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Effects of trimethylsilane (TMS) plasma coating on the hydrophobicity of denture base resin and adhesion of *C. albicans* on resin surfaces

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Abstract

Statement of problem—Candida-associated denture stomatitis (CADS) is the most common oral mucosal lesion among denture wearers. TMS plasma coating may inhibit the growth of *Candida albicans* (*C. albicans*) on denture surfaces.

Purpose—The purpose of this in vitro study was to investigate whether trimethylsilane (TMS) plasma coatings can effectively reduce *C. albicans* adhesion on denture base acrylic resin surfaces.

Material and methods—Sixty denture base acrylic resin disks (Lucitone 199) with smooth and rough surface were prepared and were either left untreated (control group) or coated with TMS monomer (experimental group) by using plasma. Contact angles were measured immediately after

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TMS plasma coating. The morphology of *C. albicans* adhesion was observed with scanning electron microscopy (SEM). Energy-dispersive spectroscopy (EDS) was used to characterize the elemental composition of the specimen surface. An adhesion test was performed by incubating the resin disk specimens in *C. albicans* suspensions (1×10^7 cell/mL) at 37°C for 24 hours and further measuring the optical density (OD) of the *C. albicans* using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay kit. One-way ANOVA and 2-way ANOVA were followed by a post hoc test analysis ($\alpha=.05$).

Results—The group with TMS coating exhibited a more hydrophobic surface than the control group. EDS analysis revealed successful TMS plasma coating. The difference in the mean contact angles between the uncoated group and the TMS coated group was statistically significant ($P<.05$), 79.0 ± 2.9 degrees versus 105.7 ± 1.5 degrees for the smooth surface and 90.2 ± 7.6 degrees versus 131.5 ± 2.1 degrees for the rough surface. In SEM analysis, the *C. albicans* biofilm was found to grow more on the surface of the denture base resin without the TMS coating than on the surfaces of the experimental group. In the adhesion test, the amount of *C. albicans* adhering to the surface of denture base resin with the TMS coating was significantly less than that on the surfaces without TMS coating ($P<.05$).

Conclusions—TMS coating significantly reduced the adhesion of *C. albicans* to the denture base resin and might reduce denture stomatitis.

INTRODUCTION

Candida-associated denture stomatitis (CADS),¹ characterized as the inflammation and erythema of the oral mucosal areas covered by a denture, is a common recurring disease that affects approximately 50% to 60% of denture wearers.² In a healthy human host, *C. albicans* remains relatively stable as a part of the normal microbial flora that colonizes the mucocutaneous surfaces of the oral cavity. However, *C. albicans* transforms into an opportunistic pathogen when immune defense is compromised or the normal microflora balance is disrupted. For instance, wearing a dental prosthesis provides a solid base favorable to yeast adhesion and colonization and results in an inflammatory process in the oral soft tissues, especially in the subjacent area covered by a complete or partial denture.^{1,3–5} Without treatment, CADS might progress into a more severe infection that can be difficult to manage.²

Clinically, strategies^{2,6–8} that target biofilm formation on the prosthesis and treatment of a fungal infection have been developed to treat CADS. Strategies include antifungal therapy, disinfectants and cleansers, oral hygiene instructions, placement of resilient liner/tissue conditioner, laser treatment, microwave disinfection, and a combination of 2 or more of these. Topical antifungal agents (miconazole 2% and nystatin) or systemic antifungal agents such as fluconazole (FLZ) and ketoconazole⁹ have become common treatments, depending on the condition's severity. Although successful in clinical use, antifungal agent therapy has disadvantages, including side-effects, antimicrobial resistance,¹⁰ and high cost to the patient. Therefore, an antifungal denture with long-term efficiency has been sought in the last 2 decades.

Antifungal agents (nystatin, amphotericin B, and chlorhexidine) have been incorporated into denture base acrylic resin.¹¹ The reduction in biofilm formation on the resin containing chlorhexidine was up to 98%, followed by nystatin with a range of 70% to 80% and amphotericin B with a range of 50% to 60%.¹¹ Instead of incorporating chemical antifungal agents, other authors^{12,13} have introduced poly (methacrylic acid) into polymethyl methacrylate (PMMA) to generate a negative charge on the denture resin surface. Their results show that the surface area of adherent *C. albicans* biofilm decreased with the increasing ratio of incorporated poly (methacrylic acid) to PMMA.^{12,13} Muttagi and Subramanya¹⁴ stated that the addition of *C. anthelminticum* and *O. sanctum* oils into soft liners produced complete inhibition at 72 hours in their diameter of inhibition zone (DIZ) experiment. Studies have also reported that when a phosphate-containing monomer was synthesized and combined into PMMA, a significant decline in *C. albicans* colonization occurred because of the enhanced adsorption of histatin 5 (a type of antimicrobial peptide in saliva) in a phosphate density-dependent manner.^{15,16} In addition, self-bonding polymer coatings¹² and hydrophilic coatings¹⁷ have been used to reduce *C. albicans* adhesion to an antifungal denture.

Although these methods are effective in reducing *C. albicans* adhesion to denture resin surfaces, concerns remain regarding the biocompatibility and physical properties of these modified polymers and long-term durability. Plasma-based coatings^{18,19} have been considered a versatile method of modifying the surface of polymeric materials in many fields. A recent study has demonstrated the possibility of applying trimethylsilane (TMS) plasma coatings to 316 L stainless steel and grade 5 titanium alloy.¹⁸ The purpose of the present research was to assess the effects of TMS coating on *C. albicans* adhesion to PMMA denture resin surfaces. The hypothesis tested was that the TMS plasma coating on PMMA denture surfaces would not affect the adhesion of *C. albicans*.

MATERIAL AND METHODS

A denture base resin (Lucitone 199; Dentsply Intl Inc) was prepared according to the manufacturer's instructions. The *C. albicans* strain of the American Tissue Culture Collection (ATCC) 18804 was cultured with yeast broth (Sabouraud Liquid Broth, Becton; Dickinson and Co). The viability of *C. albicans* was tested with a (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay kit (MTT assay kit; Thermo Fisher Scientific).

Trimethylsilane (TMS) plasma coating was carried out as described in a previous publication.¹⁸ The morphology of coated and noncoated denture surfaces and the elemental composition were investigated with a scanning electron microscope (SEM) (Quanta 600 FEI; Thermo Fisher Scientific). The hydrophobicity of the denture resin surface was studied with a computer-aided video contact angle system (VCA-2500XE; AST Products Inc).

Sixty disk-shaped specimens (10×2 mm) were fabricated from Lucitone 199 acrylic resin according to the conventional technique—flasking and pressure-pack. In order to simulate the polished and intaglio surfaces of the dentures, one half of these specimens (30 disks) were finished to a smooth surface and the other half was prepared as a rough surface. Using

a web-based computer-generated random table (<https://www.randomizer.org>), 15 disks from the group with smooth surfaces and 15 disks from the group with rough surfaces were assigned to the experimental group that received the TMS coating. The rest of the specimens were assigned to control groups without the TMS coating (Smooth Control, Rough Control). The smooth surface was prepared with laboratory acrylic resin trimming burs and polished with a rag wheel and pumice (Green Dental Laboratories).

Specimens in the experimental groups (Smooth TMS, Rough TMS) were coated with TMS monomer by using plasma in an 80-liter bell jar-type reactor as described in a previous paper.¹⁸ Briefly, pure oxygen (Praxair Inc) was used as the working gas. The plasma reactor was sealed and evacuated to a base pressure (<2 millitorrs) using a mechanical pump and booster pump in turn. Pure oxygen was then introduced into the reactor at a flow rate of 1 standard cubic centimeter per minute (SCCM) using an MKS mass flow controller (MKS Instruments Inc) and an MKS247C readout (MKS Instruments Inc) to set the flow rate. The pressure inside the plasma reactor was set at 50 millitorrs and monitored using an MKS pressure controller (MKS Instruments Inc). The oxygen was then excited with the DC power supply at 20 W to generate a plasma arc to clean the specimen surface. The time taken to remove organic contaminants from the specimen surfaces was 2 minutes. The reactor was then evacuated to the base pressure again. TMS (Gelest Inc) was introduced into the reactor at 1 SCCM. The reactor pressure was allowed to reach 50 millitorrs, and the TMS was excited to form plasma by the DC power supply at 5 W. The TMS plasma coating on the denture specimen was kept for 15 seconds.

The changes in the resin surfaces after TMS coating and in the morphology of *C. albicans* incubated for 24 hours on the denture resin surfaces were assessed using SEM (Quanta 600, FEI; Thermo Fisher Scientific). Representative specimens from the 4 groups were placed on metallic stubs and gold sputter coated, keeping the treated surface face-up. The microscope was operated at an accelerating voltage of 15 kV, with a working distance of between 15 and 27 mm. Energy-dispersive spectroscopy (EDS) equipped with the SEM was used for identifying and quantifying the elemental composition of the specimen areas. EDS spectra were obtained under 20 kV accelerating voltage, 100 mA beam current, 100-second acquisition time, and 30% to 40% dead time. A quantitative analysis of the percentage weight concentration of the probed elements was performed with nonstandard analysis using atomic number, absorbance, and fluorescence (ZAF) correction methods.

To characterize the hydrophobicity of the specimen surfaces, the contact angle was measured immediately after surface preparation using the sessile drop method with a contact angle measurement apparatus. This device had a charge-coupled device (CCD) camera that recorded the drop image on the specimen surface and image-analysis software that determined the right and left contact angles of the drop after 5 seconds. One microliter deionized (DI) water was used during the contact angle measurement. The mean static contact angle values were obtained from multiple specimens (n=6).

The effect of TMS plasma coating on the adhesion of *C. albicans* on both smooth and rough simulated denture surfaces was studied with an MTT assay kit. *C. albicans* strain (ATCC 18804) was cultured overnight in yeast broth. Sterilized resin specimens were placed into

24-well sterile culture plates, and a 500 μL 1×10^7 cell/mL colony-forming unit (CFU) *C. albicans* suspension was added. After 24-hour incubation at 37°C, nonadherent cells were washed away with phosphate buffered saline (PBS) by washing under shaking twice, 5 minutes for each time. Immediately after washing, 400 μL of PBS and 50 μL MTT solution were added according to the manufacturer's instructions. The absorbance was measured in a microplate reader (Bio-Rad 680; Hercules) at a wavelength of 670 nm, where % viability = (optical density of the experimental specimens/optical density of the control group) $\times 100$. The specimens were analyzed in triplicate, and 3 independent experiments were performed.

The values were expressed as the mean $\pm$ standard deviation of the mean. One-way analysis of variance (ANOVA) and 2-way ANOVA were carried out with statistical software (IBM SPSS Statistics v20; IBM Corp). Statistically significant differences between groups were determined by post hoc test analysis ($\alpha = .05$).

RESULTS

Two typical EDS spectra in the energy range of 0 keV to 2.0 keV from the group with/without TMS coating are presented in Figure 1. The red solid line represents the groups coated with TMS and the black dashed line represents the groups without TMS coating. In comparison with the black dashed line, the red solid line has a clear peak at 1.75 keV, revealing that trimethylsilane was coated successfully on the denture base resin surface. Except for oxygen, carbon, and silicon peaks, no other peak appeared in the whole spectra range (0–20.0 keV, data after 2.0 keV not shown), indicating that the TMS-coated surface was clean.

The means and standard deviation of the contact angle obtained from the 4 groups and the results from 1-way ANOVA analysis are presented in Table 1. Compared with the 2 control groups, the contact angles of the 2 experimental groups coated with TMS were significantly higher (1-way ANOVA F-value 171.6, $P < .001$). The post hoc Tukey test indicated a statistically significant difference in the order of Smooth Control, Rough Control, Smooth TMS, and Rough TMS ($P < .001$).

The result of *C. albicans* adhesion from the 2-way ANOVA analysis is reported in Table 2. The absorbance intensity (OD at 670 nm) of Smooth TMS group was 0.21 ± 0.05 , while the value was 0.45 ± 0.05 for the Smooth Control group. The difference was statistically significant ($P < .05$). Similarly, the absorbance value of the Rough TMS group was 0.21 ± 0.08 , which was significantly less than that of the Rough Control group, 0.43 ± 0.12 ($P < .01$). On both smooth and rough TMS-coated surfaces, about 60% of *C. albicans* was inhibited from attaching to the denture base material according to the MTT assay. Two-way ANOVA analysis showed that TMS coating was a statistically significant factor for *C. albicans* adhesion ($P < .001$), while surface characteristics (smooth or rough), after controlling for all other factors ($P > .05$), was not. No significant interaction was found between TMS coating and surface characteristics ($P > .05$). The adjusted R squared value was 0.67, which indicated the model could explain 67% of variation in the response variable of *C. albicans* adhesion (Table 2).

The distribution of *C. albicans* and the morphology of the biofilm formed by *C. albicans* on various surfaces from the SEM images are presented in Figure 2. Mature *C. albicans* biofilms were found on the untreated smooth surface of denture resin disk, and both hyphal and yeasts were visualized (Fig. 2A). Like the smooth surface, large number of yeasts dominated the untreated rough denture resin surface, whereas hyphal forms of *C. albicans* were scarcely observed (Fig. 2C). The result confirmed, in some degree, the MTT results where the untreated smooth surface showed a higher OD absorption than the untreated rough surface. However, similar to the coated smooth surface, *C. albicans* was scattered on the TMS treated rough surface (Fig. 2D).

DISCUSSION

After TMS plasma coating of the PMMA denture surface, the microbial adhesion decreased by about 60% in the TMS plasma coated group compared with the noncoated group and only sporadic *C. albicans* were found on the TMS-treated surface. After TMS plasma coating, the contact angles on both smooth surface and rough surfaces increased dramatically, showing indirectly that TMS was successfully coated on the denture resin surface. This is consistent with the results from a previous study.²⁰ The silicon peak in the EDS spectrum confirmed this effect. The contact angle study indicated that TMS coating on the denture resin surface significantly increased the hydrophobicity of the denture surface, which lowered its wettability. Although the contact angle values of both the uncoated smooth surface and uncoated rough surface were slightly higher than the values reported in previous studies,^{18,21} the trend that a rough surface showed a higher contact angle was consistent with the previous study.²¹

The sporadic growth of *C. albicans* yeasts on the smooth denture resin surface coated with TMS revealed that TMS coating efficiently restricted the growth of *C. albicans* and biofilm formation. This result was confirmed by the microbial adhesion test that showed only small numbers of *C. albicans* can adhere and grow on a TMS-coated resin surface. According to adhesion test results, the inhibitory efficacy equals that obtained by using Amphotericin B¹¹ and silver nanoparticles²² but was slightly less than that obtained with Nystatin¹¹, indicating the potential use of TMS plasma coating in combatting denture stomatitis, especially as a nonmedication treatment.

The low wettability of the denture resin surface after TMS coating could partially explain the inhibitory effects in the *C. albicans* adhesion test. The process of bacterial adhesion is complicated and is influenced by many factors,²³ for example, the bacterial properties, the material surface characteristics, and the environmental factors, including the presence of serum proteins and the associated flow conditions. The results from EDS and contact angles suggest that the surface properties of both smooth and rough surfaces changed after TMS coating, and this was due to the changes in chemical composition and hydrophobicity induced by the TMS coating. The chemical groups of -C=O, C-O-C, and -CH₃ in poly methyl methacrylate (PMMA) were covered, at least partially, by groups of -Si-CH₃, -Si-H, and -Si-Si- in layers of TMS coating (EDS). Consequently, the hydrophobicity increased as revealed by the contact angle change from 79 degrees to 105 degrees for smooth surfaces and from 90 degrees to 131 degrees for rough surfaces. Therefore, the reduction of *C.*

albicans adhesion was probably due to the change of surface hydrophobicity promoted by TMS coating.

C. albicans were reported to be hydrophilic because the contact angle of *C. albicans* cells lawn was less than 20 degrees when measured with water.²⁴ The amount of *C. albicans* attached to a hydroxyapatite surface was significantly higher than that attached to an autopolymerized acrylic resin surface in the presence of water because of the different chemical compositions of 2 types of surfaces. Meanwhile, the contact angle of the hydroxyapatite surface was significantly lower than that of the autopolymerized acrylic resin surface (53 ± 4 degrees versus 75 ± 3 degrees). The results from our study confirmed that hydrophobicity of substrate was a nonnegligible factor influencing *C. albicans* colonization and biofilm formation.

Roughness has been thought to affect microbial adhesion, and some studies^{25,26} have found that an increase in surface roughness facilitated yeast retention. Thus, in this study, the specimens were processed to have either rough or smooth surfaces. The metabolic values (OD absorption) from smooth surfaces were higher than those from rough surfaces, especially for the control group (without TMS treatment), although such a difference is not statistically significant.

Several limitations in the study must be acknowledged. Only 1 strain of *C. albicans* and 1 heat/pressure-polymerized denture base acrylic resin—Lucitone 199 were studied. The effects of TMS coating on other types of denture resin are not known and need to be evaluated in future. Also, only immediate effects after TMS coating were evaluated in the present study, and long-term effects are not understood. During the TMS plasma coating, TMS monomer is induced by plasma and polymerized to form a thin layer on the denture resin surface. The stability of TMS coating is an important parameter for future clinical application because it influences the lifespan of an antimicrobial denture in the oral cavity. Therefore, studies on the stability of the TMS denture coating are needed.

The results of TMS plasma coating in previous studies^{18,27} showed good antimicrobial activity against a number of common pathogens. As a microbicide, the TMS plasma coating is an interesting candidate for clinical application because of its effectiveness and minimal toxicity. Particularly, the gas temperature in nonthermal plasma can remain as low as room temperature so that the integrity of polymer-based materials can be preserved. This is critical for denture base resins because the heating may cause dimensional changes and affect the fit of denture bases to the supporting tissues.

Overall, compared with other methods, TMS plasma coating has apparent advantages and is a promising candidate in the management of Candida-associated denture stomatitis.

CONCLUSIONS

Based on the findings of this in vitro study, the following conclusions were drawn:

1. The hydrophobicity (high water contact angle) of the denture acrylic resin surface was increased by the TMS plasma coating.

2. The adhesion of *C. albicans* was significantly reduced by TMS plasma coating on both smooth and rough surfaces.

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CLINICAL IMPLICATIONS

Trimethylsilane (TMS) plasma coatings may modify the hydrophobicity of acrylate-based denture resin, thereby decreasing *C. albicans* adhesion and the occurrence of *Candida*-associated denture stomatitis.

Figure 1. EDS spectra of specimens with/without TMS coating. Silicon peak appeared at around 1.75 keV on spectra from TMS-coated specimens.

Figure 2 A

Figure 2 B

Figure 2 C

Figure 2 D

Figure 2. Scanning electron micrographs of *C. albicans* adhesion. A, Uncoated smooth surface. B, TMS-coated smooth surface. C, Uncoated rough surface. D, TMS coated -rough surface. TMS: trimethylsilane.

Table 1

One-way ANOVA comparison of contact angles.

Group	N	Mean $\pm$ SD (degree)	Range (degree)	Tukey Grouping
Smooth Control	6	79.0 $\pm$ 2.8	76.0–83.0	A
Smooth TMS	6	105.7 $\pm$ 1.5	104.0–108.0	B
Rough Control	6	90.2 $\pm$ 7.5	82.0–101.0	C
Rough TMS	6	131.5 $\pm$ 2.1	129.0–135.0	D

Note: ANOVA Test, $F = 171.6$, $P < .001$. Different letter means that the variables are significantly different from one another.

Table 2

Two-way ANOVA of *C. albicans* adhesion to denture surfaces

Variables	Sum of Squares	Degree of freedom	Mean Square	F value	P
Surface characteristics: smooth or rough	0.002	1	0.002	0.24	.62
Coating: no TMS or TMS	0.63	1	0.63	99.7	<.001
Interaction: surface characteristics × coating	0.001	1	0.001	0.23	.63
Adjusted R squared	0.64	3	0.21	33.4	<.001

Note: Adjusted R squared is 0.67. R squared is a statistical measure defined as the percentage of the response variable variation that is explained by a regression model.