Candida albicans Adherence to Surface-Modified Denture Resin Surfaces

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Abstract

Purpose: The purpose of this study was to investigate two innovative methods in reducing adhesion of Candida albicans to denture base resins through modification of the surface characteristics of denture resin by incorporation of surface charge and application of a self-bonding polymer on denture resins.

Materials and Methods: Three groups were tested [Group 1: control, pure poly(methyl methacrylate) (PMMA); Group 2: modified PMMA (mPMMA) with 16% methacrylic acid; Group 3: pure PMMA coated with self-bonding polymer (SBP)]. Twenty resin specimens for each group were polymerized, and four experimental subgroups for each surface type were devised, consisting of 2, 4, 6, and 12 days of incubation in C. albicans suspension. The surface area of adherent C. albicans stained with Gram’s crystal violet was examined under a light microscope at 400× magnification. Four areas were photographed on each block, one on each quadrant. The images were analyzed using Scion Image 1.63 software to calculate the percent surface area containing adherent C. albicans. Kruskal–Wallis test and Tukey’s honest significant difference (HSD) procedure were used to compare the groups.

Results: At day 2, the modified resin had statistically significantly lower levels of Candida than both the control group and the SBP group (p < 0.036). Both the mPMMA group and SBP group had statistically significantly lower levels of Candida accumulation at days 4, 6, and 12, compared to the control.

Conclusions: The amount of C. albicans adhering to the resin surfaces reduced significantly with modification of surface charge and application of self-bonding polymer. Modification of surface characteristics of polymeric biomaterials is an effective method in reducing adhesion of C. albicans to PMMA surfaces.

Candida albicans is a prevalent, opportunistic fungal pathogen in the oral cavity resulting in a multitude of Candida infections. Chronic atrophic candidiasis, also known as denture stomatitis, is a common form of oral candidiasis, associated with the adherence of C. albicans to denture base surfaces.1-3 Healthy individuals commonly exhibit the presence of C. albicans in the oral cavity; however, higher salivary yeast counts are found in full denture wearers than in dentate individuals.4 Predisposing systemic and local factors induce the transformation of this commensal organism to pathogen. Candidiasis is more susceptible in immunocompromised patients and in patients being treated with radiation or chemotherapy.5 6

In denture wearers, candidiasis is aggravated by the adhesion of C. albicans to the tissue-fitting surface of a maxillary denture base, which serves as an effective reservoir of microorganisms.7-10 Large accumulations of hyphae and inflammatory cells have been found to be present in 65% of denture wearers with denture stomatitis and only in 14% in clinically normal palatal mucosa.11 The initial attachment of Candida on the palatal mucosa and mucosal surface of the denture is an essential step in colonization and pathogenesis.12-14 Attempts have been made to inhibit candidal adhesion and subsequent colonization on the denture resin surface through the use of a wide range of antifungal agents; however the efficacy of this method of treatment is transient and does not offer a long-term effect.15

Surface characteristics resulting from chemistry are significant in the initial adherence of Candida to the denture resin and offer an opportunity for further bonding and colonization. Polymeric biomaterials feature an inherent advantage with their capacity for modification, and denture bases made today are constructed from poly(methyl methacrylate) (PMMA). Understanding the effect of electrostatic interactions in the adhesion of Candida to PMMA, negatively charged denture base materials have been suggested to prevent adhesion of Candida and to reduce the development of denture-induced stomatitis.16 The
The purpose of this study was to investigate two innovative methods in reducing adhesion of *C. albicans* to denture base resins through surface modification. The project was composed of two specific aims: (1) to examine the effect of surface-charged denture resins in reducing the adhesion of *C. albicans*; (2) to identify the effect of SBP on denture resins in reduction of candidal adhesion.

### Materials and methods

The study evaluated three groups (Group 1: control, Group 2: mPMMA, Group 3: SBP). Resin specimens in Group 1 were made of pure PMMA (0% methacrylic acid:100% MMA) and had no surface coatings applied to the surfaces. Resin specimens were modified with methacrylic acid in a ratio of 16% methacrylic acid:84% MMA for Group 2. Resin specimens in Group 3 were prepared using pure PMMA coated with KISS-CARE® Concentrated Gel (KISS-COTE, Inc., Tampa, FL) to the testing surfaces. For each resin specimen, 10 mg of the KISSCARE® concentrated gel was applied to spread completely over the specimen surfaces. Any excess of the material that could be removed was wiped off with gauze. Only light pressure was required to ensure that the surface was thoroughly wetted with the gel.

### Preparation of resin specimens

Twenty resin specimens for each group were polymerized using chemicals from Sigma-Aldrich (St. Louis, MO) as indicated in Table 1. Resin specimen fabrication for the mPMMA group involved incorporation of 16% of methacrylic acid in polymerization, whereas the other groups used 100% MMA. Resins were mixed with a powder:liquid ratio that would create an equivalent working consistency. All specimens were produced in our lab and formed in an 11 × 5 mm² mold (Polysciences, Niles, IL) with highly polished surfaces to ensure reproducible and consistent results. Polymerization of the specimens was carried out in water at 55 ± 1°C under air pressure of 20 psi for 15 minutes. The specimens were rinsed and stored in sterile distilled water for 24 hours before use to remove any residual monomer after polymerization.

### Incubation with *C. albicans*

Sabouraud dextrose broth (Sigma-Aldrich) was prepared using sterile water and autoclaved for use as a growth medium. *C. albicans* (#28366) was obtained from ATCC (Manassas, VA). The yeast was initially precultured in Sabouraud dextrose broth at 36°C for 48 hours after rehydration. Four experimental subgroups for each surface type were devised, consisting of 2, 4, 6, and 12 days of incubation in *C. albicans* suspension. Resins were added to four separate six-well plates, with one well of each plate containing five resins from each experimental group. Care was taken to place the resin blocks with the smooth surface facing upward. *C. albicans* culture was suspended in the broth with a ratio of 1:3 and then injected into the six-well plates until the resins were submerged completely below the surface of the suspension. The plates were then covered and placed in an incubator set at 36°C to simulate the ambient temperature of the oral cavity. The plates were oscillated at a rate of 90 rpm to keep the suspension from settling. At 48 hours, the day 2 samples were removed from the incubator for fixing and staining. The remaining samples had approximately 50% of their suspension replaced with fresh Sabouraud dextrose broth. This procedure was repeated at days 4, 6, and 8. After day 8, the remaining samples were left undisturbed until day 12, when they were stained.

### Surface area of adherent *C. albicans* analysis

Nonadherent yeast was removed by gently rinsing the blocks with phosphate-buffered saline (PBS). The blocks were then submerged in a PBS solution containing 1.5% glutaraldehyde (Sigma-Aldrich) for 1 hour to allow fixation of the adherent *C. albicans*. The resins were gently rinsed with sterile, deionized water to remove the fixing agent and allowed to air dry. The blocks were momentarily submerged in Gram’s crystal violet (Sigma-Aldrich) and allowed to set on the table for 1 minute. The blocks were gently rinsed with sterile, deionized water from a squeeze bottle until the runoff was clear, dipped in Gram’s iodine (Sigma-Aldrich), allowed to set for 1 minute, and then rinsed. This procedure was repeated for the remaining samples at days 4, 6, and 12.

### Table 1: Polymerization of resin samples

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Action</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMMA:MMA</td>
<td>Polymer:monomer</td>
<td>3:1 by weight</td>
</tr>
<tr>
<td>mPMMA (16% methacrylic acid)</td>
<td>Polymer:monomer</td>
<td>3:1.5 by weight</td>
</tr>
<tr>
<td>Benzoyl peroxide</td>
<td>Initiator</td>
<td>1% weight of PMMA</td>
</tr>
<tr>
<td>2-Hydroxyethyl methacrylate</td>
<td>Cross-linker</td>
<td>0.5% volume of MMA</td>
</tr>
<tr>
<td>N,N dimethylaniline</td>
<td>Activator</td>
<td>0.5% volume of MMA</td>
</tr>
</tbody>
</table>
Table 2  Candida density scores (% surface area) by surface type

<table>
<thead>
<tr>
<th>Day</th>
<th>Control</th>
<th>mPMMA</th>
<th>SBP</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>8.80 ± 5.72</td>
<td>1.93 ± 2.05</td>
<td>5.39 ± 4.32</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4</td>
<td>7.97 ± 7.97</td>
<td>3.01 ± 8.65</td>
<td>6.40 ± 7.35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6</td>
<td>17.44 ± 11.67</td>
<td>2.20 ± 4.40</td>
<td>6.82 ± 6.27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>12</td>
<td>23.32 ± 17.97</td>
<td>2.65 ± 3.97</td>
<td>5.71 ± 4.70</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Five observations per group, per day.
**p-values computed using the Kruskal–Wallis test.

The resin blocks were examined under a light microscope (Optiphot-2, Nikon, Tokyo, Japan) at 400× magnification. Four areas were photographed on each block, one on each quadrant, and examined for the entire sample. The images were analyzed using Scion Image 1.63 software (Frederick, MD) to calculate the percent surface area containing adherent C. albicans.

Statistical analyses

Data were collected over the course of the study as described above and entered into a statistical database (SPSS v.11.0, SPSS, Inc., Chicago, IL). General comparisons of candidal density were made using the Kruskal–Wallis test, given the small sample sizes and lack of normality in the data. The Tukey’s honest significant difference (HSD) procedure was used to identify specific differences between groups. For all statistical analyses, a p-value <0.05 was considered statistically significant.

Results

Each group consisted of five blocks that had density measurements of surface Candida taken at four time periods (2, 4, 6, and 12 days following incubation with C. albicans). The mean surface density measurements are summarized in Table 2. The mean surface density scores for the control, mPMMA, and SBP groups at day 2 were analyzed. Multiple comparisons testing (Table 3) demonstrated that at day 2 the modified resin had statistically significantly lower levels of Candida than both the control group and the SBP group (p < 0.036); however, the mPMMA group and SBP group were not different from each other for the remaining time points (days 4, 6, 12; p > 0.162). Both the mPMMA group and SBP group had statistically significantly lower levels of Candida accumulation at days 6 and 12, compared to the control. The results are summarized in Figure 1.

Table 3  Multiple comparisons testing for Candida density scores*

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>mPMMA</th>
<th>SBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>*</td>
<td>&lt;0.001</td>
<td>0.039</td>
</tr>
<tr>
<td>mPMMA</td>
<td>&lt;0.001</td>
<td>*</td>
<td>0.036</td>
</tr>
<tr>
<td>Day 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>*</td>
<td>0.132</td>
<td>0.810</td>
</tr>
<tr>
<td>mPMMA</td>
<td>0.132</td>
<td>*</td>
<td>0.380</td>
</tr>
<tr>
<td>Day 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>*</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>mPMMA</td>
<td>&lt;0.001</td>
<td>*</td>
<td>0.162</td>
</tr>
<tr>
<td>Day 12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>*</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>mPMMA</td>
<td>&lt;0.001</td>
<td>*</td>
<td>0.652</td>
</tr>
</tbody>
</table>

*Multiple comparisons were computed using the Tukey’s HSD procedure. Statistically significant differences are indicated in bold typeface.

Discussion

The results of the present study revealed that both the mPMMA and SBP groups had significantly lower amounts of adherent C. albicans on the resin surfaces compared to the control. A statistically significant difference was found between the mPMMA group and SBP group at day 2, where the mPMMA surface exhibited less Candida adhesion; however, as the incubation period increased to 4, 6, and 12 days, the difference, although present, was not statistically significant. This association could be explained by the understanding that the rate of Candida attachment at day 2 could have reached a point of saturation and was able to maintain a state of equilibrium on subsequent days. When comparing the initial rate of Candida attachment to the clean resin surfaces, the mPMMA group showed a significantly lower amount of Candida than the SBP group; however, as the incubation period was extended, the existing Candida colony on the resin surfaces showed less differential ability to prevent adhesion. Considering that in denture wearers daily cleansing of the denture surface is recommended as part of the home care regimen, modifying PMMA with methacrylic acid could pose an important advantage in reducing the initial attachment rate of adhesion.

Many methods can be used to determine the extent of fungal adhesion to biomaterials and comparison of the various methods is difficult, as they present different limitations. For the purpose of the present study, surface area of adherent fungal colonization was measured, because growth of Candida...
involves multicellular strands and colony-forming units, thus restricting the ability to assay single cell forms.18 Staining the fungal cells provided sufficient contrast, and adherent Candida was easy to visualize by microscopy because of their large size and high refractivity.19 To eliminate the subjective nature of this method, computer techniques using image analysis software were used. An optical method allowed accurate visual determination of distribution of attached cells and eliminated erroneous interpretation caused by porosity.

The results of this study are in agreement with our previous research where an in vitro system was designed to assess the adhesion of C. albicans to surface-charged PMMA surfaces.15 The results showed that as the ratio of incorporated methacrylic acid to PMMA increased, the surface area of adherent C. albicans decreased. Analysis of data revealed a significant decrease in Candida adhesion to the resin blocks (p < 0.05) when the methacrylic acid was present at 5% of the PMMA. The exact mechanism by which C. albicans initially attaches itself to the polymeric surfaces has not been determined; however, physicochemical forces, such as hydrophobic interactions and electrostatic forces, are shown to be significant.16 The result of this study could be explained by the contribution of electrostatic repulsion through the negative–negative charge interactions between C. albicans, which has a net negative surface charge, and negatively charged polymer.20

The physical strength of these PMMA resins modified with increasing ratios of methacrylic acid must be investigated in order to be accepted for daily clinical use. The ratio of 16% methacrylic acid to 84% methacrylate was used for the mPMMA group. The question remains whether the mPMMA resins could withstand the various challenges in compressive, tensile, and wear tests. Biological concerns regarding leaching of unpolymerized monomer of these modified polymers must be investigated, as leaching could possibly induce hypersensitivity of the oral mucosa. Studies have shown that the residual MMA content is affected by methods of polymerization and curing processes. Increasing the curing temperature of autopolymerized denture base resins from 30 to 60°C significantly decreased the residual MMA content.21 Curing in water was a critical determinant for reducing the amount of residual monomer as well as increasing surface microhardness compared to curing in air.22 In addition, storing the resin specimens in distilled water at 37°C for at least 1 day to reduce the residual monomer was shown to be an effective method.23 In the present study, we attempted to minimize the quantity of monomer elution by following the aforementioned conditions.

In another study conducted in our lab, the efficacy of SBPs in reducing extrinsic stains was evaluated in an in vitro study model.16 Results showed the SBP group exhibited the least discoloration compared to the control and the sealer groups (p < 0.05). Application of SBPs has been a highly effective method of surface coating in reducing staining of restorative resins, especially in groups with poor oral hygiene procedures. The feasibility of the duration of the coating still needs to be explored to determine whether multiple applications are required, and with what frequency, to maintain the optimal continuing effect.

The effects of saliva, pH, and the presence of a multitude of microorganisms that coexist within the oral environment and their possible association on Candida adhesion have been investigated. Research has shown that whole saliva contains factors for detachment of Candida cells to material surfaces.24,25 Whole saliva and secretory immunoglobulin A (sIgA) showed an inhibitory effect on the adhesion of C. albicans to resin restorative material.26 Candida biofilms on oral surfaces and prosthetic devices may also contribute to increased resistance to antifungal agents and protection from the host defense mechanisms.27–30 Including these potential contributing factors may give new insights into the adhesion of C. albicans to surface-modified denture resins.

The results also show that patients with poor oral hygiene could benefit greatly by using these surface-modified resins to reduce the occurrence of denture stomatitis. This was an accelerated study to investigate methods of surface modification to reduce the adhesion of C. albicans on denture resin surfaces. The long-term effect of both of these techniques must be evaluated as to whether this positive effect could carry on semi-permanently throughout the life of the denture prior to clinical application.

**Conclusion**

The amount of C. albicans adhered to the resin surfaces reduced significantly with modification of surface charge and application of SBP. Modification of surface characteristics of polymeric biomaterials is an effective method in reducing adhesion of C. albicans to PMMA surfaces.

**Acknowledgments**

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**References**