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Antifungal Effect of Acrylic Resin Containing Apatite-coated TiO_2 Photocatalyst

Takeshi SHIBATA[1], Nobushiro HAMADA[2], Katsuhiko KIMOTO[1,3], Tomofumi SAWADA[1], Tomoji SAWADA[1], Hidefumi KUMADA[2], Toshio UMEMOTO[2] and Minoru TOYODA[1]

[1]Division of Prosthetics, Department of Oral and Maxillofacial Rehabilitation, Kanagawa Dental College, 82 Inaoka-cho, Yokosuka-shi, Kanagawa 238-8580, Japan

[2]Division of Microbiology, Department of Infection Control, Kanagawa Dental College, 82 Inaoka-cho, Yokosuka-shi, Kanagawa 238-8580, Japan

[3]Research Center of Brain and Oral Science, Kanagawa Dental College, 82 Inaoka-cho, Yokosuka-shi, Kanagawa 238-8580, Japan

Corresponding author, Takeshi SHIBATA; E-mail: shibata@kdcnet.ac.jp

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The purpose of this study was to develop an acrylic resin with antifungal properties by leveraging the photocatalytic activity of apatite-coated titanium dioxide (Ap-TiO_2). *Candida albicans* was used for antifungal activity assay of the specimen plates under ultraviolet A (UVA) with a black light source. Statistically significant decreases in cell viability in acrylic resins containing 5 wt% and 10 wt% Ap-TiO_2 were observed after irradiation for two, four, and six hours ($P<0.01$), when compared to the control. As for the flexural strength and modulus values of acrylic resins mixed with Ap-TiO_2 and TiO_2 particles, they varied before and after irradiation. Among the tested specimens, a 5 wt% content of Ap-TiO_2 in acrylic resin exceeded the requirements of ISO 1567. It was thus suggested that acrylic resin containing 5 wt% Ap-TiO_2 could exert antifungal effects on *C. albicans*, while at the same time maintain adequate mechanical properties for clinical use.

Keywords: *Candida albicans*, Apatite, TiO_2 photocatalysis

INTRODUCTION

With an ageing population on the increase in many modern day societies, it has also become increasingly important to achieve early control of oral infections and recovery of oral function[1]. In the same vein, when an elderly person loses teeth due to caries or periodontal diseases, oral function is generally recovered with dentures. In dentistry, acrylic resin is widely used as a denture base material — amongst its many other dental applications.

Plaque is a mass of bacteria protected by a biofilm, and it adheres to dentures with a wider adhesion area than to natural teeth[2,3]. Most microorganisms colonizing on the surface are found as complex-structured microbial communities and which may irritate the underlying tissue[4-6]. A recent study demonstrated that denture plaque control is essential for the prevention of denture stomatitis associated with *Candida albicans*[5]. In addition, the fitted surfaces of dentures have been shown to be reservoirs of *C. albicans*, which is associated with stomatitis and disseminated fungal infections[7]. For elderly denture wearers, microorganisms on dentures would therefore pose a high risk of *Candida*-induced aspiration pneumonia[8]. This is because it has been reported that oral microorganisms, *C. albicans* in particular, cause denture stomatitis[9,10] and are closely associated with pulmonary candidiasis and aspiration pneumonia[8,11] — both of which are fatal diseases in the elderly.

Denture stomatitis is a common and recurring problem in denture wearers. Yeast infections[12] and many general predisposing factors[13] are associated with the development of denture stomatitis. Fungi, mostly *C. albicans*, have been recognized as one of the contributing factors of denture stomatitis[14,15]. Upon accumulation of *Candida* in the unique microenvironment between denture and the oral mucosa, a strong immunological reaction occurs and denture stomatitis develops[16]. Treatment of denture stomatitis should start with educating patients on meticulous daily washing of their dentures. It has been reported that mechanical cleaning methods are insufficient for a complete reduction of microorganisms on denture bases[17]. Since prevention of biofilm formation is important for oral hygiene, this study set out to evaluate the antifungal effects of a denture base acrylic resin mixed with titanium dioxide (TiO_2).

TiO_2 exerts antimicrobial, antifouling, and deodorant effects in response to light irradiation. As such, it is being actively studied as an environment purification material[18]. TiO_2 is white, inexpensive, and nontoxic. When it is illuminated, TiO_2 can photooxidize organic chemical materials in water and air[18-20]. A careful examination of the effect of the crystal structure of TiO_2 on photocatalytic properties resulted in clarifying that the anatase phase showed high photocatalytic activity[21].

TiO_2 is used in the treatment of wastewater, purification of air, and as an antibacterial material. Concerning its antibacterial use, TiO_2-coated tiles have been utilized in the sterilization of *Escherichia* *coli* and methicillin-resistant *Staphylococcus aureus* (MRSA)[22]. Due to these beneficial properties, TiO_2 has also been tested in the prosthodontic field by mixing TiO_2 with the denture base acrylic resin before polymerization.

A TiO_2 photocatalyst can decompose only organic materials and bacteria existing on the surface of TiO_2. On the other hand, apatite is reportedly[23,24] useful in absorption of bacteria, viruses, NO_X, and ammonia. On this account, apatite and TiO_2 can be combined into a composite that possesses the favorable attributes of both. The resultant composite may then be a good antibacterial and environmental purification material, with the ability to absorb and decompose bacteria and other organic materials.

In a previous study, a titanium dioxide coated with apatite (Ap-TiO_2) was evaluated[25]. It has been shown that Ap-TiO_2 did not decompose acrylic resin (with the latter acting as a medium), since there was no direct contact between TiO_2 and acrylic resin. This was because the surface of TiO_2 particles was covered with apatite, which acted as a spacer. In other words, by adding Ap-TiO_2 to acrylic resin, it is possible to manufacture a composite material that has semi-permanent antifungal, antifouling, and deodorant properties.

The purpose of the present study, therefore, was to investigate the antifungal effects of an acrylic resin with Ap-TiO_2 photocatalyst by examining its effect on the viability of *C. albicans*.

MATERIALS AND METHODS

Antifungal property of powdered TiO_2 and Ap-TiO_2

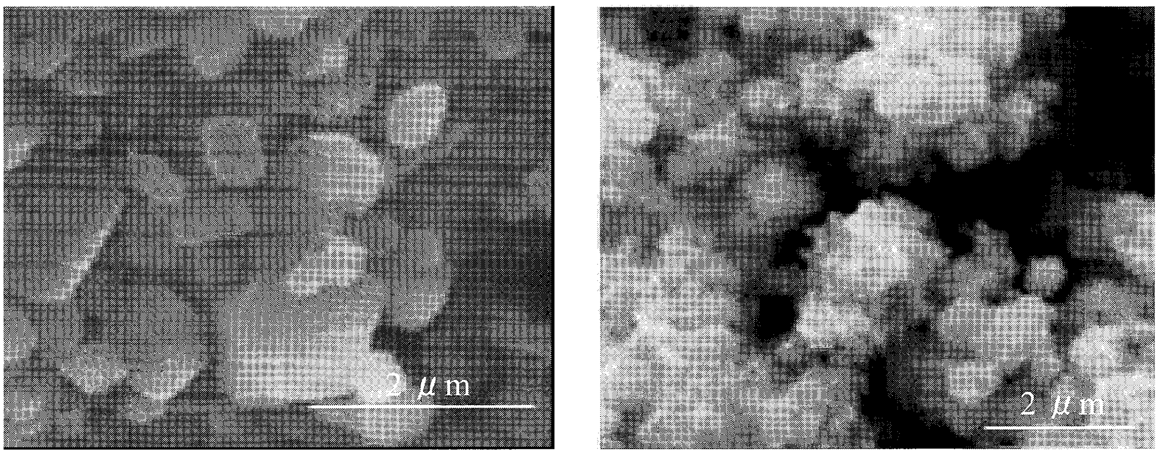
The TiO_2 photocatalyst used in this study was the anatase type of TiO_2 (Nonami Science Co., Aichi, Japan) with a diameter of approximately 400 nm. Ap-TiO_2 was formed by precipitating apatite onto the surface of TiO_2 particles, so that part of each particle was covered, as shown in Fig. 1.

C. albicans ATCC 1002 was used for antifungal activity assay of powdered TiO_2 and Ap-TiO_2. *C. albicans* cells were cultured aerobically at 37℃ for 24 hours in brain heart infusion broth (BHI; Difco Laboratories, Detroit, MI). They were harvested by centrifugation at 12000 rpm for five minutes and then suspended in saline to a concentration of 1.0×10^9 cell/mL. Then, 100 mg of powdered TiO_2 (or Ap-TiO_2) was added to 1 mL of the fungal solution in a 24-well plate. UVA from a black light source (FPL27BLB27W, Sankyo Denki Co., Kanagawa, Japan; wavelength: 360 nm) was selected as the light source for catalytic excitation. Distance between the light source and 24-well plate was set to 20 cm for irradiation. The 24-well plate was irradiated with UVA from the black light bulb for six hours, while being stirred (Micromixer MX-5, Sanko Junyaku Co., Tokyo, Japan) to prevent precipitation of the powder. A fungal suspension without TiO_2 and Ap-TiO_2 was irradiated as a control.

Viable cells in culture were determined with a homogeneous method (BacTiter-Glo™ Microbial Cell Viability Assay, Promega Corp., Madison, WI). This method is based on quantity of ATP present. ATP is an indicator of metabolically active cells. One hundred microliters of BacTiter-Glo™ Reagent was added to 100 μL of the medium containing cells in a 96-well plate. The contents were mixed briefly with Micromixer MX-5, incubated for five minutes, and luminescence was recorded (Multimode Detector DTX880, Beckman Coulter Inc., Fullerton, CA).

Antifungal activity test of resin plates containing Ap-TiO_2

Denture base resin (Natural Resin, Nisshin Co.,



TiO_2 Ap-TiO_2

Fig. 1 SEM photographs of TiO_2 and Ap-TiO_2 particles. TiO_2: Titanium dioxide; Ap-TiO_2: Apatite-coated titanium dioxide.

Kyoto, Japan) was used as the basic resin body for specimen plates. Resin polymers containing 1, 5, and 10 wt% of Ap-TiO_2 powder was polymerized with the monomer according to manufacturer's instructions. Surfaces of the plates were ground with 600-grit waterproof paper. Following which, specimen plates were randomly divided into four groups as follows: (1) acrylic resin as a control (0 wt% Ap-TiO_2); (2) acrylic resin containing 1 wt% Ap-TiO_2; (3) acrylic resin containing 5 wt% Ap-TiO_2; and (4) acrylic resin containing 10 wt% Ap-TiO_2. Specimen plates were formed into ellipsoids (30×20×1.5 mm) with a metal mold.

C. albicans ATCC 1002 was cultured aerobically at 37℃ for 24 hours in BHI broth. *C. albicans* cells were harvested by centrifugation at 12000 rpm for five minutes and then suspended in saline to a concentration of 1.69×105 cell/mL. One milliliter of the fungal solution was dropped onto the specimen plate. After irradiation, 100 μL of the fungal solution was collected from the surface of the specimen. The suspension was diluted using a 10-step dilution procedure. Diluted suspension of *C. albicans* was placed onto BHI agar medium and incubated aerobically for 48 hours. After which, viable cells in each test group (n=4) were counted according to the number of colony-forming units (CFUs).

1. Cell viability by the distance between light and specimen

Distance between UVA black light source and specimen plate was set to 10 and 20 cm for irradiation. Specimens were then irradiated for four hours. In this experiment, cell viability of each specimen was calculated as a percent of the cell viability of a non-irradiated specimen.

2. Number of viable cells based on irradiation time

Distance between UVA black light source and specimen plate was set at 20 cm for irradiation. Specimens were then irradiated for two, four, and six hours.

Mechanical properties

Each specimen was tested for flexural strength (*Fs*) and flexural modulus (*Fm*) according to ISO 1567[26]. Specimen plates were formed into a rectangle (64×10×3.3 mm) with a metal mold (n=5). Rectangular specimens were then divided into seven groups as follows: (1) acrylic resin as a control; (2) acrylic resin containing 1 wt% Ap-TiO_2; (3) acrylic resin containing 5 wt% Ap-TiO_2; (4) acrylic resin containing 10 wt% Ap-TiO_2; (5) acrylic resin containing 1 wt% TiO_2; (6) acrylic resin containing 5 wt% TiO_2; and (7) acrylic resin containing 10 wt% TiO_2.

Surfaces of all the specimens were polished with 1500-grit waterproof paper. They were irradiated with UVA black light in water for 360 hours. Distance between the light source and specimen was set at 20 cm for irradiation. The test was conducted with a universal testing machine (EZ Test 500N, Shimadzu, Kyoto, Japan) at a crosshead speed of 5 mm/min.

Fs was calculated in megapascals (MPa) using the following equation:

$$Fs = 3Fl/2bh^2$$

where F is the maximum load in newtons (N) exerted on the specimen; l is the distance between the supports (50 mm); b is the width (mm) of specimen; and h is the height (mm) of specimen.

Fm (MPa) was calculated using the following equation:

$$Fm = F_1 l^3/4bh^3 d$$

where F_1 is the load (N) at a convenient point in the straight-line portion of the trace; d is the deflection (mm) at load F_1; l, b, and h are the same as the equation of *Fs*.

Surface analysis

Surface morphologies of the acrylic resins containing 5 wt% Ap-TiO_2 and TiO_2 particles were observed using a scanning electron microscope (SEM) (EPMA 8705, Shimadzu, Kyoto, Japan) in secondary electron image (SEI) (Fig. 6(a)) and composition image (CPI) (Fig. 6(b)) modes. An area scan (400×400 μm) was carried out at an accelerating voltage of 20 kV after being coated with gold.

Statistical analysis

Data were statistically analyzed using StatView Version 4.58 for Windows (Abacus Concepts Inc., USA). The number of viable cells in each group was analyzed with ANOVA followed by multiple comparison test (Scheffe's test). Statistical significance was set at $P<0.01$.

RESULTS

Photocatalytic and antifungal effects of powdered TiO_2 and Ap-TiO_2

Post-irradiation cell viability of suspended TiO_2 and Ap-TiO_2 are shown in Table 1. It was determined that TiO_2 and Ap-TiO_2 showed strong photocatalytic and antifungal actions on *C. albicans*, although there were no statistical differences between TiO_2 and Ap-TiO_2.

Antifungal activity of resin plates containing Ap-TiO_2

1. Cell viability by the distance between light and specimen

Cell viability as a function of distance after irradiation for 0 wt% Ap-TiO_2 and 5 wt% Ap-TiO_2 are shown in Fig. 2. There were statistically significant differences in cell viability based on the distance between light and specimen. There were also statistically significant differences in cell viability in 5 wt% Ap-TiO_2 specimens irradiated at 10 cm and 20 cm when compared with 0 wt% Ap-TiO_2.

2. Number of viable cells based on irradiation time

Figure 3 shows the number of viable cells after irradiation for each group. Decrease in the number of viable cells in the control group with passage of time was assumed to be due to the influence of UVA black light. In the 1 wt% Ap-TiO_2 group, the number of viable cells tended to be less than that of control; however, no statistically significant differences were found. In the 5 wt% Ap-TiO_2 and 10 wt% Ap-TiO_2 groups, there were statistically significant decreases in the number of viable cells after irradiation for two, four, and six hours ($P<0.01$), when compared with the control.

Mechanical properties

1. Flexural strength

Figure 4 shows the mean scores and standard deviations of the *Fs* value before and after

Table 1 Cell viability of *Candida albicans* in solutions of suspended TiO_2 and Ap-TiO_2. Values with the same superscript letter are not statistically different at $P<0.01$

Specimen	Cell viability (%)
Control	100 ± 22.3[a]
TiO_2	0.0503 ± 0.0201[b]
Ap-TiO_2	1.76 ± 0.164[b]

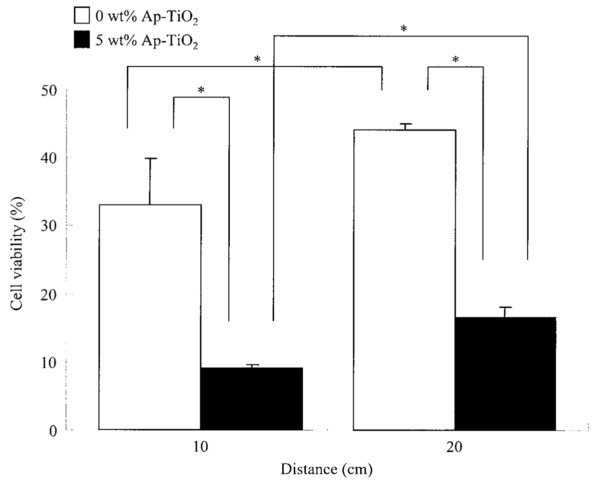


Fig. 2 Cell viability of *Candida albicans* by the distance between light and specimen plates. Cell viability (%) = CFUs at irradiation time of 4 hours/CFUs of non-irradiated groups × 100. Mean values and standard deviations were calculated for each irradiation distance and specimen (*: $P<0.01$).

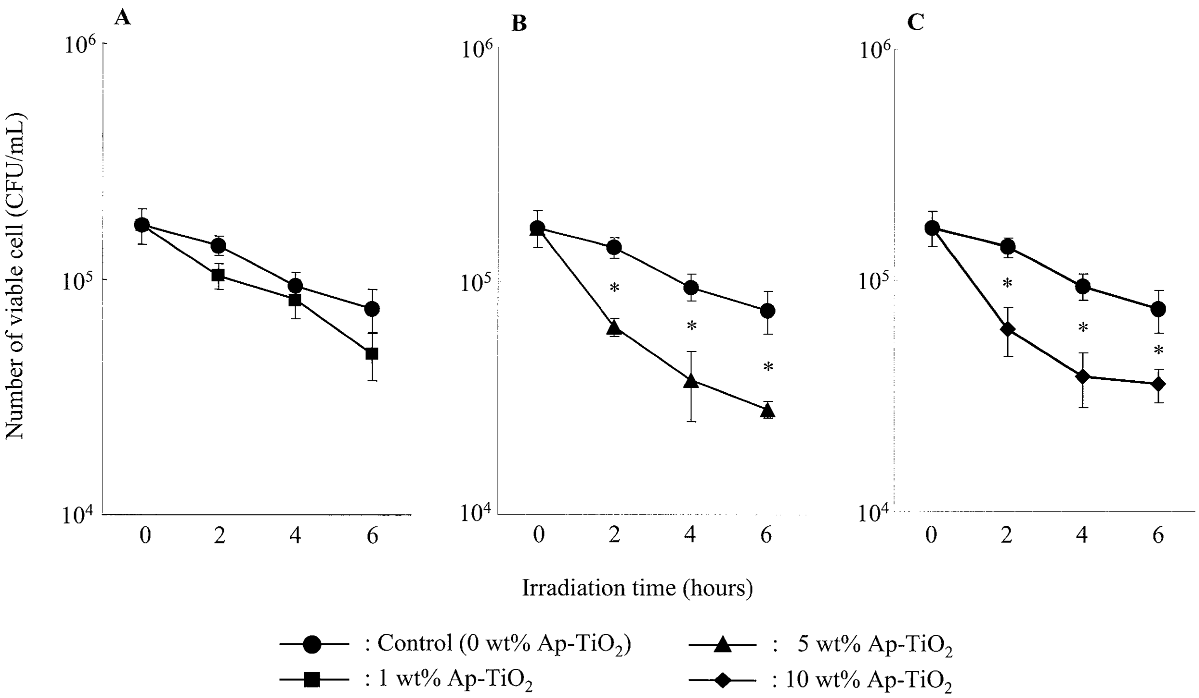


Fig. 3 Viable cell numbers of *Candida albicans* on the specimen plates. Mean values and standard deviations were calculated for each irradiation time (*: $P<0.01$). A: Control compared with 1 wt% Ap-TiO_2; B: Control compared with 5 wt% Ap-TiO_2; C: Control compared with 10 wt% Ap-TiO_2.

irradiation by UVA black light. It could be seen that *Fs* value decreased with increase in the content of Ap-TiO_2 and TiO_2 particles in acrylic resin. ANOVA showed significant differences between the contents of Ap-TiO_2 and TiO_2 particles in acrylic resin before irradiation ($F=162.8$; $P<0.001$) and after irradiation ($F=66.3$; $P<0.001$), and between Ap-TiO_2 and TiO_2 particles before irradiation ($F=24.01$; $P<0.001$) and after irradiation ($F=9.72$; $P=0.0047$). *Post hoc* tests showed that significant mean differences between Ap-TiO_2 and TiO_2 particles existed in 1 wt%, 5 wt%, and 10 wt% before irradiation ($P<0.005$), and in 5 wt% and 10 wt% after irradiation ($P<0.004$).

All specimens except pre-irradiation 10 wt% TiO_2 passed the requirements of ISO 1567 regarding

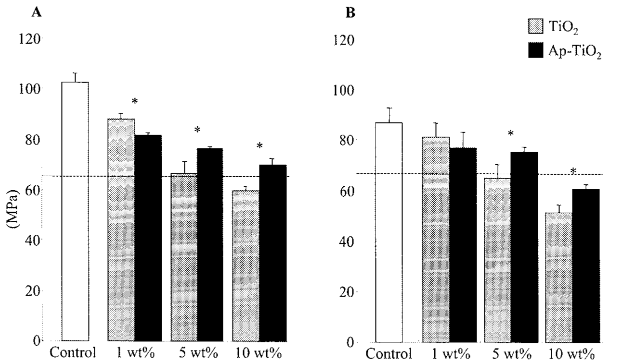


Fig. 4 Means and standard deviations of flexural strength of specimens. A: Before irradiation by UVA; B: After irradiation by UVA. Contents of TiO_2 and Ap-TiO_2 particles in acrylic resin were 1 wt%, 5 wt%, and 10 wt%. Control was conventional acrylic resin without TiO_2 and Ap-TiO_2 particles. -----: ISO 1567 (>65 MPa). Asterisks have been placed above pairs of groups for which the difference in flexural strength was statistically significant (*: $P<0.01$).

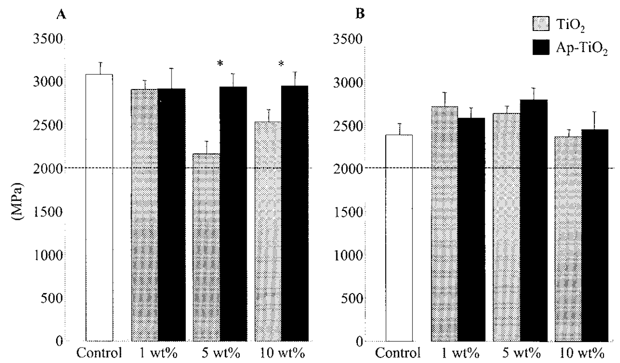


Fig. 5 Means and standard deviations of flexural modulus of specimens. A: Before irradiation by UVA; B: After irradiation by UVA. Contents of TiO_2 and Ap-TiO_2 particles in acrylic resin were 1 wt%, 5 wt%, and 10 wt%. Control was conventional acrylic resin without TiO_2 and Ap-TiO_2 particles. -----: ISO 1567 (>2000 MPa). Asterisks have been placed above pairs of groups for which the difference in flexural modulus was statistically significant (*: $P<0.01$).

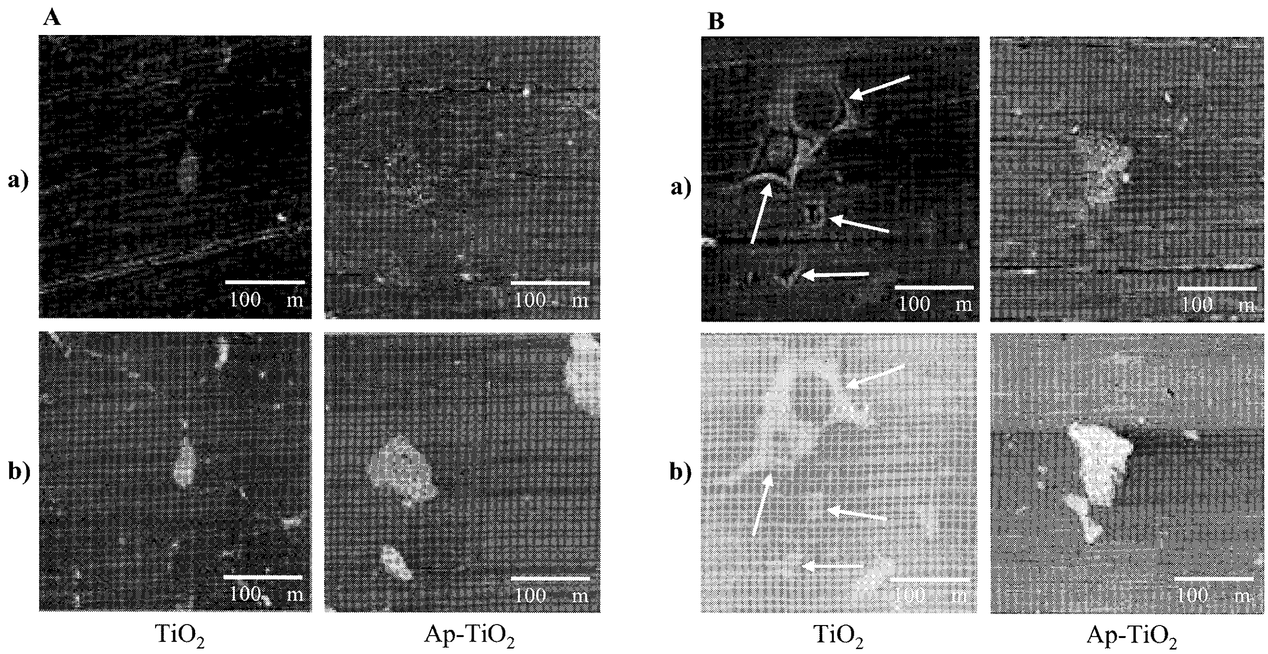


Fig. 6 SEM photographs of the surface of specimens containing 5 wt% TiO_2 and Ap-TiO_2 particles. A: Before irradiation by UVA; B: After irradiation by UVA. (a) Secondary electron images (SEI); (b) Composition images (CPI).

Fs testing (>65 MPa). Meanwhile, post-irradiation 5 wt% TiO_2, 10 wt% TiO_2, and 10 wt% Ap-TiO_2 did not fulfill the requirements.

2. Flexural modulus

Figure 5 shows the mean scores and standard deviations of the *Fm* value before and after irradiation by UVA black light. ANOVA showed significant differences in *Fm* with the contents of Ap-TiO_2 and TiO_2 particles in acrylic resin before irradiation (F=12.0; P=0.002) and after irradiation (F=13.6; P=0.001), and between Ap-TiO_2 and TiO_2 particles before irradiation (F=42.9; P<0.001). *Post hoc* tests showed that significant mean differences in *Fm* between Ap-TiO_2 and TiO_2 particles existed in pre-irradiation 5 wt% and 10 wt% TiO_2 (P<0.05).

Although the *Fm* value varied with the contents of Ap-TiO_2 and TiO_2 particles in acrylic resin, all pre- and post-irradiation test specimens passed the requirements of ISO 1567 regarding *Fm* testing (>2000 MPa).

Surface analysis

Figure 6 shows, in SEI and CPI modes, the SEM photomicrographs of acrylic resins containing 5 wt% TiO_2 and Ap-TiO_2 before and after irradiation. In SEI mode, some pores were observed on the post-irradiation surface of acrylic resin containing TiO_2 (arrowhead), whereas there were no pores on the pre-irradiation surface of acrylic resin containing TiO_2. As for Ap-TiO_2, no pores were observed both before and after irradiation.

In CPI mode, dark and black areas reflect an element of lower atomic weight (e.g., Si) or voids, while bright and white areas are made up of elements of higher atomic weight (e.g., Ti). In this study, it was observed that some pores were distributed in bright and white areas of Ti.

DISCUSSION

With regard to keeping dentures adequately plaque- and *Candida*-free, chemical cleansers have been touted as an important means[27]. However, it has been shown that neither denture cleansers nor a placebo were effective in reducing candidal colonization or plaque reaccumulation[28]. In addition, regular, routine use of denture cleansers may be unaffordably prohibitive in cost, especially among geriatric and handicapped denture wearers.

Against this backdrop of functional and cost-related reservations toward denture cleansers, several researchers have attempted to mix acrylic resins with antifungal agents, antiseptics, a silver inorganic system, and organic materials for additional antibacterial effects[29-32]. These previous studies showed that the added antifungal and antiseptic agents were rapidly released from denture bases. As a result, the amount of antifungal and antiseptic agents required for routine use would again pose the problem of cost *versus* affordability to the denture wearers. Furthermore, such a level of agent release may be harmful to elderly people. As for silver ions, it should be highlighted that besides their beneficial effect on antibacterial activity, silver-based alloys were found to undergo frequent corrosion and tarnishing under oral conditions[33].

In contrast, TiO_2 is biocompatible, nontoxic, and inexpensive. Powdered TiO_2 has been reported to be able to kill *Streptococcus mutans*, *E. coli*, and *C. albicans*[34,35]. However, the antifungal effect of powdered Ap-TiO_2 or acrylic resin containing Ap-TiO_2 remains unclear. In this study, we sought to investigate the antifungal effects of acrylic resin containing Ap-TiO_2, as powdered Ap-TiO_2 showed strong photocatalytic antifungal actions on *C. albicans* nearly equivalent to that of TiO_2. Unfortunately, acrylic resin containing Ap-TiO_2 was not able to eradicate *C. albicans* completely, although we have reported that it killed *E. coli* completely within four hours[36]. With regard to the *Candida* species, the oral cavity is a niche whereby *they* frequently inhabit as commensals. These yeasts can also develop on the surfaces of prostheses and medical devices, thereby developing and exhibiting resistance to antifungal agents as compared to their free-living (planktonic) counterparts[37,38]. This is likely to be the cause of recalcitrant persistence of *Candida* on inert surfaces. Hence, it was impossible to eradicate the fungus completely even after six hours of irradiation by virtue of the photocatalytic effect of Ap-TiO_2.

When Ap-TiO_2 was applied to acrylic resin in clinical situations, we instructed the patients to wash their dentures before going to bed and then let the dentures be irradiated all through the night with a dedicated light irradiator. If they were able to do so every night, the denture base surface would not become a breeding place for the growth of microorganisms. Expectedly, the number of microorganisms would steadily decrease day by day. Nonetheless, further investigation is needed on how to eradicate the fungus completely in clinical applications.

With regard to flexural strength, the *Fs* values of acrylic resins with different contents of Ap-TiO_2 and TiO2 differed — regardless of irradiation condition. In particular, *Fs* value was reduced significantly with increase in the content of Ap-TiO_2 and TiO_2 particles in acrylic resin. Based on these obtained flexural strength results, two drawbacks were clearly manifested. The first one pertained to the degree of conversion of acrylic resin. Some studies reported that the *Fs* value decreased with increase in the content of other compounds added to acrylic resin[30,39]. In the context of the present study, the degree of conversion of acrylic resin might be adversely affected because of the addition of other material — namely TiO_2 or Ap-TiO_2. If this were so, it would lead to an increase in residual monomer amount on acrylic resin, which is considered to act as a plasticizer. Taken together, the decrease in degree of conversion of acrylic resin and the increase in residual monomer amount would cause a loss in mechanical properties.

As for the other drawback that was manifested through this study, it pertained to the lack of chemical bonding between inorganic substances such as TiO_2 or Ap-TiO_2 and acrylic resin. It is well known that acrylic resin does not bond well chemically with inorganic substances. To solve this problem, adhesive monomers such as 4-methacryloxyethyl trimellitate anhydride (4-META) and γ-methacryloxypropyltrimethoxysilane (γ-MPS) have been used to improve bonding between metal and resin[39-41]. In the context of the present study, the physical and mechanical properties of acrylic resin containing TiO_2 or Ap-TiO_2 might be impaired by adding an adhesive monomer. For further clarification on these speculations, more detailed studies should be undertaken to elucidate these effects.

With regard to the effect of irradiation condition, ANOVA results showed that the *Fs* and *Fm* values of Ap-TiO_2 and TiO_2 differed significantly before and after irradiation. According to the *post hoc* test, acrylic resins containing Ap-TiO_2 tended to be higher in mechanical properties than TiO_2. A previous study has already noted that Ap-TiO_2 did not decompose the medium (e.g., acrylic resin), since there was no direct contact between TiO_2 and the medium due to the presence of apatite[42]. On this note, some pores were seen under SEM on the surface of acrylic resin with TiO_2 particles after irradiation, whereas no pores were observed with Ap-TiO_2. Interestingly, these pores were consistent with the distribution of Ti.

Based on the findings in the present study, it could be said that acrylic resin on the surface of TiO_2 was destroyed by the photocatalytic effect, whereas Ap-TiO_2 prevented decomposition due to the presence of apatite. In other words, it could be suggested that acrylic resin containing Ap-TiO_2 inhibited the loss of mechanical properties as compared with TiO_2 alone. On this point, it should be noted that although the mechanical properties of specimens — particularly the *Fs* value — were reduced by increasing the contents of Ap-TiO_2 and TiO_2 particles in acrylic resin, 5 wt% of Ap-TiO_2 exceeded the requirements of ISO 1567 after irradiation for 360 hours by UVA. Hence, an adequate amount of Ap-TiO_2 in acrylic resin for clinical applications would be 5 wt% — considering the antifungal effect and *Fs* test results.

In the present study, the experimental design followed the guidelines of ISO 1567 regarding *Fs* and *Fm* testing. It should be highlighted that if parameters — such as water immersion and exposure to oral temperature — were modified, the *Fs* and *Fm* values of denture base resin might be affected. Therefore, further study is needed before clinical application can be realized.

CONCLUSIONS

Adding Ap-TiO_2 to acrylic resin was expected to produce antifungal effects. However, it was found that excessive amount of Ap-TiO_2 should be avoided because of its detrimental influence on mechanical properties. It was thus suggested that acrylic resin containing 5 wt% of Ap-TiO_2 could exert antifungal effect on *C. albicans*, while at the same time maintain adequate mechanical properties for clinical use.

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