Temporal Changes of Denture Plaque Microbiologic Composition Evaluated In Situ

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This study assessed how biofilm composition is affected by both time and denture material in complete denture wearers. Biofilm was formed during two phases of 14 days on acrylic resin and denture liner specimens mounted on the buccal surface of the mandibular dentures of 21 patients. Specimens were removed randomly on days 2, 7, and 14. Higher counts of *Candida glabrata*, total streptococci, *Actinomyces*, total microorganisms, and percentage of *Actinomyces* were observed after 7 and 14 days ($P < .05$). *C glabrata* was the only species to show progressively rising counts from day 2 to 14, while no difference was found in biofilm composition among the materials tested. *Int J Prosthodont 2010;23:239–242.*

Poorly fitting dentures, continuous denture wearing, the use of denture liners, and poor oral hygiene facilitate plaque formation and *Candida* colonization on complete dentures.[1,2] This in situ study assessed how biofilm composition is affected by time and denture material, and if the materials' surface roughness is also affected by time.

Materials and Methods

This crossover, split-mouth, short-term in situ study was approved by the ethics committee of the Faculty of Dentistry of Piracicaba (protocol 040/2006). Forty-eight patients wearing complete dentures had their mouths and dentures swabbed for *Candida* species. Inclusion and exclusion criteria for enrollment in this study are listed in Table 1. Samples and saliva collected were plated in CHROMagar (Difco) *Candida* and incubated at 37°C for 24 to 48 hours. Forty-three patients were identified as *Candida* carriers and 21 agreed to participate (16 women, 5 men; mean age: 65.5 ± 13.6 years).

During two phases of 14 days, volunteers wore their dentures with six acrylic resin and six soft or hard denture liner specimens (depending on the experimental phase) inserted randomly in recesses created in their mandibular denture's flange, leaving 1 mm for biofilm accumulation, and protected with a plastic mesh. Since they were *Candida* carriers but did not suffer from candidosis, no food elimination or diet recommendations were planned. Those taking any antifungal agents, mouthwashes, or medications known to predispose patients to oral candidosis, or had any predisposing medical condition, were excluded. Volunteers were instructed to wear the dentures at all times and to brush their dentures three times per day after the main mealtimes with a soft toothbrush and toothpaste (provided by the researchers), except for the area containing the specimens, where only the slurry from the toothpaste was spread gently with the manual toothbrush.

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Table 1 Criteria for Patient Selection

Inclusion criteria	Exclusion criteria
• Adults of both sexes • Have worn complete denture for at least 6 months • Not presenting signs or symptoms of candidosis but carrying *Candida* species • Normal salivary flow rate • Good health • Ability to comply with the experimental protocol • Not using any type of intraoral device	• Use of antifungal agents or antiseptic mouthwashes • Use of any medication or medical condition (eg, diabetes mellitus or iron and vitamin deficiencies) known to predispose to oral candidosis • Use of antibiotics during the 2 months prior to the study and during the pre-experimental and experimental periods • Temporomandibular joint disorder

Table 2 Surface Roughness (μm) According to Denture Material and Time

	Baseline	2 days	7 days	14 days
Acrylic resin	0.21 ± 0.12^{a}	$0.41 \pm 0.33^{a*}$	$0.48 \pm 0.54^{a*}$	$0.40 \pm 0.38^{a*}$
Hard liner	0.68 ± 0.49^{b}	0.71 ± 0.57^{b}	0.83 ± 0.62^{b}	$1.7 \pm 3.7^{b*}$
Soft liner	1.37 ± 1.33^{b}	1.6 ± 1.8^{b}	2.0 ± 2.2^{b}	$2.8 \pm 2.7^{b*}$

Different letters show differences in surface roughness among materials at baseline and after removal at each time point (analysis of variance, $P < .05$).
*Differences between surface roughness at baseline and after the specimens were subjected to the clinical trial, fixing the evaluation time points (paired *t* test or Wilcoxon signed rank test, $P < .05$).

Specimens (4 × 4 × 2 mm) were prepared according to manufacturers' recommendations (Acron MC, Kooliner, and Coe Soft, GC America) and assessed for surface roughness.[1] Acrylic resin and hard liner specimens were ground using progressively smoother aluminum oxide papers (320-, 400-, and 600-grit) in a horizontal polisher. For mechanical polishing, a brush wheel with pumice slurry and a felt cone with chalk powder were used. For the soft denture liner, surface roughness was standardized by the contact with the glass slides. In both experimental phases, two specimens of each substratum type (acrylic resin or denture liner) were selected randomly (using a randomization table) to be removed on day 2, 7, or 14; on day 2, four specimens had their biofilm collected with a plastic spatula after removing the acrylic mesh with a scalpel. After this procedure, the specimens were removed from the denture and stored until surface roughness reassessment. The same procedure was done on days 7 and 14.

Specimens were not reinserted and the recess was cleaned and filled with wax. Biofilm samples were suspended in a phosphate buffer solution (1 mL/mg, % by weight), sonicated at 40 W and 5% amplitude with six pulses of 9.9 seconds each, serially diluted and inoculated on specific media, and incubated at 37 °C in 10% carbon dioxide (mitis salivarius bacitracin/mitis salivarius agar), anaerobiosis (blood agar/acriflavine tellurite) or aerobiosis (CHROMagar *Candida*) for 24 to 96 hours. A randomized block design was used for the statistical analyses, considering the subjects as statistical blocks and time points and materials as factors under study ($\alpha = .05$) (Tables 2 to 4).

Results

To the authors' knowledge, temporal changes on denture material characteristics and their association with biofilm formation and *Candida* colonization has never been reported. Three different time points were chosen for biofilm collection to ensure that initial and late colonizers would be identified, while a maximum of 14 days was related to the recommended time of use by the manufacturers.

All subjects presented at least two *Candida* species, with *C albicans* always present. Two withdrawals were recorded. Surface roughness increased mainly after 14 days ($P < .05$), except for the acrylic resin, which was smoother than the denture liners ($P < .001$) (Table 2). Higher counts in total streptococci, *Actinomyces,* and total microorganisms were observed after 7 and 14 days ($P < .05$, Table 3). The percentage of all *Candida* species in relation to total microorganisms rose from day 2 to 7, while *C glabrata* counts rose from day 2 to days 7 and 14 ($P < .05$, Table 4).

Table 3 Microbiologic Results for Bacteria in the Biofilm According to the Experimental Conditions (n = 19)

Time point/ material	Mutans streptococci (CFU/mg × 10^4)	Total streptococci (CFU/mg × 10^6)	*Actinomyces* (CFU/mg × 10^6)	Total microorganisms (CFU/mg × 10^7)	% mutans streptococci/ total streptococci	% mutans streptococci/ total microorganisms	% *Actinomyces*/ total microorganisms
Day 2							
AR1	0.57 ± 1.21	3.03 ± 4.07	0.77 ± 1.94	0.64 ± 0.55	0.45 ± 0.88	0.12 ± 0.19	7.88 ± 14.75*
HL	0.27 ± 0.57	2.17 ± 3.55	0.89 ± 1.80	1.12 ± 2.24	0.28 ± 0.55	0.18 ± 0.44	11.48 ± 19.61*
AR2	2.07 ± 6.64	9.08 ± 21.78	0.58 ± 1.63	1.04 ± 1.36	0.62 ± 1.90	0.21 ± 0.61	3.40 ± 8.13*
SL	3.37 ± 11.02	4.40 ± 6.19	1.53 ± 2.84	2.53 ± 3.20	1.06 ± 2.49	0.37 ± 0.97	7.32 ± 14.82*
Day 7							
AR1	0.33 ± 0.71	7.08 ± 8.47*	1.74 ± 6.22*	2.48 ± 3.39*	0.28 ± 0.86	0.03 ± 0.09	2.99 ± 7.42
HL	0.24 ± 0.45	12.58 ± 14.92*	3.06 ± 7.28*	2.95 ± 3.49*	0.26 ± 0.82	0.03 ± 0.07	7.14 ± 14.86
AR2	2.74 ± 7.46	7.47 ± 13.22*	0.66 ± 1.73*	1.4 ± 1.39*	0.54 ± 1.11	0.51 ± 1.58	2.98 ± 5.56
SL	4.02 ± 7.64	7.13 ± 5.74*	1.15 ± 2.46*	1.79 ± 1.62*	1.81 ± 5.36	0.37 ± 0.83	6.55 ± 12.35
Day 14							
AR1	2.90 ± 6.53	17.57 ± 18.11*	5.73 ± 9.50*	4.23 ± 4.36*	0.39 ± 0.98	0.17 ± 0.53	18.40 ± 26.80*
HL	8.35 ± 19.02	85.08 ± 268.67*	2.99 ± 5.42*	3.66 ± 3.83*	0.83 ± 2.32	0.33 ± 0.71	16.13 ± 30.45*
AR2	0.63 ± 1.69	9.59 ± 10.67*	3.68 ± 13.33*	1.73 ± 1.13*	0.62 ± 2.38	0.06 ± 0.18	28.65 ± 94.00*
SL	0.49 ± 0.92	7.45 ± 4.97*	1.96 ± 3.16*	2.01 ± 1.60*	0.25 ± 0.84	0.09 ± 0.29	13.69 ± 22.33*

AR = acrylic resin (AR1 and AR2 represent acrylic resin specimens used in the first and second phase, respectively); HL = hard denture liner; SL = soft denture liner.
*Differences among time points ($P < .05$).

Table 4 Microbiologic Analysis for *Candida* Species in the Biofilm (n = 19)*

Time point/ material	*C albicans* (CFU/mg × 10^3)	*C glabrata* (CFU/mg × 10^3)	*C tropicalis* (CFU/mg × 10^3)	*C krusei* (CFU/mg × 10^3)	Other *Candida* species (CFU/mg × 10^3)	% *C albicans*/ total microorganisms	% *Candida* species/ total microorganisms
Day 2							
AR1	1.06 ± 3.73	0.06 ± 0.26	2.69 ± 10.40	16.71 ± 48.51	4.07 ± 16.25	0.04 ± 0.12	0.65 ±1.99[a]
HL	0.82 ± 2.17	0.08 ± 0.29	3.24 ± 12.91	1.87 ± 7.06	5.32 ± 21.25	0.05 ± 0.12	0.30 ± 0.67[a]
AR2	0.19 ± 0.43	1.74 ± 7.06	1.11 ± 4.71	1.80 ± 4.01	0.001 ± 0.004	0.01 ± 0.01	0.07 ± 0.15[a]
SL	0.18 ± 0.33	0.08 ± 0.30	1.25 ± 5.00	0.82 ± 2.99	0.84 ± 3.33	0.01 ± 0.01	0.04 ± 0.10[a]
Day 7							
AR1	0.05 ± 0.18	3.14 ± 12.95	0.10 ± 0.40	49.99 ± 172.88	1.20 ± 5.11	0.003 ± 0.01	0.13 ± 0.32[b]
HL	0.76 ± 2.18	19.49 ± 78.42	0.03 ± 0.08	14.27 ± 56.38	0.20 ± 0.65	–	1.35 ± 4.42[b]
AR2	0.11 ± 0.23	12.22 ± 36.73	0.09 ± 0.39	14.76 ± 46.08	6.49 ± 27.50	0.002 ± 0.01	0.36 ± 0.87[b]
SL	1.09 ± 3.30	17.59 ± 74.64	0.09 ± 0.39	40.72 ± 124.90	–	0.01 ± 0.03	0.50 ± 0.99[b]
Day 14							
AR1	8.03 ± 30.07	98.73 ± 229.56†	–	4.77 ± 15.15	–	0.05 ± 0.19	0.79 ± 1.82[a,b]
HL	10.01 ± 34.39	22.36 ± 13.12†	–	17.16 ± 51.69	–	0.09 ± 0.32	0.46 ± 0.91[a,b]
AR2	0.38 ± 1.24	13.12 ± 53.74†	2.94 ± 12.13	56.48 ± 146.50	6.18 ± 25.47	0.007 ± 0.02	0.58 ± 0.74[a,b]
SL	0.58 ± 1.34	13.18 ± 55.78†	0.83 ± 3.54	41.74 ± 126.19	2.04 ± 8.64	0.002 ± 0.01	0.43 ± 0.87[a,b]

AR = acrylic resin; HL = hard denture liner; SL = soft denture liner; – = not detected.
Different letters represent statistical differences ($P < .05$) among time points regarding percentage of *Candida* species in relation to total microorganisms.
*Morphology and biochemical tests of sugar fermentation were used to confirm *Candida* species (the ones not differentiated were considered as other *Candida* species).
†Differences among time points of biofilm formation ($P < .05$).

Discussion

Surface roughness is a crucial factor in the entrapment of microorganisms, protecting the denture from shear forces in the initial *Candida* and other microorganism adherence.[1,3,4] However, the formation of salivary pellicle may be more important for biofilm formation, since saliva decreases surface roughness,[1,3] explaining the similar results for different microorganism counts in all materials tested in this study.

It is known that a denture provides many sites where protection from salivary flow and mechanical removal forces in the mouth allow plaque to accumulate. In these stagnant areas, denture plaque is likely to be more acidogenic and therefore favors *Candida* species and streptococci development, with these later supporting the growth of *C albicans*.[1,2]

The presence of *Candida* in the oral environment does not mean that the individual will develop candidosis. However, a shift in disease-associated *Candida*

species has been found from *C albicans* towards non-*albicans* species,[1] supporting the claim that lengthy biofilm accumulation could be a predisposing factor to candidosis. The current results suggest that *C glabrata* is more competitive in the biofilm community and is more complex since it exhibited higher denture surface adherence[5] and was the most prevalent species found after 14 days. Proportions of pathogenic microorganisms are dictated by environmental changes, turning health to disease. As the elderly population is rising, together with their need for dental treatment, studies considering biofilm shifts as a consequence of improper oral hygiene are of the utmost importance.

Conclusion

Since lengthy biofilm accumulation, a result of a lack of hygiene, could be a predisposing factor to candidosis development, these findings highlight the need for effective physical removal of denture plaque, especially considering emerging pathogens such as *Candida glabrata*.

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Literature Abstract

Formation of TiO_2 nano-network on titanium surface increases the human cell growth

Titanium readily forms a protective oxide layer when it comes into contact with air. When a titanium implant is placed in the body, cells come into contact with the titanium oxide layer and cell behavior is influenced by the properties of this surface. Numerous surface modification techniques have been developed to improve the interaction between the cell layer and the implant surface. Here, electrochemical techniques have been explored as an alternative surface modification approach. This paper uses an electrochemical anodization treatment to create a structured nanonetwork titanium dioxide surface and investigates the response of human mesenchymal stem cells on this surface both in vitro and in vivo. Commercially available pure titanium discs were polished. Anodic currents, I^1 and I^2 ($I^1 < I^2$), were applied to the discs in a 5 M NaOH solution at 25°C. The specimens were designated I^1 and I^2, respectively, and untreated polished discs were designated I^0 where no current was applied. Each of the 24 specimens per group was sterilized under ultraviolet light for 1 hour before cell culturing. Adult human bone marrow mesenchymal stem cells (hMSCs) were used for the cell culture. The hMSCs were transfected with a gene encoding green fluorescent protein (GFP) and this was used to evaluate the growth of the hMSCs. For in vitro cell growth, 5×10^4 labeled hMSCs were seeded on each specimen and the fluorescence intensity was followed for 9 days. In vivo cell growth was evaluated by seeding the GFP labeled hMSCs onto the titanium specimens (I^0, I^1, and I^2) and inserting them subcutaneously under the back skin of 8-week-old nude mice. I^0 specimens without hMCSs were used as a negative control. The results showed a higher GFP signal, indicating better cell growth, on the anodized specimens compared to the control after 6 days, with I^1 showing stronger GFP fluorescence than I^2. There was also significantly faster cell growth on the test specimens compared to the control, with I^1 growing faster than I^2.

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