Short Communication

In Vitro *Candida* Colonization on Acrylic Resins and Denture Liners: Influence of Surface Free Energy, Roughness, Saliva, and Adhering Bacteria

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This study aimed to determine the influence of surface roughness (Ra), surface free energy (SFE), saliva, and bacteria on *Candida* adhesion to denture materials. The Ra and SFE of 2 acrylic resin specimens and 2 denture liner specimens were measured and assayed in a flow chamber for bacteria culture perfusion plus *Candida albicans* or *C glabrata* cultures. Adhesion was determined by counting under light microscopy. *Candida* adhesion showed significant differences depending on the factors involved. The overall colonization was significantly decreased by saliva and influenced by bacteria. *Candida* adhesion was strongly affected by Ra, saliva, and bacteria, but not by SFE. *Int J Prosthodont 2007;20:308–310.*

Denture plaque plays a causative role, together with *Candida* species, in the development of denture stomatitis. It is composed mainly of bacteria,[1] of which streptococci and *Actinomyces* species are the prominent members.[2] Moreover, the influence of saliva on bacteria and yeast's initial colonization and biofilm formation is poorly understood. Although *C albicans* is by far the predominant isolate in denture stomatitis, other non-*albicans* species such as *C glabrata* are frequently isolated.[3]

The use of denture liners in prostheses is advantageous in several clinical situations, even though the presence of *Candida* colonization on and within the material still exists. Additionally, a material's physicochemical surface properties may alter the denture pellicle composition and initial adherence of microorganisms to these surfaces. However, limited attention has been paid to the interactions between yeast, denture material surfaces, saliva, and bacteria.[4,5]

The purpose of this study was to determine the influence of surface free energy (SFE), surface roughness, saliva, and bacteria on the adhesion of 2 species of *Candida* to denture materials.

Materials and Methods

Three hundred twenty specimens (2.5 × 1.2 × 0.2 cm) were prepared from 2 acrylic resins (1 hot water bath–cured and 1 microwave-cured) and 2 denture liners (1 hard [Kooliner, Clássicos Artigos Odontológicos] and 1 soft [CoeSoft, GC America]) according to the manufacturers' instructions. The denture liners were prepared by placing glass slides over them, separating the glass slides after curing, and then cutting any excess material. All samples except for those using soft denture liner were finished using an automatic grinder and polished with pumice slurry and chalk powder.

Surface roughness was measured using a profilometer (Surfcorder SE 1700, Kozaka Industry). Three readings were made for each specimen and a mean value was calculated. SFE was calculated using cosine θ of contact angles, by dispensing a droplet (15 μL) of deionized distilled water on the specimen surface. This procedure was carried out 3 times for each specimen. Photographs of the droplets were taken immediately and contact angles were measured. Specimens were randomly assigned to one of the experimental conditions (n = 10 for each condition). The contaminants

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were removed by sonication for 20 minutes. Human whole saliva from a healthy donor was collected during masticatory stimulation and clarified by centrifugation at 10,000g for 10 minutes at 4°C. The samples were then rested for 30 minutes to form a pellicle.

C albicans (ATCC90028) and *C glabrata* (ATCC2001) were incubated in Sabouraud dextrose broth (Difco) for 24 hours at 37°C. Cells were harvested, washed with phosphate-buffer saline (PBS), and standardized to 5×10^6 cells/mL obtained spectrophotometrically. *Streptococcus mutans* (UA159) and *Actinomyces naeslundii* (ATCC12104) were subcultured overnight in 1% glucose-enriched tryptic soy broth (Difco) in 10% carbon dioxide. Growth curves were previously established to ascertain a microbial concentration of 10^8 bacteria/mL.

Specimens were tested in a flow chamber, using uncoated specimens and lack of exposure to bacteria as controls. First, bacteria were allowed to adhere to the sample surfaces from a flowing suspension (30 minutes), followed by a yeast-flowing suspension to allow yeast adherence (2 hours). Samples were then removed, washed in PBS, fixed with 80% ethanol, stained with crystal violet, and washed again with PBS.

Adherent yeast cells in each sample (15 fields, 0.25 mm^2/field) were enumerated under light microscopy at 400× magnification (Leitz-Ortholux, Leitz), and the results were expressed as cells/mm^2. Data of surface roughness and yeast counts were transformed by log and analyzed using analysis of variance (ANOVA) and the Tukey test. SFE data were assessed by using ANOVA on ranks ($\alpha = .05$).

Results

The surface roughness statistically differed among the materials (Table 1), except for between the acrylic resins, which presented the smoothest surfaces ($P < .0001$). The soft denture liner showed higher yeast counts and surface roughness in all conditions, which suggests that soft denture liners have a supporting effect on fungal growth, thus indicating the ability of the yeasts to penetrate the deepest confines of the materials. SFE values for the acrylic resins and hard denture liner were similar to each other but not to the soft liner, which showed the lowest values ($P < .0001$) (Table 1). Moreover, SFE seemed to have no direct influence on the adhesion of *Candida* species, which is in agreement with another study where no relationship between SFE and number of retained yeast cells was found.[5]

Table 1 Surface Roughness (Ra) (μm) and Surface Free Energy (SFE) (erg cm^{-2}) of the Tested Materials (Mean ± SD)*

Material	Ra	SFE
Hot water bath–cured acrylic resin	0.15 ± 0.06^{a}	42.77 ± 3.75^{a}
Microwave-cured acrylic resin	0.15 ± 0.06^{a}	42.42 ± 3.45^{a}
Hard liner	0.56 ± 0.17^{b}	42.37 ± 3.58^{a}
Soft liner	1.49 ± 0.43^{c}	39.82 ± 4.57^{b}

*Values followed by different lowercase letters are statistically different ($P < .05$).

Table 2 *Candida albicans* Adhesion in the Presence or Absence of Saliva Coating and Bacteria (Mean Yeast/mm^2 ± SD)*

	No saliva		Saliva-coated	
Material	No bacteria	Bacteria	No bacteria	Bacteria
Hot water bath–cured acrylic resin	87.0 ± 113.5^{abA}	84.6 ± 66.5^{aA}	10.8 ± 7.4^{aB}	25.4 ± 13.2^{aB}
Microwave-cured acrylic resin	91.0 ± 70.6^{aA}	56.6 ± 35.0^{aB}	26.5 ± 27.2^{aD}	39.9 ± 43.5^{aC}
Hard liner	91.6 ± 72.7^{aA}	48.2 ± 35.6^{aAB}	62.8 ± 41.6^{abAB}	27.0 ± 21.6^{aB}
Soft liner	195.9 ± 174.9^{bA}	185.2 ± 144.9^{bA}	63.4 ± 24.5^{bC}	84.6 ± 86.7^{bB}

*Values followed by different letters are statistically different from each other ($P < .05$). Lowercase letters show differences among materials and uppercase letters show differences between the presence/absence of bacteria and uncoating/coating with saliva.

Table 3 *Candida glabrata* Adhesion in the Presence or Absence of Saliva Coating and Bacteria (Mean Yeast/mm^2 ± SD)*

	No saliva		Saliva-coated	
Material	No bacteria	Bacteria	No bacteria	Bacteria
Hot water bath–cured acrylic resin	255.2 ± 230.5^{aA}	149.5 ± 86.7^{aC}	50.0 ± 47.4^{aD}	195.6 ± 432.8^{abB}
Microwave-cured acrylic resin	266.2 ± 228.5^{aA}	192.7 ± 208.5^{aB}	60.3 ± 44.1^{aD}	123.2 ± 246.7^{aC}
Hard liner	416.2 ± 322.9^{abA}	355.5 ± 458.7^{aB}	49.0 ± 46.1^{aD}	60.5 ± 78.0^{aC}
Soft liner	483.4 ± 141.7^{bA}	131.6 ± 137.6^{aD}	236.6 ± 234.8^{bC}	270.9 ± 373.0^{bB}

*Values followed by different letters are statistically different from each other ($P < .05$). Lowercase letters show differences among materials and uppercase letters show differences between the presence/absence of bacteria and uncoating/coating with saliva.

The adhesion of *C albicans* and *C glabrata* ranged from 3.2 to 564.4 cells/mm^2 and 3.2 to 1,400.4 cells/mm^2, respectively. *C glabrata* showed higher counts than *C albicans* in most of the experimental conditions and materials, in accordance with previous findings.[3]

The overall colonization on all materials was significantly decreased by the presence of saliva ($P < .05$). Although bacteria precolonization increased the yeast adhesion in the presence of saliva, these counts were not higher than in the absence of saliva ($P < .05$), thus supporting the antimicrobial properties of saliva (Tables 2 and 3).

Conclusions

1. *C glabrata* showed the highest counts in most experimental conditions, which may indicate higher virulence.
2. Adhesion of *Candida* species was affected by the presence of saliva and bacteria precolonization. There was a decrease in adherence of both *Candida* species with saliva precoating.
3. Surface roughness influenced adhesion, as the roughest surface material exhibited higher yeast counts. None of the materials appeared to have an inhibitory influence on the growth of either *Candida* species.
4. In clinical terms, the selection of appropriate materials for a given function may affect *Candida* adhesion and should be carefully considered in treatment protocols.

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Literature Abstract

Displacement of implant components from impressions to definitive casts

Four kinds of displacement of implant components can be introduced when making a definitive cast: *(1)* displacement of the impression coping on the surface of the implant or the abutment within the machining tolerance, *(2)* displacement of the impression coping caused by the impression material or the impression technique, *(3)* displacement of the impression coping on the surface of the implant or the abutment analog within the machining tolerance, *(4)* displacement caused by dimensional changes in the dental stone. This study evaluated the displacement of implant components using 2 impression techniques (evaluating the second and fourth kinds of displacement). Nonsplinted open tray and light-cured resin-splinted open tray techniques were used. A resin cast was fabricated with 5 parallel implants (4 × 11 mm, Nobel Biocare), and 5 casts were made using each technique. For the splinted group, 5 light-cured (Triad) blocks attached to the impression analogs on a preliminary cast were splinted on the patient's cast using adhesive resin (Palavit). Final impressions were made with polyether and definitive casts with type IV dental stone. Each sample underwent 5 measurement phases (from patient cast to definitive cast) using a computerized coordinate-measuring machine. Seven sets of data were obtained for each sample. The Mann-Whitney test was used to determine any differences. The average displacements while connecting the impression coping and abutment analogs were 31.3 μm (SD: 15.5) and 30.4 μm (SD: 15.6), respectively. The nonsplinted group showed smaller distortion than the splinted group during impression taking (possible influence of resin shrinkage). The splinted group showed smaller distortion than the nonsplinted group during fabrication of the definitive cast (possible influence of the splinted impression coping, which reduced the effect of dental stone expansion). Excluding displacements produced during component connection, no significant difference was noted between the 2 techniques.

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