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ORIGINAL ARTICLE

Adherence of *Candida albicans* to denture base acrylics and silicone-based resilient liner materials with different surface finishes

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Abstract This study evaluated the surface roughness and *Candida albicans* adherence on denture base acrylic resins and silicone-based resilient liners with different surface finishes. Four commercial denture base acrylic resins (three heat polymerized and one room temperature polymerized) and five silicone-based liner materials (two heat polymerized and three room temperature polymerized) (10×10×2 mm) were tested in this study. The materials were processed against glass or plaster or finished with a tungsten carbide bur. Surface roughness measurements were made using a profilometer with an optical scanner probe. All specimens were ultrasonically cleaned in water for 15 s, autoclave sterilized, and contaminated with *C. albicans* solution for adherence assay evaluation. The materials processed against the glass surface showed significantly lower surface roughness values (0.11±0.1–1.66±1.1 μm) than those of the materials processed against the dental plaster (2.61±0.2–6.12±2.8 μm) or roughening with a bur (1.48±0.2–7.05±1.2 μm; $p<0.05$, one- or two-way analysis of variance). Also, the materials processed against the glass surface showed lower *C. albicans* adhesion (mean ranks 120.36) than those of the materials processed against the dental plaster (mean ranks 139.77) or roughening with a bur (mean ranks 143.06), but the differences were not statistically significant ($p>0.05$, Kruskal–Wallis and Mann–Whitney). In all types of surface finishes, *C. albicans* adhesion on denture base acrylics was significantly less (mean ranks 90.18–90.40) than those of silicone liners (mean ranks 119.38–205.18; $p<0.01$, Kruskal–Wallis).

Keywords *Candida albicans* · Denture resin · Resilient liner · Stomatitis · Surface roughness

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Introduction

Although bacteria and other yeast could be pathogen in some cases, it has been proven that *Candida albicans* is the primary microbial factor in denture stomatitis [1–6, 8, 16, 17, 20, 22]. There are many studies concerning the adhesion mechanisms of *C. albicans* to denture base materials as well as factors affecting these mechanisms [6, 7, 9–13, 18, 19, 21, 23]. Because the adhesion of microorganisms to a surface is prerequisite for the colonization at that surface, the denture may function as a reservoir of infection [2, 11, 19, 21].

C. albicans has been found on both hard denture base acrylic resins and silicone-based resilient liner materials in vivo and in vitro [19]. Adherence of microorganisms to hard surfaces occurs in a two-stage process. While the initial interactions between two surfaces are nonspecific and reversible, the secondary phase is caused by specific

intermolecular interactions. Many theories have been made to explain the initial adherence of microorganisms to the substrate surfaces [1, 19]. The thermodynamic approach is one of those that describes the adhesion of microorganisms to surfaces due to the surface-free energies of the surfaces and the microorganisms. The second phase of adhesion process involves specific adhesion–receptor interactions. The microorganisms carry adhesives that bind to complementary receptors on the surface stereochemically. This stage is necessary for the tight binding of the microorganism to the specific substrate that permits colonization [19]. Other factors affecting the adherence of yeasts to surfaces include material type [7, 9, 16, 17, 19, 24], surface roughness [12, 13, 17, 19, 24], presence of salivary proteins and serum [6, 17, 19], presence of other adherent microorganisms, strain variability [19], concentration [23, 24], consumption of carbohydrate-rich diet [19], and culture conditions [5, 21–23].

There have been many studies in the dental literature on the adhesion of *C. albicans* to denture base acrylic resin and silicone-based resilient liner materials [7, 9, 13, 14, 16, 17, 19]. However, the substrates in these studies tend to have smooth and transparent surfaces or were not representative of technical processing of these materials or the in vivo environment. The objectives of this study, therefore, were to evaluate the surface roughness and adherence of *C. albicans* to different denture base acrylic resins and silicone-based resilient liner materials with different surface finishes simulating the processed fitting surfaces of the dentures representing the clinical situations.

Materials and methods

Preparation of test specimens

Four commercial denture base acrylic resins, three of which were heat polymerized and the other room temperature polymerized, as well as five silicone-based resilient liner materials, two of which were heat polymerized and the other three room temperature polymerized, were tested in this study. The brand names, types, manufacturers, and abbreviations of the materials used for the experiments are listed in Table 1.

To prepare the specimens, pink modeling wax (10×10×2 mm) (Cavex® Set Up Modeling Wax, Haarlem, The Netherlands) was placed in hard dental plaster (Moldano, Heraeus Kulzer GmbH, Hanau, Germany) in a two-part mold using a standard dental flask. Two types of molds were prepared in such a manner that in the first type one part of the mold was hard dental plaster and the other was surface glass, and in the second type both parts of the molds were vacuum-mixed hard dental plaster. The wax was eliminated under running hot water and plaster surfaces were sealed with one coat of sealant (Impact, Dental Exports of London, Watford, England). All materials were mixed and processed according to each manufacturer's instructions.

For surface roughness and for *C. albicans* measurements, five (N=45) and ten specimens (N=90), respectively, were made per material and randomly assigned for different surface finishes. In the first group, the materials were processed against the glass to achieve surfaces as smooth as possible. This group acted as the control group. In the second group, while the denture base acrylic resins were roughened with fine grit cross-cut tungsten carbide bur (Batch no. H79EFL, Komet, Paris, France), the silicone-based resilient liner materials were roughened with the burs supplied by their manufacturers. The choice of rotary instruments was determined according to those commonly used in clinical prosthetic dentistry. The procedure for finishing the surfaces with burs was standardized by ensuring that the rotary instrument cut along the surface of the specimen only in one direction. Minimal pressure was applied as it would be the case in clinical practice when adjusting the surface of a denture base [19]. In the third group, the materials were processed against the hard dental plaster. All specimens were prepared by the same operator (EN).

Table 1 The brand names, types, manufacturers, and abbreviations of the materials used for the experiments

Material	Type	Manufacturer
Lucitone 199 (L)	Heat-polymerized denture base acrylic resin	Dentsply International, York, USA
Paladent (P)	Heat-polymerized denture base acrylic resin	Heraeus Kulzer GmbH, Hanau, Germany
Impact (I)	Heat-polymerized denture base acrylic resin	Dental Exports of London, Watford, England
Fortex (F)	Room-temperature-polymerized denture base acrylic resin	International Dental Surgical and Industrial Polymer Suppliers, London, England
Ufi Gel P (UP)	Room-temperature-polymerized silicone-based resilient liner	VOCO, Cuxhaven, Germany
Ufi Gel SC (USC)	Room-temperature-polymerized silicone-based resilient liner	VOCO, Cuxhaven, Germany
Mollosil (M)	Room-temperature-polymerized silicone-based resilient liner	DETAX GmbH, Ettingen, Germany
Molloplast (MB)	Heat-polymerized silicone-based resilient liner	DETAX GmbH, Ettingen, Germany
Luci-Sof (LS)	Heat-polymerized-silicone-based resilient liner	Dentsply International, York, USA

After preparation of the specimens, their surfaces were washed with water steam under pressure to remove the possible contaminants present on the surfaces. The specimens were then stored in distilled water at 37°C for 7 days and the water was changed every 24 h [17]. After drying the specimens on the bench, surface roughness measurements were made.

Surface roughness measurements

Average surface roughness (μm) (R_a) was measured at four areas of each specimen yielding to 12 measurements from each specimen using a profilometer (Perthen Perthometer S&P, Göttingen, Germany) with an optical scanner probe (Facodyne, Göttingen, Germany). Because the measurements were made with an optical scanner probe instead of a diamond stylus, it was also possible to measure the surface roughness of the silicone-based resilient liner materials without damaging the surface.

Subsequently, all specimens were cleaned ultrasonically in distilled water for 15 s (Quantrex 90, L&R Ultrasonics, Kearny, NJ, USA), autoclave sterilized (Charisma Vacuum TD, S.r.1, Mediline, Amersfoort, The Netherlands) for 18 min at 1.2 bar, 121°C, and stored in distilled water at 37°C for 24 h prior to *C. albicans* contamination and adhesion assay.

C. albicans contamination

C. albicans strain ATCC 2091 was obtained as a stock culture (KÜKENS study group, Department of Microbiology, University of Istanbul, Turkey) and incubated on Sabouraud dextrose agar slope (Delta Medical and Chemical Materials Trading, Istanbul, Turkey) at 37°C for 48 h. Standard amounts of this culture were inoculated into 2 ml of liquid Sabouraud dextrose agar and incubated at 37°C for 24 h. The culture was then centrifuged (Function Line, Labofuge 400 R, Hereaus Instruments, Germany) at 3,000 rpm for 10 min and the resultant cell pellet was washed twice with phosphate-buffered saline solution (0.15 M, pH 7.3; Delta Medical and Chemical Materials Trading). After dilution with this solution, a final yeast suspension of approximately 10^6 *C. albicans* per milliliter was prepared.

Adherence assay

All specimens were contaminated with *C. albicans* at the same time according to a method described elsewhere [19, 23].

Statistical analysis

The statistical analysis was performed with the SPSS software package (version 11.5; SPSS, Chicago, IL, USA). Data obtained from surface roughness measurement and adherence assay were evaluated with one- and two-way analysis of variance (ANOVA). When significant differences were found between or within groups, Scheffé F test was used to determine the differences. Because the data for *C. albicans* adherence was not normally distributed, Kruskal–Wallis and Mann–Whitney nonparametric tests were used. Correlation between the surface roughness and *C. albicans* adherence was determined using repeated measures for ANOVA. In all comparisons, statistical significance was declared if the p value was less than or equal to 0.05.

Results

Surface roughness measurements

Surface finish types significantly affected the surface roughness values of the tested materials ($p<0.05$). The mean surface roughness values of the same material groups showed statistically significant differences depending on the surface finish type ($p<0.05$). The materials that were processed against the glass surface showed significantly lower surface roughness values (0.11±0.1–1.66±1.1 μm) than those of the materials processed against the dental plaster (2.61±0.2–6.12±2.8 μm) or roughening with a bur (1.48±0.2–7.05±1.2 μm; $p<0.05$, one- or two-way ANOVA).

Denture base acrylic resins demonstrated significantly lower surface roughness values (2.07±1.6 μm) than those of the silicone-based resilient liners (3.84±2.5 μm) regardless of the polymerization method ($p<0.05$; Table 2). Polymerization type for silicone-based resilient liners, on the other hand, did not affect the surface roughness values for these two types of materials ($p>0.05$) except when they were finished with tungsten burs ($p<0.05$; Table 3).

Table 2 Mean (±standard deviation) of surface roughness (R_a) (μm) values for the denture base acrylic resins and silicone-based resilient liners regardless of the polymerization type, depending on the surface finishes

Material	Surface roughness values (R_a) (μm)		
	Glass surface	Plaster surface	Bur surface
Acrylic-based resin	0.28±0.2	3.54±1.5	1.66±0.3[a]
Silicone-based resilient liners	0.75±0.8	4.08±2.1	5.45±1.8[a]

[a] Statistically significant differences at a level of $p<0.05$

Table 3 Mean (±standard deviation) of surface roughness (R_a) (μm) values for the room-temperature-polymerized or heat-polymerized silicone-based resilient liners depending on the surface finishes and the polymerization type

Material	Surface roughness values (R_a) (μm)		
	Glass surface	Plaster surface	Bur surface
Heat-polymerized resilient liners	0.62±0.6	4.48±1.7	3.62±0.4[a]
Room-temperature-polymerized resilient liners	0.83±1.0	3.81±2.3	6.66±1.3[a]

[a] Statistically significant differences at a level of $p<0.05$

C. albicans adhesion

C. albicans adherence levels for the test materials depending on the surface finishes are listed in Table 4. Surface finish type did not influence the *C. albicans* adhesion regardless of the material type ($p>0.05$), showing the lowest when the surfaces were processed against glass (mean ranks 120.36) and the highest when the surfaces were finished with tungsten carbide burs (mean ranks 143.06; Table 4). Regardless of the surface finishes, in all groups, the *C. albicans* adherence was higher for room-temperature-polymerized resins than heat-polymerized ones. However, there was no statistical difference ($p>0.05$; Table 5).

In all types of surface finishes, *C. albicans* adhesion on the denture base acrylic resins was significantly less (median ranks 90.18–90.40) than those of the silicone-based resilient liners (median ranks 119.38–205.18; $p<0.05$).

Although room-temperature- and heat-polymerized resilient liners did not show significant differences in surface roughness (3.26±1.89 and 4.22±2.8 μm, respectively; $p>0.05$), the latter showed significantly less *C. albicans* adhesion (mean ranks 119.38) than those of room-temperature-polymerized ones (mean ranks 205.18, $p<0.05$; Table 5).

Surface roughness values and *C. albicans* adhesion rate showed correlation for the glass surface, plaster surface, and bur processed specimens as $r=0.54$, 0.05, and 0.43, respectively (ANOVA for repeated measures).

Table 4 *C. albicans* adherence levels according to surface finish type (Kruskal–Wallis test)

Surface finish	Number of finishes	Mean rank
Glass surface	88	120.36
Plaster surface	90	139.77
Bur surface	90	143.06
Total	268	

Table 5 *C. albicans* adherence levels according to the material (Kruskal–Wallis test)

Material type	Number of materials	Mean rank
Heat-polymerized silicone	60	119.38
Room-temperature-polymerized silicone	88	205.18
Heat-polymerized denture base acrylic	90	90.18
Room-temperature-polymerized denture base acrylic	30	90.40
Total	268	

Discussion

C. albicans existence presents a high significance in the etiology of prosthesis stomatitis and is reported to be found on surfaces of hard and resilient acrylic resin materials in vivo [17]. Among many studies concerning the adhesion mechanisms of *C. albicans* to denture base materials and factors affecting their mechanisms, surface roughness [6, 16, 17, 19, 22] and type of materials [9, 14] are known to be two major factors for the adherence mechanism directly. The yeasts, being a part of the prosthesis plaque, adhere and accumulate on the surface of the prosthesis that plays a storing role for them [14, 19–21]. Clinically, materials exhibit the exterior properties of the surface on which they are finished [12]. During the fabrication of removable dentures, acrylic resin denture base materials and silicone-based resilient liners are processed and finished onto the dental plaster surface and smoothened by a tungsten carbide bur. This trimming procedure surely creates rougher surfaces [15]. Materials with rough surfaces make the cleaning of the prosthesis and mechanical removal of the microorganisms difficult. Also, they cause discoloration of the denture base materials [12, 14, 19, 24]. To compare the material performance in terms of surface roughness and *C. albicans* adherence, such prosthetic materials have been processed on glass surfaces for experimental purposes [19, 22, 24]. Unfortunately, in real clinical situations, denture resins and resilient liners could not be processed on glass. Therefore, a real smooth surface could not be achieved when these materials are processed on plaster and thereafter finished with tungsten carbide burs. For this reason, the results in the literature could not be considered as simulating the clinical situation completely.

In the dental literature, surface roughness (R_a) values found for heat-polymerized acrylic resin base materials range from 0.02 to 7.6 μm, for light-polymerized hard liner materials 0.7 to 2.6 μm, for room-temperature-polymerized hard liners 2.9 to 4.4 μm, for light-polymerized resilient liners 0.7 to 3.5 μm, for room-temperature-polymerized resilient liners 2.8 to 4.2 μm, for heat-polymerized resilient liners 1.3 to 7.9 μm, and for tissue conditioners 1.8 to 7.8 μm [12, 14, 17, 19]. In our study, the mean surface

roughness (R_a) values for denture base acrylic resin materials was 2.07 μm and for resilient liners it was 3.84 μm regardless of whether they were heat or room temperature polymerized. These results are in compliance with the findings of some previous studies [12, 14, 17, 19]. In the study of Radford et al. [17], the mean surface roughness (R_a) value for heat-polymerized acrylic resins that were finished on glass surface was found to be 1.6 μm and for resilient liners 1.3 to 1.5 μm. Our results were lower than these results. The reasons for the differences could be partially attributed to the material variations used in these studies. The other reason could be related to the differences in measurement methods between this study and those of previous studies. Conventional surface roughness measurement techniques often require surface contact with the object being measured; this could potentially damage the surface. Evaluation of surface roughness through surface contact involves the use of a stylus that is drawn over the specimen to detect and record variations in surface irregularity. A primary limitation of this technique is that the stylus must be drawn perpendicular to the surface. Noncontact methods such as the one used in this study without diamond stylus should be considered in future studies to avoid surface damage during measurements.

In this study, silicone-based resilient liners showed surface roughness values ranging from 0.11 to 7.12 μm, depending on the surface that they were finished onto. Resilient liners finished onto glass surface exhibited lower surface roughness than those finished onto dental plaster or finished with burs. Except after bur finishing, room-temperature- and heat-polymerized resilient liners did not show significant differences in terms of surface roughness. This could be explained by the differences in wear resistance of the materials, namely, that heat-polymerization would increase the cross-linking of residual monomers, resulting in a harder, more wear-resistant surface.

In all surface finish comparisons, *C. albicans* adherence was significantly higher in resilient liners than those of denture resins. Our findings also support the findings of previous studies with similar findings [7, 16, 17]. The results of this study, however, also showed that glass-surface-finished materials accumulated less *C. albicans* compared to plaster surface or bur surface finishing, with the latter type having the most. In contrast to these findings, Wright et al. [23] found no relation between surface properties of resilient liner materials and *C. albicans* adhesion. Independent from surface roughness characteristics, the electrochemical reaction that occurs between *C. albicans* and resilient liners may be the reason for the significant differences between the denture base resins. Therefore, in future studies, not only the surface roughness or porosity levels but also factors like material hydrophobicities, chemical compositions, and surface energies should be taken into consideration. In another study, Minagi et al. [12] tested 21 different acrylic base materials to evaluate the effect of hydrophobicity of these materials on *C. albicans* and *Candida tropicalis* adhesion. They found that with the increase in surface energy of the tested materials, *C. albicans* adhesion increased but *C. tropicalis* adhesion decreased. It was also emphasized that *C. albicans* adhesion on hydrophobic materials was low. The results of this study with higher *C. albicans* adherence on silicone-based liners could be due to the fact that acrylic resin denture base materials are considered more hydrophobic than silicone-based resilient liners.

Interestingly, even though the surface roughness values of heat-polymerized resilient liners were found to be less than those of room-temperature-polymerized ones, yet not significant, *C. albicans* adherence was significantly higher on the room-temperature-polymerized ones in all surface finish types. The chemical composition of the room-temperature-polymerized resilient liners and the difference in surface energies or higher hydrophilicity could be the reasons for this condition [10]. Further investigations should be made to investigate the differences between such materials considering the effect of saliva and electrostatistical reactions for *Candida* binding.

Because the adhesion of *C. albicans* on resilient liners were higher than on denture resin materials, clinically, hygiene instructions and maintenance programs for patients with relined dentures should be performed strictly to avoid stomatitis.

References

1. Arendorf TM, Walker DM (1987) Denture stomatitis: a review. J Oral Rehabil 14:217–227
2. Baysan A, Whiley R, Wright PS (1998) Use of microwave energy to disinfect a long-term soft lining material contaminated with *Candida albicans* or *Staphylococcus aureus*. J Prosthet Dent 79:454–458
3. Budtz-Jorgensen E (1974) The significance of *Candida albicans* in denture stomatitis. Scand J Dent Res 8:1–47
4. Budtz-Jorgensen E, Bertram U (1970) Denture stomatitis I. The etiology in relation to trauma and infection. Acta Odontol Scand 28:71–92
5. Douglas LJ (1984) Surface composition and adhesion of *Candida albicans*. Biochem Soc Trans 13:467–469
6. Edgerton M, Scannapieco FA, Reddy MS, Levine MJ (1993) Human submandibular–sublingual saliva promotes adhesion of *Candida albicans* to polymethylmethacrylate. Infect Immun 61:2644–2652
7. Ellepola ANB, Samaranayake LP (1998) Adhesion of oral *Candida albicans* isolates to denture acrylic following limited exposure to antifungal agents. Arch Oral Biol 43:999–1007
8. Graham BS, Jones DW, Burke J, Thompson JP (1991) In vivo fungal presence and growth on two resilient denture liners. J Prosthet Dent 65:528–532
9. Gruber RG, Lucatarto FM, Molnar EJ (2001) Fungus growth of tissue conditioners and soft denture liners. JADA 73:641–648

10. Loney RW, Moulding MB (1993) The effect of finishing and polishing on surface roughness of a processed resilient denture liner. Int J Prosthodont 6:390–396
11. Loney RW, Price RBT, Murphy DG (2000) The effect of polishing on surface roughness of tissue conditioners. Int J Prosthodont 13:209–213
12. Minagi S, Miyake Y, Inagaki K, Tsuru H, Suginaka H (1985) Hydrophobic interaction in *Candida albicans* and *Candida tropicalis* adherence to various denture base materials. Infect Immun 47:11–14
13. Nikawa H, Hayashi S, Nikawa Y, Hamada T, Samaranayake LP (1993) Interactions between denture lining material, protein pellicles and *Candida albicans*. Arch Oral Biol 38:631–634
14. Nikawa H, Hamada T, Yamamoto T, Kumagai H (1997) Effects of salivary or serum pellicles on the *Candida albicans* growth and biofilm formation on soft lining materials in vitro. J Oral Rehabil 24:594–604
15. Nikawa H, Hamada T, Yamashiro H, Murata H, Subiwahjudi A (1998) The effect of saliva or serum on *Streptococcus mutans* and *Candida albicans* colonization of hydroxylapatite beads. J Dent 26:31–37
16. Okita N, Orstavik O, Orstavik J, Ostby K (1991) In vivo and in vitro studies on soft denture materials: microbial adhesion and tests for antibacterial activity. Dent Mater 7:155–160
17. Radford DR, Sweet SP, Challacombe SJ, Walter JD (1998) Adherence of *Candida albicans* to denture base materials with different surface finishes. J Dent 26:577–583
18. Samaranayake LP, McFarlane TW (1980) An in-vitro study of the adherence of *Candida albicans* to acrylic surfaces. Arch Oral Biol 25:603–609
19. Samaranayake LP, McCourtie J, McFarlane TW (1980) Factors affecting the in-vitro adherence of *Candida albicans* to acrylic surfaces. Arch Oral Biol 25:611–615
20. Waltimo T, Vallittu P, Haapasalo M (2001) Adherence of *Candida* species to newly polymerized and water-stored denture base polymers. Int J Prosthodont 14:457–460
21. Waters MGJ, Williams DW, Jagger RG, Lewis MAO (1997) Adherence of *Candida albicans* to experimental denture soft lining materials. J Prosthet Dent 77:306–312
22. Williamson JJ (1968) The effect of denture lining materials on the growth of *Candida albicans*. Br Dent J 125:106–110
23. Wright PS, Young KA, Riggs PD, Parker S, Kalachandra S (1998) Evaluating the effect of soft lining materials on the growth of yeast. J Prosthet Dent 79:404–409
24. Zissis AJ, Polyzois GL, Yannikakis SA, Harrison A (2000) Roughness of denture materials: a comparative study. Int J Prosthodont 13:136–140