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Effectiveness of Coating Acrylic Resin Dentures on Preventing *Candida* Adhesion

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Abstract

Purpose: The aim of this study was to prevent the adhesion of *C. albicans* on acrylic resin dentures by modifying their surfaces.
Materials and Methods: Ninety acrylic resin plates were divided into three groups. Group I: conventionally processed acrylic resin plates. Group II: plates painted with 2-Octyl Cyanoacrylate adhesive. Group III: plates painted with Adper Single Bond Adhesive. All specimens were immersed separately in containers filled with artificial saliva that contained *C. albicans* and then incubated for 11 days at 37°C. Three methods of evaluation were used to count the adhered *Candida*: direct culture, slide count, and serial dilutions.
Results: *C. albicans* in 1/10, $1/10^2$, and $1/10^3$ dilutions showed overgrowth in group I, while overgrowth was noted only with 1/10 dilution in group III. For group III, mean colony numbers of 123, 22, 3.4, and 0 were found for the $1/10^2$, $1/10^3$, $1/10^4$, and $1/10^5$ dilutions, respectively. Regarding the slide counts, group I showed a mean fungal count of 166 compared to 40 for group III with 1/10 dilution, 21 compared to 9 with $1/10^2$ dilution, 8.6 compared to 0.7 with $1/10^3$ dilution, and 1.2 compared to 0 with $1/10^4$ dilution. No plates in group II showed any candidal colonies regardless of the method of evaluation (0%). These differences were statistically significant ($p < 0.0001$).
Conclusion: Coating the acrylic resin dentures with Adper Single Bond Adhesive was effective in reducing *C. albicans* adhesion to dentures, while coating with 2-Octyl Cyanoacrylate adhesive completely inhibited such adhesion.

Candida albicans is a common opportunistic fungal pathogen that causes a variety of *Candida* infections in the oral cavity. Denture stomatitis is a common form of oral candidiasis that develops with the adherence of *C. albicans* to denture base surfaces.[1,2]

Adhesion of microorganisms to a surface initiates biofilm formation.[3] *Candida* species that adhere to the surfaces of prostheses are essential for the pathogenesis of denture stomatitis.[4] Therefore, to control denture stomatitis, it is important to control the adhesion of *Candida* on the surface of the prosthesis.

Tissue surfaces of dentures usually show microporosities, which harbor microorganisms difficult to remove by mechanical or chemical cleaning. Such yeasts adhere to the denture surfaces and act as reservoirs of microorganisms. Several investigators have analyzed the adherence of *C. albicans* to acrylic resin surfaces.[5-9]

The first study to properly investigate the adhesion of *C. albicans* to acrylic resin in vitro was reported in 1980.[6] Maxillary denture bases can serve as good reservoirs of *C. albicans*, so among denture wearers, candidiasis had been reported to be aggravated by its adhesion to the tissue-fitting surface of these denture bases.[7-9]

The initial adherence of *Candida* on the denture-bearing mucosa and fitting surface of the denture is essential for colonization and development of denture stomatitis.[10-12] The first stage of colonization by an organism is now widely recognized to involve the adherence of the organism to a host surface.[13]

The mechanisms of microorganism adhesion have been studied extensively. The basic mechanism of microbial adhesion to either hard or epithelial surfaces is still unclear; however, four phases are normally recognized: phase 1—transport to the surface; phase 2—initial adhesion; phase 3—attachment; phase 4—colonization.

The four aforementioned stages are based on the surface free energy and surface roughness. The substratum surface energy is important in initial adhesion, although surface roughness

provides a larger surface area for attachment and a protected environment until firm attachment is completed in phase 3.[14,15]

Another factor that influences yeast adherence to acrylic resin surfaces is the hydrophilicity of the microorganism. Larger amounts of *C. albicans* have been reported to adhere to surfaces with increased surface wettability (i.e., surface energy), due to its hydrophilic nature.[5]

The contribution of both electrostatic and hydrophilic/hydrophobic forces on the adherence process varies between substrates and environments; however, these forces are important in the initial resistance or adherence of yeasts, and should adherence occur, there is an opportunity for further bonding and formation of denture plaque. Several factors such as saliva, other microorganisms, serum, differences in surface texture, and chemistry may influence this complex process.[16]

Minagi et al[5] concluded that increasing the free surface energy of the resin material increased the surface adherence of the hydrophilic species *C. albicans*, whereas it decreased the adherence of the hydrophobic species *C. tropicalis*. There was a higher adherence of microorganisms to the material when the organism had a surface free-energy closest to that of the resin.

Acrylic resin denture base materials were found to be less prone to microbial adhesion than soft lining materials.[17-19] This is because of the surface textures and the physical and chemical affinity for microorganisms of soft lining materials. Verran and Maryan[20] showed more fungal adhesion on rough surfaces than that on smooth surfaces.

Surface roughness directly influences the initial surface adherence of microorganisms, biofilm development, and colonization of *Candida* species. Materials with the roughest surfaces usually exhibit higher yeast counts.[19-22] These higher yeast counts occur because surface irregularities may act as a reservoir. Moreover, they favor increased microorganism retention and protection from shear forces that may occur even during denture cleaning. These irregularities may also allow irreversible attachment of the entrapped microbial cells to a surface.[23]

Electrostatic interactions play a role in the adhesion of *Candida* to polymethyl methacrylate (PMMA). Adhesion of *Candida* has been reported to be prevented by using negatively charged denture base materials. This in turn can prevent the development of denture-induced stomatitis.[24] Modification of the surface charge of the denture resin can be attained by the interacting polymerization of methacrylic acid to PMMA, preventing or reducing the adhesion of the microorganism to the denture surface.

Another reported alternative to prevent microbial adhesion is the application of a protective coating made of a pure poly (e.g., dimethyl) siloxane. This self-bonding polymer provides an inert and acid-resistant mono-molecular layer, which can inhibit microbial attachment and growth. This thin coating changes the surface chemistry and provides a chemically stable, nonsticky surface, which can last as long as the substrate to which it is bonded.[24,25]

Candida biofilms on oral mucosa and denture resins may lead to increased resistance to antifungal agents and provide protection from host defense mechanisms.[26-29] These potential contributing factors may offer new insights into the adhesion of *C. albicans* to denture resins with modified surfaces. The same study also showed that the occurrence of denture stomatitis in patients with poor oral hygiene could be reduced by using these surface-modified resins.

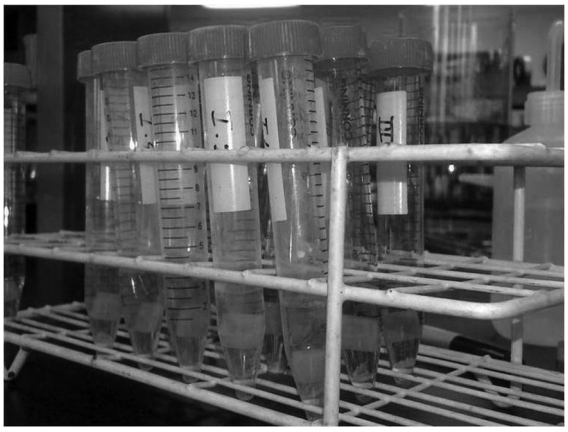

Figure 1 Acrylic resin plates of different groups in test tubes.

To date, the complete inhibition of candida adhesion to acrylic resin denture bases has yet to be reported, which served as an impetus for the present study. Therefore, the purposes of this study were to determine the effect of coating acrylic resin plates with Adper Single Bond Adhesive (3M ESPE, St. Paul, MN) on the adhesion of *C. albicans* to acrylic resin surfaces, and the effect of coating acrylic resin with 2-Octyl Cyanoacrylate (Johnson & Johnson, Ethicon, Inc., Somerville, NJ) adhesive on the adhesion of *C. albicans* in acrylic resin dentures.

Materials and methods

Ninety square acrylic resin specimens were prepared and divided into three groups of 30 plates. The specimens were fabricated at the prosthodontic laboratory of the College of Dentistry, University of Dammam, with dimensions of $10 \times 10 \times 2$ mm^3, using heat-polymerizing acrylic resin (Trevalon/Universal Clear-Dentsply, Konstanz, Germany) (Fig 1).

The specimens were divided as follows:

1. **Group I (control group):** Normal acrylic resin plates (N = 30 plates).
2. **Group II (study group 1):** Acrylic resin plates painted with 2-Octyl Cyanoacrylate adhesive (N = 30 plates).
3. **Group III (study group 2):** Acrylic resin plates painted with Adper Single Bond Adhesive (N = 30 plates).

All specimens in the three groups were immersed separately in containers filled with artificial saliva containing 2,000,000 cells of *C. albicans* (ATCC 10231) and were then incubated for 11 days at 37°C. All specimens were washed with tap water for 1 minute and each incubated in test tubes with 2 ml of Sabouraud's dextrose broth (SD broth- Acumedica Co., Manufacturers, Inc., Lansing, MI) for 2 days. After incubation, *C. albicans* colonies were separated from the acrylic resin using a hard vortex mix for 10 minutes and then centrifuged at 4500 rpm for 5 minutes.

Evaluation

After centrifuging, the acrylic resin plates were removed from their tubes, and the concentrated pellet was collected from the tube. Three methods of evaluation were used to calculate the amount of *C. albicans* adhered to each acrylic resin specimen as follows:

1- **Direct culture test:**

One hundred microliters (100 μl) of the centrifuged pellet were taken and cultured in plates filled with SD agar and incubated for 24 hours at 37°C. After incubation, colonies of *C. albicans* were counted in the plates using a marker pen colony counter (Scienceware, Bel-Art Products, Wayne, NJ). Colonies covering the whole surface of the plate were considered to be overgrowth.

2- **Serial dilutions:**

Five tubes were filled with 900 μl of normal saline. One hundred microliters of the concentrated pellet was added to the first tube (1/10) and mixed well. A total of 100 μl was aspirated from the first tube and added to the second tube ($1/10^2$). The second tube was then mixed well, and 100 μl was aspirated from it, before being added to the third tube ($1/10^3$). The solution in the third tube was mixed, followed by aspirating 100 μl and adding it to the fourth tube ($1/10^4$), which was further mixed as well. Subsequently, 100 μl was added to the fifth tube ($1/10^5$) from the fourth tube and mixed well. Finally, 100 μl was aspirated from the fifth tube and, after thorough mixing, discarded. By this stage, all tubes contained 900 μl of diluted concentration of the broth. SD agar plates were cultured with 10 μl of diluted concentration of each tube. Plates were incubated for 24 hours at 37°C. Colonies were counted as previously mentioned in a direct culture test.

3- **Slide count:**

A total of 7.5 μl was taken from the serial dilution of the concentrated pellet and added to 2.5 μl of trypan blue 0.4% solution in phosphate (MP Biomedicals, Santa Ana, CA). The new 10 μl solution was placed on a slide count (Neubauer Slide Counter Chambers, Marienfeld, Lauda-Königshofen, Germany) for microscopic evaluation. Trypan blue stain enabled the counting of *Candida* numbers under a light microscope at 10× magnification. A slide count contained 4 main squares, with each divided into 16 smaller squares. *Candida* colonies were counted on the two main squares and multiplied by 2 to obtain the total numbers of *Candida* on each slide.

Statistical analysis

Data obtained in this study were analyzed using SPSS, PC Statistical Package (SPSS Inc., Chicago, IL). The statistical analysis was performed with a chi-square test. A p-value < 0.05 and a CI at 95% were considered statistically significant; however, when an expected cell value according to the chi-square test was < 5, a Fisher test was used.

Table 1 Group I—serial dilution and microscopic count

Evaluation	Serial dilutions	N	Minimum	Maximum	Mean	SD
Serial dilution count	1/10	30	Φ	Φ	Φ	<0.001
	$1/10^2$	30	Φ	Φ	Φ	<0.001
	$1/10^3$	30	Φ	Φ	Φ	<0.001
	$1/10^4$	30	64	212	107.67	53.929
	$1/10^5$	30	3	53	17.23	15.950
Microscopic count	1/10	30	143	190	166.27	11.030
	$1/10^2$	30	15	28	21.13	3.126
	$1/10^3$	30	4	12	8.63	1.752
	$1/10^4$	30	1	2	1.23	0.430
	$1/10^5$	30	0	0	0.00	<0.001

Φ: overgrowth.

Table 2 Group III—serial dilution and microscopic count

Evaluation	Serial dilutions	N	Minimum	Maximum	Mean	SD
Serial dilution count	1/10	30	Φ	Φ	Φ	<0.001
	$1/10^2$	30	104	151	123.53	13.980
	$1/10^3$	30	4	38	22	7.202
	$1/10^4$	30	1	7	3.40	1.714
	$1/10^5$	30	0	0	0.00	<0.001
Microscopict count	1/10	30	31	52	40.27	6.716
	$1/10^2$	30	5	14	9	2.464
	$1/10^3$	30	0	2	0.70	0.702
	$1/10^4$	30	0	0	0.00	<0.001
	$1/10^5$	30	0	0	0.00	<0.001

Φ: overgrowth.

Results

Direct culture count

All plates in groups I and III revealed an overgrowth of *C. albicans* colonies, while no plates in group II showed any *C. albicans* colonies. Differences between group II and the other groups were found to be highly significant statistically ($p < 0.0001$).

Serial dilution count

Three dilutions (1/10, $1/10^2$, $1/10^3$) for group I showed an overgrowth of *C. albicans* colonies, while overgrowth was detected only in the 1/10 dilution for group III. The mean number of colonies in the $1/10^2$, $1/10^3$, $1/10^4$, and $1/10^5$ dilutions for group III were recorded as 123.53, 22, 3.4, and 0, respectively, while the mean number of colonies in the $1/10^4$ and $1/10^5$ dilutions for group I were recorded as 107.67 and 17.23, respectively. These differences were statistically significant ($p < 0.05$). No plates in group II showed any candidal colonies for any of the dilutions (0%). These differences were highly significant statistically ($p < 0.0001$) (Tables 1, 2, Fig 2).

Slide count

Light microscopic examination showed that the mean numbers of *C. albicans* in group I were 166.27, 21.13, 8.63, 1.23, and

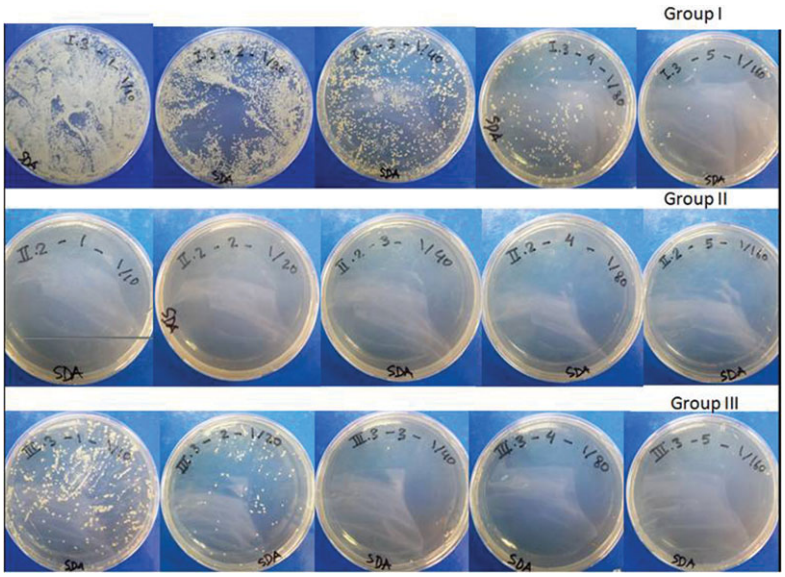

Figure 2 Plate count of different dilutions in groups I, II, and III.

0 for the 1/10, $1/10^2$, $1/10^3$, $1/10^4$, and $1/10^5$ dilutions, respectively. For group III, the mean numbers were 40.27, 9, 0.7, 0, and 0 for the 1/10, $1/10^2$, $1/10^3$, $1/10^4$, and $1/10^5$ dilutions, respectively. These differences were statistically significant ($p < 0.05$). No slides in group II showed any *Candida* for any of the dilutions (0%). These differences were highly significant statistically ($p < 0.0001$) (Tables 1, 2).

Discussion

Denture stomatitis is a common disorder affecting denture wearers. Many factors play a role in the etiology of denture stomatitis; however, all of these factors have been shown to increase the ability of *C. albicans* to colonize on both the denture and oral mucosal surfaces.[30] It is widely accepted that the development of denture stomatitis is associated with the pathogenic overgrowth of *C. albicans* on denture surfaces and on oral mucosa.[1,2,30] Although antifungal treatment can eradicate *C. albicans* contamination, dentures must be decontaminated, and their cleanliness maintained. Without proper management, the recurrence of stomatitis is very likely after antifungal therapy is discontinued. Thus, many researchers are exploring other modalities for the prevention and management of denture stomatitis.[4,31,32]

As denture materials differ in the ability of oral bacteria and yeast to form biofilms and colonize them, they may reflect greater or lesser susceptibility for the occurrence of denture stomatitis.[30] Therefore, new developments related to denture materials have focused on ways to decrease the development of adherent biofilms. These developments may be effective in reducing bacterial and yeast colonization, which may lead to potential reductions in denture stomatitis.[30]

Research has also examined the role of denture surfaces in reducing the ability of *C. albicans* to adhere to them and form biofilms, which lead to denture stomatitis.[4,32] Such studies have focused on modifying the surface of acrylic resin base denture materials to make them more resistant to adhesion by *Candida*. The majority of previous studies have focused on smoothening and polishing of different acrylic resin base materials, while this current study proposed a different technique in which the denture surface was modified by painting it with a biocompatible adhesive that formed a very thin and smooth layer. The results of this study showed that surface modification of the acrylic resin base denture material by coating it with 2-Octyl Cyanoacrylate adhesive or Adper Single Bond Adhesive reduced the ability of *C. albicans* to adhere to its acrylic resin base surface.

A comparative analysis of results between group III and the control group (I) showed that painting acrylic resin base dentures with Adper Single Bond Adhesive reduced the number of *Candida* adhered to the acrylic resin base dentures. Furthermore, comparing the results between group II and the control group (I) indicated that painting with 2-Octyl Cyanoacrylate adhesive completely prevented *Candida* from adhering to the acrylic resin base dentures.

The mechanism via which the coating agents used in this study reduced adhesion by *C. albicans* may be that these agents smooth the surface and fill cracks on the acrylic resin surface and/or modify the surface hydrophilicity/hydrophobicity of the acrylic resin. Ramage et al used scanning electron microscopy to demonstrate the ability of *Candida* biofilms to adhere along irregularities on denture surfaces.[33] Filamentous forms of *Candida* species were found to become deeply embedded within these deformities. Other studies have also supported this hypothesis and confirmed that surface cracks and surface roughness favor the adherence of microorganisms and development of biofilms.[4] Such cases can be prevented when dentures are painted with 2-Octyl Cyanoacrylate adhesive, which can fill cracks and smooth rough surfaces. On the other hand, surface hydrophobicity has been proposed to selectively increase the propensity of hyphal forms of *C. albicans* to colonize denture surfaces.[32] Adhesive agents used in this study can coat acrylic resin dentures with a thin and glossy layer, which tends to

modify the contact angle and affect the hydrophobicity of the surface to reduce adhesion by *C. albicans*.

Differences in the results of this study between group II and group III may be associated with the ability of the 2-Octyl Cyanoacrylate coating agent to fill cracks on acrylic denture bases and smooth the surfaces more effectively than does Adper Single Bond Adhesive. Similarly, the 2-Octyl Cyanoacrylate coating agent may decrease the hydrophobicity of the acrylic resin surface better than can Adper Single Bond Adhesive.

Morgan and Wilson concluded in their in vivo study that smooth acrylic resin surfaces could lead to reduced bacterial biofilm formation. This finding again may explain the reduction of *Candida* adhesion in groups II and III of this study, as coating the acrylic resin plates with 2-Octyl Cyanoacrylate and Adper Single Bond Adhesive smoothed the surfaces of the acrylic resin.[34] Other in vitro studies have shown that rough surfaces are able to promote the adhesion of *C. albicans* and other microorganisms to denture base materials.[19,20,35] However, Yamauchi et al[35] demonstrated an increase in *C. albicans* adherence to smoothening-treated surfaces compared to polished surfaces. This finding indicates that coating of acrylic resin surfaces in this study may have mimicked the effect of polishing but not of smoothened surfaces.

From a clinical perspective, smoothing or polishing of the intaglio surfaces of dentures affects the fitting of the denture; however, 2-Octyl Cyanoacrylate adhesive forms a very thin biocompatible layer, which does not interfere with the denture fit.

Conclusion

With regard to the limitations of the current study, it might be concluded that coating acrylic resin dentures with Adper Single Bond Adhesive effectively reduces the number of adherent *C. albicans*, while coating acrylic resin dentures with 2-Octyl Cyanoacrylate adhesive inhibits the adhesion of *C. albicans*. Coating with 2-Octyl Cyanoacrylate adhesive is very easy and effective in reducing *C. albicans* adhesion; however, the durability of these agents and the effectiveness of periodic recoating of dentures with the same agents warrants further investigation.

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