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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 ($p \leq 0.036$). 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

surface charge of the denture resin is modified by the interacting polymerization of methacrylic acid to PMMA to prevent or reduce adhesion of the microorganism to the denture surface. This material has a negative charge incorporated by copolymerization of methacrylic acid to methyl methacrylate to create a modified PMMA (mPMMA).

Another suggested solution to prevent the microbial adhesion to restorative materials is the application of a protective coating made of a pure poly(dimethyl siloxane). This self-bonding polymer (SBP) provides a mono-molecular layer of an inert and acid-resistant finish to discourage microbial attachment and growth. The result is a chemically stable, nonstick surface, which will last as long as the substrate to which it is bonded. It is a thin coating, which changes the surface chemistry of the surface to which it is applied, but provides no mechanical protection.[17]

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 mm^2 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

Chemical	Action	Ratio
PMMA:MMA	Polymer:monomer	3:1 by weight
mPMMA (16% methacrylic acid)	Polymer:monomer	3:1.5 by weight
Benzoyl peroxide	Initiator	1% weight of PMMA
2-Hydroxyethyl methacrylate	Cross-linker	0.5% volume of MMA
N,N dimethylaniline	Activator	0.5% volume of MMA

Table 2 *Candida* density scores (% surface area) by surface type

Day*	Control	mPMMA	SBP	p-value**
2	8.80 ± 5.72	1.93 ± 2.05	5.39 ± 4.32	<0.001
4	7.97 ± 7.97	3.01 ± 8.65	6.40 ± 7.35	<0.001
6	17.44 ± 11.67	2.20 ± 4.40	6.82 ± 6.27	<0.001
12	23.32 ± 17.97	2.65 ± 3.97	5.71 ± 4.70	<0.001

*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 ($p \leq 0.036$); however, the mPMMA group and SBP group were not different from each other for the remaining time points (days 4, 6, 12; $p \geq 0.162$). 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*

	Control	mPMMA	SBP
Day 2			
Control	*	**<0.001**	**0.039**
mPMMA	**<0.001**	*	**0.036**
Day 4			
Control	*	0.132	0.810
mPMMA	0.132	*	0.380
Day 6			
Control	*	**<0.001**	**<0.001**
mPMMA	**<0.001**	*	0.162
Day 12			
Control	*	**<0.001**	**<0.001**
mPMMA	**<0.001**	*	0.652

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

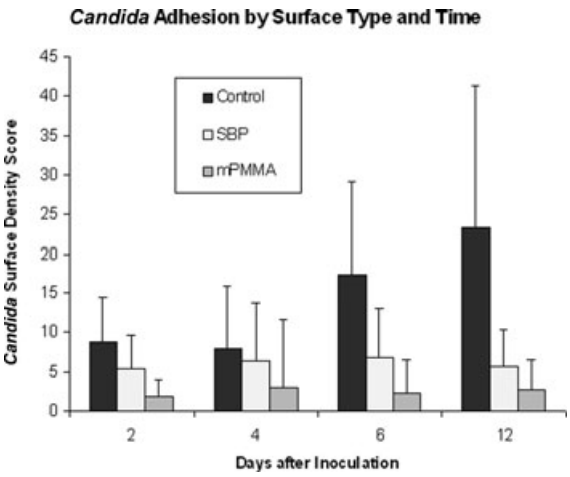

Figure 1 Surface areas of adherent *C. albicans* on different surfaces (control, mPMMA, and SBP) upon incubation period of 2, 4, 6, and 12 days.

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* adhesion 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. [8]

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 adherence 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

1. Regezi JA, Sciubba J: White Lesions, in: Oral Pathology: Clinical-Pathologic Correlations (ed 2). Philadelphia, PA, Saunders, 1993, pp. 93-135
2. McMullan-Vogel CG, Jude HD, Ollert MW, et al: Serotype distribution and secretory acid proteinase activity of *Candida albicans* isolated from the oral mucosa of patients with denture stomatitis. Oral Microbiol Immunol 1999;13:183-189
3. Budtz-Jorgensen E: The significance of *Candida albicans* in denture stomatitis. Scand J Dent Res 1974;82:151-190
4. Parvinen T: Stimulated salivary flow rate, pH and lactobacillus and yeast concentrations in persons with different types of dentition. Scand J Dent Res 1984;92:412-418
5. Slots J, Taubman MA: Oral Microbiology: Infections of Oral Mucosa and Submucosa, in: Contemporary Oral Microbiology and Immunology (ed 5). St. Louis, MO, Mosby, 1992, pp. 476-499
6. Samaranayake LP, Holmstrup P: Oral candidiasis and human immunodeficiency virus infection. J Oral Pathol Med 1989;18:554-564
7. Budtz-Jorgensen E: Oral mucosal lesions associated with the wearing of removable dentures. J Oral Pathol 1981;10:65-80
8. Samaranayake LP, McCourtie J, McFarlane TW: Factors affecting the *in vitro* adherence of *Candida albicans* to acrylic surfaces. Arch Oral Biol 1980;25:611-615

9. Vasilas A, Molina L, Hoffman M, et al: The influence of morphological variation on *Candida albicans* adhesion to denture acrylic in vitro. Arch Oral Biol 1992;37:613-622
10. Budtz-Jorgensen E: Oral mucosal lesions associated with the wearing of removable dentures. J Oral Pathol 1981;10:65-80
11. Budtz-Jorgensen E, Stenderup A, Grabowski M: An epidemiologic study of yeasts in elderly denture wearers. Community Dent Oral Epidemiol 1975;3:115-119
12. Fukazawa Y, Kagaya K: Molecular bases of adhesion of *Candida albicans*. J Med Vet Mycol 1997;35:87-99
13. Gibbons RJ, Houte JV: Bacterial adherence in oral microbial ecology. Annu Rev Microbiol 1975;29:19-44
14. Shibl AM: Effect of antibiotics on adherence of microorganisms to epithelial cell surfaces. Rev Infect Dis 1985;7:51-65
15. Ehara A, Torii M, Imazato S, et al: Antibacterial activities and release kinetics of a newly developed recoverable controlled agent-release system. J Dent Res 2000;79:824-828
16. Park SE, Raj PA, Loza J: Effect of surface-charged poly (methyl methacrylate) on the adhesion of *Candida albicans*. J Prosthodont 2003;12:249-254
17. Park SE, Weber HP, Ishikawa-Nagai S: Self-bonding polymers as surface-coatings of restorative resins to prevent staining. J Clin Dent 2006;17:134-137
18. Christensen GD, Baldassarri L, Simpson WA: Methods for studying microbial colonization of plastics. Methods in Enzymol 1995;253:477-499
19. Hazen KC, Glee PM: Adhesion of fungi. Methods in Enzymol 1995;253:414-424
20. Klotz SA, Drutz DJ, Zajic JE: Factors governing adherence of *Candida* species to plastic surfaces. Infect Immun 1985;50:97-101
21. Vallittu PK, Ruyter IE, Buykuilmaz S: Effect of polymerization temperature and time on the residual monomer content of denture base polymers. Eur J Oral Sci 1998;106:588-593
22. Lee SY, Lai YL, Hsu TS: Influence of polymerization conditions on monomer elution and microhardness of autopolymerized polymethyl methacrylate resin. Eur J Oral Sci 2002;110:179-183
23. Bayraktar G, Guvener B, Bural C, et al: Influence of polymerization method, curing process, and length of time of storage in water on the residual methyl methacrylate content in dental acrylic resins. J Biomed Mater Res B Appl Biomater 2006;76:340-345
24. Kamagata-Kiyoura Y, Abe S, Yamaguchi H, et al: Detachment activity of human saliva in vitro for *Candida albicans* cells attached to a plastic plate. J Infect Chemother 2003;9:215-220
25. Maza JL, Elguezabal N, Prado C, et al: *Candida albicans* adherence to resin-composite restorative dental material: influence of whole human saliva. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;94:589-592
26. Elguezabal N, Maza JL, Ponton J: Inhibition of adherence of *Candida albicans* and *Candida dubliniensis* to a resin composite restorative dental material by salivary secretory IgA and monoclonal antibodies. Oral Dis 2004;10:81-86
27. Ramage G, Vandewalle K, Wickes BL, et al: Characteristics of biofilm formation by *Candida albicans*. Rev Iberoam Micol 2001;18:163-170
28. Hawser SP, Douglas LJ: Resistance of *Candida albicans* biofilms to antifungal agents in vitro. Antimicrob Agents Chemother 1995;39:2128-2131
29. Gilbert P, Das JR, Jones MV, et al: Assessment of resistance towards biocides following the attachment of micro-organisms to, and growth on, surfaces. J Appl Microbiol 2001;91:248-254
30. Gilbert P, Das J, Foley I: Biofilm susceptibility to antimicrobials. Adv Dent Res 1997;11:160-167