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Original Article

Antifungal Effects of a Tissue Conditioner Coating Agent with TiO_2 Photocatalyst

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Tissue conditioners are susceptible to colonization by microorganisms. Therefore, the prevention of biofilm formation are important for oral hygiene. However, mechanical and chemical cleaning methods may cause clinical problems such as deformation or surface degradation of tissue conditioners. The objective of this study is to evaluate the antifungal effects of coating agents with a TiO_2 photocatalyst. Photocatalytic antifungal effects on *C. albicans* biofilms and photodegradation effects of adsorbed protein were measured by colorimetric assays. Scanning electron microscopy was used to examine morphological changes in *C. albicans*. Viscosities of coating agents increased with incorporation of TiO_2. However, both of coating agents with TiO_2 were acceptable to the application by brush. The antifungal and protein degradation effects increased with the concentration of TiO_2 in the coating agents. These effects also increased with radiation time. After 90 min radiation, the viability of *C.albicans* was reduced to 16.2±3.3 %. Scanning electron microscopy observation showed *C. albicans* remained on the coated surfaces even after 90 min radiation. These results suggest that coating agents with TiO_2 photocatalyst can be effective for the maintenance of tissue conditioners when dentures are removed; during sleep.

Key words: tissue conditioner, coating agent, TiO_2 photocatalyst, antifungal effect, *Candida* biofilm

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Introduction

Tissue conditioners that improve the health of abused denture-bearing tissues are more susceptible to colonization by microorganisms than denture base resins[1]. Most microorganisms colonizing on the surface are found as complex-structured microbial communities (biofilms) and may irritate the underlying tissue[2-4]. Therefore, maintenance of tissue conditioners and the prevention of biofilm formation are important for oral hygiene. However, mechanical and chemical cleaning methods may cause clinical problems such as deformation or surface degradation of tissue conditioners[5,6].

Several studies have attempted to incorporate antibacterial agents into tissue conditioners for plaque control and treatment of denture stomatitis. However, the elution behavior of antibacterial agents has not been completely clarified and the salivary pellicles may inhibit the antibacterial agents from contacting with microbes[7-10].

A TiO_2 photocatalyst, which produces free radicals on its surfaces by adequate light activation, has come to utilization such as self-cleaning[11], antibacterial[12], and waste water purification[13] component. Additionally, a TiO_2 photocatalyst is chemically and physically stable and safe enough to be used as an additive for foods

and cosmetics. These advantages have drawn much attention recently in the field of dental materials and some studies have been performed concerning the antifungal effect[14-16]. However, antifungal effects on biofilms that show much stronger resistance to antibacterial agents than the planktonic form[17-20] have not yet been reported in the previous studies.

We incorporated a TiO_2 photocatalyst into a coating agent for a tissue conditioner because it is important to expose a great number of TiO_2 particles on the surface of the tissue conditioner. In addition, coating agents are an effective method to reduce plaque accumulation of tissue conditioners[21,22]. The purpose of this study was to investigate the antifungal effects of coating agents with a TiO_2 photocatalyst on biofilms. In the successful colonization and development of pathogenesis, the adherence of *C. albicans* to polymers such as soft lining materials is essential and necessary first step, followed by the growth of adherent cells or the co-adhesion of floating cells to adherent ones[23,24]. Therefore we examined the viability of biofilm-grown *C. albicans* and observed *C. albicans* on the radiated coating surfaces by scanning electron microscopy (SEM). Moreover, we investigated the degradation of adsorbed protein that promotes microorganism accumulation[25].

Materials and Methods

Specimen preparation

The TiO_2 was anatase type (rutile type was less 50 %) and average particle size 10 nm (SHOWA DENKO, Tokyo, Japan). The TiO_2 powder was incorporated with polyethyl methacrylate based coating agents (TOP COAT; NISSIN, Kyoto, Japan). Three coating agents with different concentration of TiO_2 were prepared (Table 1). Tissue conditioner (FICTIONER; NISSIN, Kyoto, Japan) was processed according to the manufacturer's direction and prepared as disk-shape specimens (20 mm in diameter, 1 mm in thickness). Each specimens was painted with coating agents one times on all surfaces and then stored in the dark for 15 min at room temperature.

Table 1. Component of coating agents
PEMA: polyethyl methacrylate

	TiO_2 (g)	PEMA (g)	ethyl acetate (g)
Coating agent 1	0	2	20
Coating agent 2	2	2	20
Coating agent 3	3	2	20

Yeast strain and growth condition

C. albicans strain (JCM 1542; Physico-chemistry Institute, Saitama, Japan) was used as the biofilm forming yeast. Cells were grown for 24 h at 37 °C in a yeast nitrogen base medium (YNB medium; Difco Laboratories, Detroit, USA) supplemented with 50 mM glucose. Cells were harvested, washed with phosphate-buffered saline (PBS, pH 7.2), and adjusted to 1×10^7 cells/mL.

Artificial candidal biofilm formation

Artificial candidal biofilms were formed on specimens according to Chandra J. et al.[20] with some modifications. Specimens were placed in each well of 12-well tissue culture plates and incubated for 2 h at 37 °C in 1 mL of 1 % bovine serum albumin (BSA; Sigma Aldrich Japan, Tokyo, Japan). After this pretreatment, 80 μL of *C. albicans* cell suspension was applied to the surfaces of specimens placed in 12-well tissue culture plates. Cells were allowed to adhere for 90 min at 37 °C (adhesion phase). Specimens were then immersed in 4 mL of YNB medium with 50 mM glucose and incubated for 48 h at 37 °C (biofilm growth phase).

Antifungal assay

After incubation, the YNB medium was removed and 4 mL of PBS was added to each well of the plates. The specimens with biofilms were radiated with an ultraviolet-A light source (365 nm, 1 mW/cm^2) for 60 and 90 min. Subsequently, the plates were placed in an ultrasonic bath (45W, VC-1; AS ONE, Osaka, Japan) for 15 min to collect *C. albicans* cells from the specimens.

The viability of collected cells was determined by a Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan), which is based on bioreduction of 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium (WST-8) that produces a water-soluble formazan dye. The formazan dye decreases with reduced viability of biofilm-grown *C. albicans*. Ninety-six-well plates containing 50 μL of the collected cell suspension per well were incubated with 50 μL of the YNB medium with 50 mM glucose and 10 μL of CCK-8 prepackaged solution for 5 h at 37 °C. After incubation, absorbance at 450 nm was measured using a microplate reader (VersaMax; Molecular Devices Japan, Tokyo, Japan). The viability percentage was normalized to the 100 % conversion observed in nonradiated specimens.

Scanning electron microscopy observation

Specimens were incubated for 24 h at 37 °C in suspension of *C. albicans*. After incubation, the specimens that were nonradiated or radiated with an ultraviolet-A light for 90 min were examined by SEM (JSM-5400; JEOL, Tokyo, Japan). Briefly, the specimens were placed in 2.5 % glutaraldehyde overnight, then rinsed in PBS and placed in 1 % osmium tetraoxide for 3 h. Specimens were subsequently rinsed in distilled water, freeze-dried with liquid nitrogen and then coated with gold/palladium. After processing, specimens were observed using SEM.

Protein degradation assay

In previous artificial candidal biofilm formation process, BSA was used to substitute the salivary protein on *in vivo* biofilm formation. The effect of TiO_2 on BSA degradation was also evaluated.

One hundred μL of 0.5 % BSA was applied to the surfaces of specimens placed in 12-well tissue culture plates. The specimens were radiated with an ultraviolet-A light source (365 nm, 1 mW/cm^2) for 60 and 90 min. After radiation, the plates with 1 mL of 0.5% sodium dodecyl sulfate per well were placed in the ultrasonic bath for 15 min to collect residual BSA and decomposition products of BSA from the specimens.

The collected residual BSA and decomposition products were measured by a Protein Quantification Kit-Wide Range (PQK; Dojindo, Kumamoto, Japan), which is based on the reduction of tetrazolium salts. Absorbance has a correlation with a concentration of protein. The colorimetric assay was performed according to the manufacturer's direction and absorbance at 650 nm was measured using the microplate reader.

Statistical analysis

The effects of TiO_2 concentration and illumination time were analyzed using two-way ANOVA and Tukey's test at a 0.05 probability level . Each experiment was repeated five times. Two independent variables were TiO_2 concentration and illumination time. The dependent variables were the data of antifungal assay and protein degradation assay respectively.

Results

Viscosities of coating agents increased with incorporation of TiO_2. However, both of coating agents with TiO_2 could be applied to tissue conditioners by brush. Incorporated TiO_2 particles were found on the coated surface (Figure 1).

Antifungal assay

Figure 2 shows relations between the viability of *C. albicans* in biofilms and radiation time. The viability significantly decreased with increasing the concentration of TiO_2 in the coating agents ($P < 0.05$). The viability also significantly decreased with radiation time ($P < 0.05$). After 60 min radiation, coating agent 2 showed no significantly antifungal effects in comparison with coating agent 1 (coating agent 1: 88.8±4.5 %, coating agent 2: 88.6±1.8 %). However, significant decreases

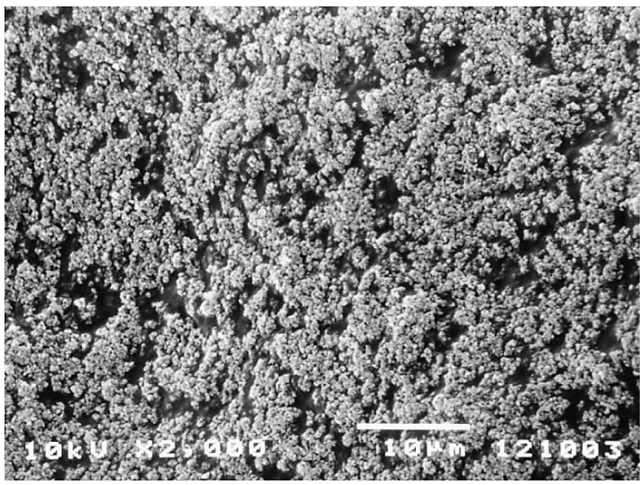


Fig. 1. SEM image of coating agent 3 (×2000)
TiO_2 particles were observed on the surface of coated tissue conditioner.

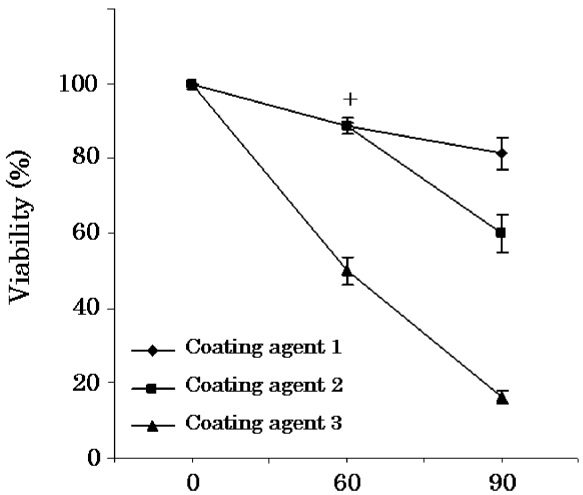
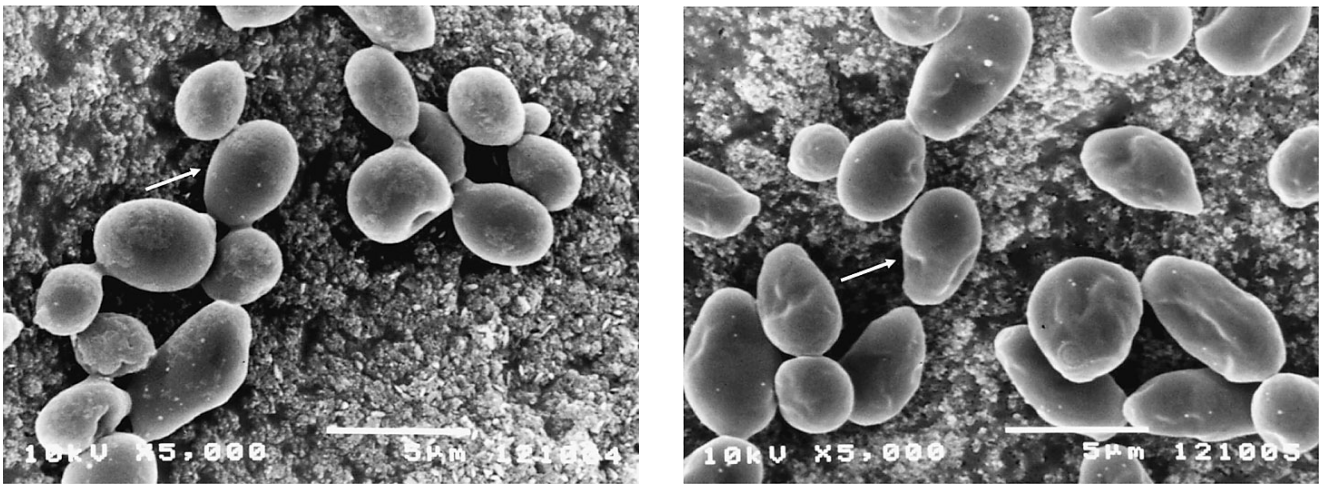


Fig. 2. Viability of *C. albicans* in antifungal assay
Error bar indicates ± 1 SD. A single plus indicates no significantly difference between the coating agent 1 and 2 ($P > 0.05$).



a b

Fig. 3. SEM of *C. albicans* on coating agent 3 (×5000)
a. Image of *C. albicans* on the coated surface before radiation (the arrow).
b. Image of *C. albicans* on the coated surface after 90 min radiation (the arrow).

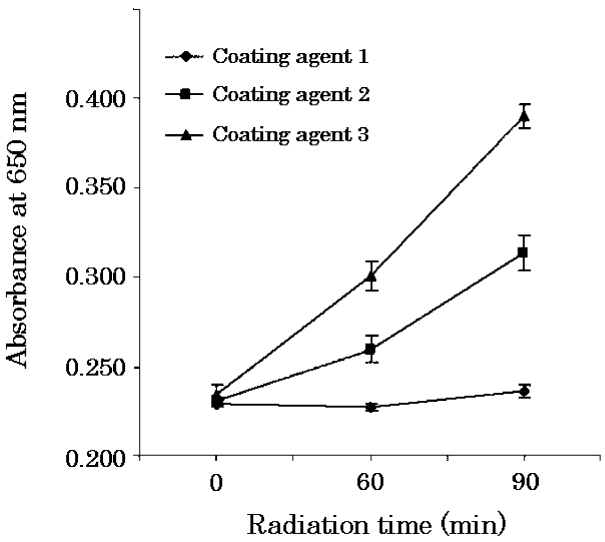


Fig. 4. Absorbance in protein degradation assay
Error bar indicates ± 1 SD.

were observed after 90 min in radiated coating agent 2 (coating agent 1: 81.4± 8.5 %, coating agent 2: 60.1±10.5 %, $P < 0.05$). Coating agent 3 showed a significant greater decrease of the viability of *C. albicans* than other coating agents ($P < 0.05$). After 90 min radiation, the viability of *C. albicans* was reduced to 16.2±3.3 %.

Scanning electron microscopy observation

Figure 3 shows SEM analysis of *C. albicans* on non-radiated and radiated coated surfaces of coating agent 3, which showed most marked antifungal effect in this study. After 90 min radiation, fungous bodies of *C. albicans* remained on the coated surfaces of tissue conditioners. However, many *C. albicans* on the radiated surfaces had dents and irregular contours.

Protein degradation assay

Figure 4 shows the absorbance in protein degradation assay. The absorbance significantly increased with the concentration of TiO_2 in the coating agents ($P < 0.05$). The absorbance also significantly increased with radiation time ($P < 0.05$). The absorbance of coating agent 3 showed a significantly greater increase than that of coating agent 2 ($P < 0.05$).

Discussion

Coating agents with TiO_2 showed significant decreases in the viability of *C. albicans*. The photocatalytic antifungal effects result from denaturation of *Candida* cell walls because many yeast cells on the radiated surfaces had dents in SEM observation. SEM examination observed that fungous bodies of *C. albicans* remained on the radiated surfaces, suggesting that rinsing out the radiated surfaces is important to prevent more microorganisms accumulating.

A TiO_2 photocatalyst activated by UV can only decompose protein in contact with the TiO_2, and therefore, decomposition products were derived from adsorbed BSA. The decomposition products are

smaller molecular size protein than BSA because a TiO_2 photocatalyst acts on the amide bond of protein[13]. The absorbance of radiated conditions with TiO_2 significantly increased with radiation time. The results showed that the coating agents with greater amounts of TiO_2 had greater degradation effects on the adsorbed protein.

In this study, coating agent 2 that showed significant degradation of BSA indicated no antifungal effects after 60 min radiation. This might have been caused that the radicals from photocatalystic reactions were expended decomposing the extracellular matrix of the biofilm. However, the elimination of the biofilms on the coated surfaces with TiO_2 may be easy because it elevates the hydrophilicity after radiation and decomposes adsorbed protein that promote microorganism accumulation. It is of interest to establish whether the coating agents with TiO_2 are effective the maintenance of tissue conditioners by short radiation time and studies are recommended.

Antibacterial agents incorporated into coating agents in the previous studies consistently act on oral flora. However, TiO_2 shows no photocatalystic effects at nonradiation. So that means the coating agents with TiO_2 may have no antifungal effect in oral cavity environment. When dentures are removed, for instance during sleep, the photocatalystic coating agents allow for the maintenance of tissue conditioners by just radiating the coated surfaces. This will be useful in an aging society that requires simple and easy cleaning methods.

References

1. Okita N, Orstavik D, Orstavik J, et al. In vivo and *in vitro* studies on soft denture materials: microbial adhesion and tests for antibacterial activity. Dent Mater 1991;7:155-160.
2. Radford DR, Challacombe SJ, Walter JD. Denture plaque and adherence of Candida albicans to denture-base materials *in vivo* and *in vitro*. Crit Rev Oral Biol Med 1999;10:99-116.
3. Barbeau J, Seguin J, Goulet JP, et al. Reassessing the presence of Candida albicans in denture-related stomatitis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;95:51-59.
4. Ramage G, Tomsett K, Wickes BL, et al. Denture stomatitis: a role for Candida biofilms. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;98:53-59.
5. Harrison A, Basker RM, Smith IS. The compatibility of temporary soft materials with immersion denture cleansers. Int J Prosthodont 1989;2:254-258.
6. Nikawa H, Yamamoto T, Hamada T, et al. Commercial denture cleansers-cleansing efficacy against Candida albicans biofilm and compatibility with soft denture-lining materials. Int J Prosthodont 1995;8:434-444.
7. Graham BS, Jones DW, Burke J, et al. In vivo fungal presence and growth on two resilient denture liners. J Prosthet Dent 1991;65:528-532.
8. Truhlar MR, Shay K, Sohnle P. Use of a new assay technique for quantification of antifungal activity of nystatin incorporated in denture liners. J Prosthet Dent 1994;71:517-524.
9. Chow CK, Matear DW, Lawrence HP. Efficacy of antifungal agents in tissue conditioners in treating candidiasis. Gerodontology 1999;16:110-118.
10. Abe Y, Ishii M, Takeuchi M, et al. Effect of saliva on an antimicrobial tissue conditioner containing silver-zeolite. J Oral Rehabil 2004;31:568-573.
11. Fujishima A, Tryk DA, Watanabe T, Hashimoto K. Titanium oxide-coated glass: self-cleaning and anti-fogging properties and applications. Int Glass Review-Flat Glass Proc 1998;114-116.
12. Sunada K, Watanabe T, Hashimoto K. Studies on photokilling of bacteria on TiO_2 thin film. J Photochem Photobiol A: Chemistry 2003;156:227-233.
13. Muszkat L, Feigelson L, Bir L, et al. Titanium dioxide photocatalyzed oxidation of proteins in biocontaminated waters. J Photochem Photobiol B: Biology 2001;60:32-36.
14. Nagame S, Oku T, Kambara M, et al. Antibacterial Effect of the powdered semiconductor TiO_2 on the viability of oral microoganisms. J Dent Res 1989;68:1696-1697.
15. Kubo Y, Ochi M, Sawada N, et al. Antifungal Effect of titanium dioxide photocatalysis on *Candida albicans*. (in Japanease) J Jpn Dent Products. 2001;15:16-21.
16. Tanimura Y, Watari F, Uo M, et al. Visible light responsibility of titanium dioxide photocatalyst by metal ion decoration and besic research into its dental application. (in Japanease, English abstract) J J Dent Mater. 2002;21:342-350.
17. Hawser SP, Douglas LJ. Resistance of Candida albicans biofilms to antifungal agents *in vitro*. Antimicrob Agents Chemother 1995;39:2128-2131.
18. Ramage G, Vande Walle K, Wickes BL, et al. Standardized method for *in vitro* antifungal susceptibility testing of Candida albicans biofilms. Antimicrob Agents Chemother 2001;45: 2475-2479.
19. Chandra J, Kuhn DM, Mukherjee PK, et al. Biofilm formation by the fungal pathogen Candida albicans: development, architecture, and drug resistance. J Bacteriol 2001;183: 5385-5394.
20. Chandra J, Mukherjee PK, Leidich SD, et al. Antifungal resistance of candidal biofilms formed on denture acrylic *in vitro*. J Dent Res 2001;80:903-908.
21. Dominguez NE, Thomas CJ, Gerzina TM. Tissue conditioners protected by a poly (methyl methacrylate) coating. Int J Prosthodont 1996;9:137-141.
22. Malmstrom HS, Mehta N, Sanchez R, et al. The effect of two different coatings on the surface integrity and softness of a tissue conditioner. J Prosthet Dent 2002;87:153-157.
23. Verran J, Maryan CJ. Retention of Candida albicans on acrylic resin and silicone of different surface topography. J Prosthet Dent 1997;77:535-539.
24. Waters MGJ, Williams DW, Jagger RG, Lewis MAO. Adherence of Candida albicans to experimental denture soft lining materials. J Prosthet Dent 1997;77:306-312.
25. Nikawa H, Hayashi S, Nikawa Y, et al. Interactions between denture lining material, protein pellicles and Candida albicans. Arch Oral Biol 1993;38:631-634.