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Original Article

Improvement of the Surface of Denture Base Resins with Straight Silicone

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Purpose: Denture plaque tends to form on the surface of the resins that are used as denture base materials, and *Candida* spp., including *Candida albicans*, are said to be causative organisms of denture stomatitis. Thus, modifying the surface properties of the resin to make it more difficult for denture plaque to adhere is very useful in terms of oral hygiene. In order to evaluate the usefulness of surface treatment with straight silicone, in this study we used heat-polymerized acrylic resin (polymethylmethacrylate [PMMA]) as the test material, and performed *C. albicans* adhesion test, a protein adsorption test and contact angle measurements with distilled water. Results: Significantly fewer *C. albicans* adhered and significantly less protein adsorbed in the coated group than in the control group or the buff-polished group (P<0.01). The angle of contact with distilled water in the coated group was significantly larger than in the control group or buff-polished group (P<0.01). Conclusion: Coating with straight silicone easily succeeded in improving the surface properties of PMMA used as the denture base material so that it became difficult for *C. albicans* to adhere, suggesting that an important property can be acquired clinically.

Key words: *Candida albicans*, denture plaque, polymethylmethacrylate, straight silicone; oral hygiene

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Introduction

Denture plaque, which consists of aggregates of microorganisms and their metabolites, tends to form on the surface of the resins that are used as denture base materials,[1] and *Candida* spp., including *Candida albicans*, are said to be causative organisms of denture stomatitis.[2] Microorganisms tend to contaminate the mucosal surface of dentures in particular,[3] because its complex topography makes it difficult to polish or clean, and the hard palate portion of the upper jaw, which is largely covered by dentures, is a common site of denture stomatitis.[4] In addition, the possibility of dental plaque being involved in aspiration pneumonia and other systemic diseases in denture wearers with low immunologic resistance has also been pointed out.[5,6,7,8,9,10,11,12,13] Thus, improving the surface of dentures so that they have properties that would make contamination by microorganisms difficult would be a very effective means of maintaining favorable oral hygiene, and it would also appear to be of help in health support and management.

In recent years silicone has been used in a variety of fields, including vehicles, ships, and the outside walls of buildings, mobile phones, food containers, etc., as a means of treating surfaces to prevent contamination of the surface of base materials and to increase durability. The silicone used in the surface treatment techniques is a material that possesses numerous excellent characteristics, including heat resistance, weather resistance, water repellence, water resistance and chemical stability. Thus, coating the surface of denture base materials with silicone can be expected not only to save labor in terms of polishing, but to improve their surface properties so that contamination by microorganisms is more difficult.

In this study we used heat-polymerized acrylic resin

(polymethylmethacrylate [PMMA]), which is widely used as a denture base resin, as the test material, and assessed its efficacy in preventing adherence by *C. albicans* in order to evaluate the usefulness of surface treatment with straight silicone.

Materials and methods

1. Preparation of the test specimens

Straight silicone(SFII, ECO24) was used as the surface treatment material, and the heat-polymerized acrylic resin(ACRON shade No. 3, GC)(powder: Lot 0604201, liquid: Lot 0611131) was used as the base resin. The test specimens were prepared by shaping the heat-polymerized acrylic resin into disks 20 mm in diameter and 1 mm thick, and after polymerizing them in a stainless metal mold according to the manufacturer's instructions, the surface was polished with abrasive paper #240 (Three Bird Brand abrasive paper). The test specimens that had been prepared were immersed in 20% straight silicone solution for 5 minutes or 30 minutes, and specimens that were thoroughly dried at room temperature were used as the coated group. Untreated specimens were used as the control group. In addition, after polishing with abrasive paper #240, then with water-resistant abrasive paper #600, and, finally, with water-resistant abrasive paper #1000 (Sankyo Rikagaku), specimens buff-polished with glossy polish for resin (Terukijin, Eikan) were used as the buff-polished group. In order to standardize the amount of water absorption in each group, all test specimens were immersed in distilled water for 72 hours and then ultrasonically washed for 5 minutes before submitting them to the tests. Five specimens were used in each group.

2. *C. albicans* adherence test

1) Preparation of the *C. albicans*-adherent specimens

Artificial candidal biofilms were formed on specimens according to Chandra J. et al. [13]with some modifications.

C. albicans (JCM1542, Physico-chemistry Institute) were adjusted to a concentration of 1.0×10^7 cells/ml with phosphate-buffered saline (PBS, pH7.2) and used as the microorganism solution that was submitted to the tests. Each specimen was allowed to stand in the bottom of the wells of a 12-well cell culture plate, and after adding 1 ml of 1.0% bovine serum albumin (BSA, Sigma Aldrich Japan), they were allowed to stand in the dark for 30 minutes at 37°C The BSA was then discarded, and after seeding a 200 μl volume of microorganism solution on the surface of each specimen and adding 2 ml of yeast nitrogen base liquid culture medium to which glucose 50 mM had been added (YNB medium, Difco Laboratories), the specimens were incubated for 48 hours at 37°C[5,14]. At the end of the incubation period, the specimens were gently washed with PBS in order to remove microorganisms that had not adhered, and they were used as the *C. albicans*-adherent specimens.

2) Determination of the number of adherent microorganisms

After dispensing 2 ml of PBS to the *C. albicans* adherent specimens that had been transferred to a 12-well culture plate, they were ultrasonically washed for 5 minutes, and the *C. albicans* that had adhered to the surface of the specimens were collected. Cell Counting Kit-8 (Dojindo), which uses a 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium (WST-8) enzymatic reducing reaction, was used to count the viable *C. albicans* that had been collected, and incubation was performed for 8 hours at 37°C. To 50 ml portions of the microorganism fluid dispensed into the wells of a 96-well microplate, 10 ml of the Cell Counting Kit-8 was added, and after incubating for 8 h at 37°C, absorbance at a wavelength of 450 nm was measured with microplate reader (MTP300, Corona Electric). The number of viable *C. albicans* was calculated from the degree of absorbance obtained.

3. Protein adsorption test

1) The specimens were allowed to stand in the bottom of a 12-well cell culture plate, and after dispensing 1 ml of 1.4% BSA, they were allowed to stand in the dark at 37°C for 30 min. After soaking in BSA, they were dipped in PBS, and the specimens from which extra BSA was removed were used as the protein adsorption specimens.

2) Measurement of adsorbed protein

The protein-adsorbed specimens were transferred to a 12-well culture plate, and after adding 1 ml of 0.5% sodium dodecyl sulfate and ultrasonically washing for 15 min, the BSA that had adhered to the surface of the specimen was recovered. A Protein Quantification Kit-Wide Range (Dojindo), which utilizes tetrazolium salt reduction reactions, was used to quantitatively measure the protein. Absorbance was measured according to the manufacturer's instructions, and the amount of adsorbed protein was calculated from the absorbance values obtained.

4. Contact angle measurements

The contact angle, θ, for distilled water was measured with a dynamic contact angle measurement kit (FTA125, First 10 Angstrom).

5. Statistical analysis

The statistical analysis was performed by one-way analysis of variance with SPSS 10.0J for Windows Base System (SPSS) statistical software, and the Tukey-Kramer multiple comparison test was performed with a significance criterion of 5% and 1%.

Results

The results of all of the tests are shown in Fig 1, Fig 2 ,Fig 3. The amount of adherent *C. albicans* was significantly lower in the buff-polished group than in the control group (P<0.01).The number of adherent *C. albicans* in the coated group was significantly lower than in the control group or the buff-polished group (P<0.01). It was less than 1/15 of the number in the control group, and less than 1/5 of the number in the buff-polished group. There were no statistically significant differences in terms of the number of adherent microorgan-

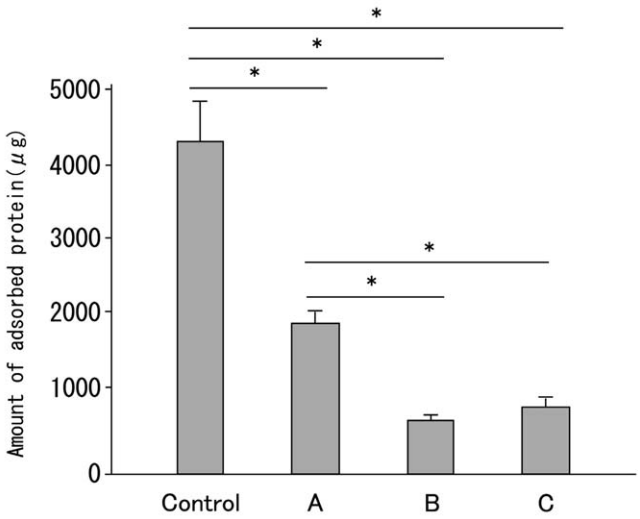

Fig. 2. Amount of adsorbed protein on samples
Data are presented as mean+SD. (N=5 for each test group)
Significantly less protein adsorbed in the coated group (B,C) than in the control group or the buff-polished group (A) (P<0.01).
The amount of adsorbed protein was significantly lower in the buff-polished group (A) than in the control group (P<0.01).
(*; P<0.01)
A: buff-polished group, B: straight silicone coating group (5minutes), C: straight silicone coating group (30minutes)

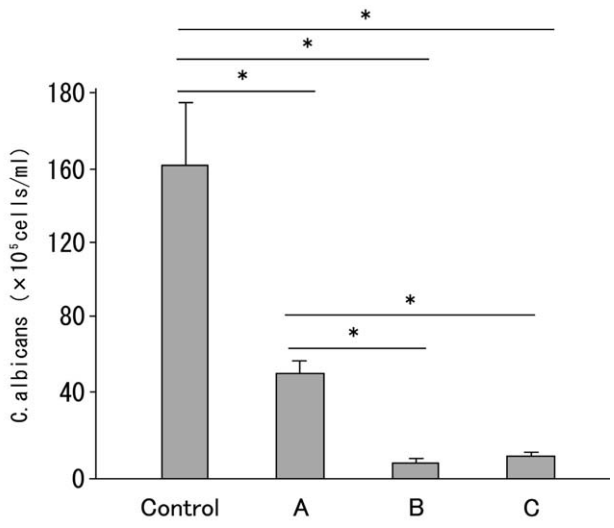

Fig. 1. *Candida albicans* adhesion
Data are presented as mean+SD. (N=5 for each test group)
Significantly fewer *C. albicans* adhered in the coated group (B,C) than in the control group or the buff-polished group (A) (P<0.01).
The amount of adherent *C. albicans* was significantly lower in the buff-polished group (A) than in the control group (P<0.01).
(*; P<0.01)
A: buff-polished group, B: straight silicone coating group (5minutes), C: straight silicone coating group (30minutes)

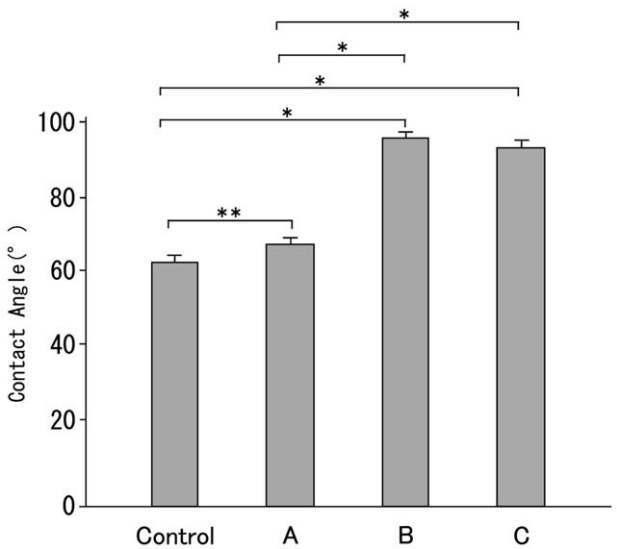

Fig. 3. Surface contact angle analysis
Data are presented as mean+SD. (N=5 for each test group)
The angle of contact with distilled water in the coated group (B,C) was significantly larger than in the control group or buff-polished group (A) (P<0.01). The angle of contact with distilled water in the buff-polished group (A) was significantly larger than in the control group (P<0.05). (*; P<0.01,**; P<0.05)
A: buff-polished group, B: straight silicone coating group (5minutes), C: straight silicone coating group (30minutes)

ism between the two coated groups.

The amount of adsorbed protein was significantly lower in the buff-polished group than in the control group ($P<0.01$).The amount of adsorbed protein in the coated group was significantly lower than in the control group or the buff-polished group ($P<0.01$). It was less than 1/6 of the amount in the control group, and less than 1/2 of the amount in the buff-polished group. There were no statistically significant differences between the two coated groups.

The contact angle for water was greater than 90° in the coated group, and was significantly larger than the less than 70° in the control group and the buff-polished group. The angle of contact with distilled water in the buff-polished group was significantly larger than in the control group ($P<0.05$). No statistically significant differences were found between the two coated groups.

Discussion

Siloxane bonds (Si-O-Si bonding) form the basic skeleton of silicone, and because of the extremely high bond energy between the Si and O, they possess such properties as chemical stability, physiological inertness, weather resistance, etc. Moreover, because of the location of methyl groups on the surface of the molecular chain, there is little molecular attraction or surface energy, and it exhibits water repellency. Silicones possess these superior properties and are classified into straight silicones, which have a basic structure in which methyl groups are arrayed on side chains, and modified silicones, in which other organic chains have been introduced on some of the side chains or at the ends of the methyl groups. Comparisons between straight silicone and modified silicone when they are used for the surface treatment have shown that straight silicone possesses such characteristics as greater hardness, greater acid resistance, and greater resistance to contamination than modified silicone. The straight silicone used in experiments is a colorless, clear 1 liquid solvent with high lubricity, and after surface treatment PMMA exhibited high brilliance, but no change in hue. It also has very little in vivo toxicity, and it may also be possible to use it for surface treatment of eating utensils and intraoral applications.

In this study we treated the surface of PMMA, a denture base material, with straight silicone and investigated its preventive effect against adherence of *C. albicans*, which are often isolated from denture plaque. The adherence of microorganisms to the solid surface is said to be due to a hydrophobic interaction and a static electrical interaction as a result of the physical properties of the surface of the material and the surface of the microorganisms.[15] It has also been pointed out that because the denture surface is covered with saliva immediately after it is inserted in the mouth, the protein adsorbed to the surface of the material is important for adhesion by microorganisms.[4,5,16,17] We therefore performed bacterial adherence tests after treating the surface of the material with protein (BSA) and measured the amount of protein that had been adsorbed. The results of the experiments showed a tendency for fewer *C. albicans* to adhere in the coated group, in which little protein adsorption had occurred, and that finding was consistent with a previous report that pointed out the importance of protein adsorption.[15] The reason for the low level of protein adsorption in the coated group appears to be the weak adsorption of water-soluble BSA to the silicone surface because of its hydrophobicity. Thus, because it was easy to remove the adsorbed protein by washing in the coated group, few microorganisms were adherent after washing with PBS, because the microorganisms whose adherence was mediated by protein adsorption were also easy to remove. *C. albicans* are also said to readily adhere to coarse surfaces,[18,19] and since the amount of adherent microorganisms was significantly lower in the buff-polished group than in the control group, it is assumed that the surface was less rough after coating than after buff-polishing, and that a smooth surface had been achieved.

Since PMMA is a water-absorbing material, its contact angle changes with the level of atmospheric humidity, and thus the contact angles were measured after allowing it to absorb distilled water. The contact angle in the coated group was significantly larger than in the control group or the buff-polished group, and thus the water-repellent property of silicone was sufficiently expressed, which made it possible to confirm that a coating layer had formed on the surface of the PMMA.

The longer the soaking time in straight silicone, the thicker the coating layer should be, but no significant differences were found between the two coated groups in any of the experiments in this study. This finding shows that silicone, which has little intermolecular attraction, easily spreads over the base material, and since it readily impregnates small defects in the surface of PMMA, it can be expected to sufficiently improve its surface properties even during a brief soaking period. Assessing the impact of surface treatment time on the durability of the coating layer will be a future task, but

since it is easy to apply additional coats to just denuded sites or sites where the mucosal surface of the denture base has been adjusted, there appear to be very few disadvantages from the standpoint of clinical use. The fact that silicone, which has high bond energy, in addition to being able to improve surfaces in a short time, never alters the quality of other organic resins that include PMMA, appears to be an important advantage from the standpoint of clinical use.

Conclusion

Coating with straight silicone is readily capable of improving the surface properties of the denture base material heat-polymerized acrylic resin so that it becomes difficult for *C. albicans* to adhere, suggesting that it will be very useful from the standpoint of oral hygiene.

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