez-Rios P, Romo-García J, Corona-Izquierdo FP, Hidalgo-Loperena H, Franco-Martínez F. Salivary pH and culture determinations in HIV infected and non-HIV infected patients with oral candidosis. Rev Iberoam Micol 2002; 19:155–160.
- Kulak-Ozkan Y, Kazazoglu E, Arikan A. Oral hygiene habits, denture cleanliness, presence of yeasts and stomatitis in elderly people. J Oral Rehabil 2002;29:300–304.
- Cannon RD, Chaffin WL. Oral colonization by Candida albicans. Crit Rev Oral Biol Med 1999;10:359–383.
- Barbeau J, Séguin J, Goulet JP, et al. Reassessing the presence of Candida albicans in denture-related stomatitis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;95:51–59.
- Zaremba ML, Daniluk T, Rozkiewicz D, et al. Incidence rate of Candida species in the oral cavity of middle-aged and elderly subjects. Adv Med Sci 2006;51:233–236.
- Samaranayake LP, Keung Leung W, Jin L. Oral mucosal fungal infections. Periodontol 2000 2009;49:39–59.
- Hitchcock CA, Pye GW, Troke PF, Johnson EM, Warnock DW. Fluconazole resistance in Candida glabrata. Antimicrob Agents Chemother 1993;37:1962–1965.
- Redding SW. The role of yeasts other than Candida albicans in oropharyngeal candidiasis. Curr Opin Infect Dis 2001;14:673–677.
- Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: A persistent public health problem. Clin Microbiol Rev 2007;20:133–163.
- 20. Radford DR, Challacombe SJ, Walter JD. Denture plaque and adherence of Candida albicans to denture-base materials in vivo and in vitro. Crit Rev Oral Biol Med 1999;10:99–116.
- Zomorodian K, Haghighi NN, Rajaee N, et al. Assessment of Candida species colonization and denture-related stomatitis in complete denture wearers. Med Mycol 2011;49:208–211.

476 | The International Journal of Prosthodontics

© 2013 BY QUINTESSENCE PUBLISHING CO, INC. PRINTING OF THIS DOCUMENT IS RESTRICTED TO PERSONAL USE ONLY. NO PART MAY BE REPRODUCED OR TRANSMITTED IN ANY FORM WITHOUT WRITTEN PERMISSION FROM THE PUBLISHER.

- Ramage G, Vande Walle K, Wickes BL, López-Ribot JL. Biofilm formation by Candida dubliniensis. J Clin Microbiol 2001;39:3234–3240.
- Torres SR, Peixoto CB, Caldas DM, et al. A prospective randomized trial to reduce oral Candida spp. colonization in patients with hyposalivation. Braz Oral Res 2007;21:182–187.
- Torres SR, Peixoto CB, Caldas DM, et al. Relationship between salivary flow rates and Candida counts in subjects with xerostomia. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;93:149–154.
- Pereira-Cenci T, Deng DM, Kraneveld EA, et al. The effect of Streptococcus mutans and Candida glabrata on Candida albicans biofilms formed on different surfaces. Arch Oral Biol 2008;53:755–764.
- Perezous LF, Stevenson GC, Flaitz CM, Goldschmidt ME, Engelmeier RL, Nichols CM. The effect of complete dentures with a metal palate on Candida species growth in HIV-infected patients. J Prosthodont 2006;15:306–315.
- Nevzatoglu EU, Ozcan M, Kulak-Ozkan Y, Kadir T. Adherence of Candida albicans to denture base acrylics and siliconebased resilient liner materials with different surface finishes. Clin Oral Investig 2007;11:231–236.

- Pereira-Cenci T, da Silva WJ, Cenci MS, Cury AA. Temporal changes of denture plaque microbiologic composition evaluated in situ. Int J Prosthodont 2010;23:239–242.
- de Souza RF, de Freitas Oliveira Paranhos H, Lovato da Silva CH, Abu-Naba'a L, Fedorowicz Z, Gurgan CA. Interventions for cleaning dentures in adults. Cochrane Database Syst Rev 2009;7:CD007395.
- Oeppen J, Vaupel JW. Demography. Broken limits to life expectancy. Science 2002;296:1029–1031.
- Lodter JP, Marty N, Andrieu S, et al. Improved oral hygiene and Candida species colonization level in geriatric patients. Oral Dis 2005;11:163–169.
- Coulthwaite L, Verran J. Potential pathogenic aspects of denture plaque. Br J Biomed Sci 2007;64:180–189.
- Marsh PD. Microbial ecology of dental plaque and its significance in health and disease. Adv Dent Res 1994;8:263–271.

Literature Abstract

Long-term clinical outcome analysis of poly-methyl-methacrylate cranioplasty for large skull defects

This retrospective study aimed to analyze the effect of poly-methyl-methacrylate (PMMA) cranioplasty used for skull defect reconstruction, usually after trauma (64%). Seventy consecutive patients with 78 cranioplasties placed from 2007 to 2010 were selected. A thorough medical record including: the mechanism of injury, location of cranioplasty, type of original repair, complications postoperatively, and follow-up time were reviewed. The same maxillofacial prosthetic technician fabricated all 78 acrylic cranioplasties. Out of the 70 patients reviewed in the study, there were 6 failures. These patients had their original PMMA cranioplasty removed and reinserted. Out of these 6 patients, there were 2 reinsertions, resulting in a total of 78 cranioplasties. The most common complication was chronic pain (14%). Nine out of 70 patients experienced postoperative infection (13%), with the main isolated organism from infected cranioplasties being staphylococcus aureus (67%). The overall complication rate (24%) obtained was comparable to other acrylic cranioplasties studies, as well as studies with the use of autogenous bone. In conclusion, PMMA cranioplasty is safe, cost effective, esthetically acceptable, and with similar complication rates compared to autogenous bone.

Jaberi J, Gambrell K, Tiwana P, Madden C, Finn R. J Oral Maxillofac Surg 2013;71:e81–e88. References: 27. Reprints: Division of Oral and Maxillofacial Surgery, MS #9109, UT Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390-9109. Email: paul.tiwana@utsouthwestern.edu—Sheralyn Quek, Singapore

Copyright of International Journal of Prosthodontics is the property of Quintessence Publishing Company Inc. and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.