PHOTOCATALYTIC ANTIFUNGAL ACTIVITY AGAINST *CANDIDA ALBICANS* BY TiO_2 COATED ACRYLIC RESIN DENTURE BASE

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Statement of problem. Proliferation of *Candida albicans* is primarily within the plaque on the fitting surface of the denture rather than on the inflamed mucosa. Consequently, the treatment of the denture is equally important as treatment of the tissue. Cleansing and disinfection should be efficiently carried-out as the organisms can penetrate into the voids of the acrylic resin and grow in them, from which they can continue to infect and reinfect bearing tissues.

Purpose. The purpose of this study was to evaluate the applicability of photocatalytic reaction to eliminate *Candida albicans* from acrylic resin denture base, and to investigate the antifungal effect with various UVA illumination time.

Materials and Methods. The specimens were cured by the conventional method following the manufacturer' s instruction using thermal polymerized denture base resin (Vertex RS; Dentimex, Netherlands). TiO_2 photocatalyst sol(LT), which is able to be coated at normal temperature, was made from the Ti-alkoxide progenitor. The XRD patterns, TEM images and nitrogen absorption ability of the TiO_2 photocatalyst sol(LT) were compared with the commercial TiO_2 photocatalyst P-25.

The experimental specimens were coated with the mixture of the TiO_2 photocatalyst sol(LT) and binder material (silane) using dip-coater, and uncoated resin plates were used as the control group. Crystallinity of TiO_2 of the specimen was tested by the XRD. Size, shape and chemical compositions were also analyzed using the FE-SEM/EDS. The

angle and methylene blue degradation efficiency were measured for evaluating the photocatalytic activity of the TiO_2 film. Finally, the antifungal activity of the specimen was tested.

Candida albicans KCTC 7629(1 ml, initial concentration 10^5cells/ml) were applied to the experiment and control group specimens and subsequently two UVA light source with 10W, 353 nm peak emission were illuminated to the specimens from 15cm above. The extracted 2 μl of sample was plated on nutrient agar plate (Bacto™ Brain Heart Infusion; BD, USA) with 10 minute intervals for 120 minute, respectively. It was incubated for 24 hours at 37℃ and the colony forming units (CFUs) were then counted.

Results. Compared the characteristics of LT photocatalyst with commercial P-25 photocatalyst, LT were shown higher activity than P-25. The LT coated experimental specimen surface had anatase crystal form, less than 20 nm of particle size and wide specific surface area.

To evaluate the photocatalytic activity of specimens, methylene blue degradation reaction were used and about 5% of degradation rate were measured after 2 hours. The average contact angle was less than 20° indicating that the LT photocatalyst had hydrophilicity. In the antifungal activity test for *Candida albicans*, 0% survival rate were measured within 30 minute after irradiation of UVA light.

Conclusion. From the results reported above, it is concluded that the UVA-LT photocatalytic reaction have an antifungal effect on the denture surface *Candida albicans*, and so that could be applicable to the clinical use as a cleaning method.

Key Words

Titanium dioxide (TiO_2), Photocatalysis, antifungal activity, Denture cleansing, Denture-related stomatitis

Denture-related stomatitis is an inflammatory process that mainly involved the palatal mucosa when it is covered by complete or partial dentures and presents as an intensely red, glistening, and slightly swollen palatal epithelium.[1,2] The prevalence has been reported as varying ranges from 9% to 97%.[3-5] It has been suggested that denture-related stomatitis has a multi-factorial cause. Nonsystemic factors are bacteria,[6] yeast,[7] ill-fitting dentures, poor denture hygiene,[1] consumption of carbohydrate- rich diet,[8] and effects arising from denture materials.[1] However, generally the etiologic factor is microbial, and the presence of the opportunistic pathogen *Candida albicans* in denture plaque is considered as an important factor in the development of this inflammation.[7,9] This dimorphic fungus exists in both yeast and mycelial (hyphal) forms. During tissue invasion, the mycelial form predominates.[10] Because of its large size, host phagocytes are unable to completely destroy it. Therefore, extracellular killing mechanisms are required to eliminate the candida. The usual treatment of denture-related stomatitis involves elimination of the source of the infectious agent (denture cleansing and disinfection) and elimination of the oral tissues (antifungal therapy).[11,12] To minimize reinfection, dentures should continue to be soaked in solutions that destroy or remove the organism. Reduced use of denture or the removal of it during the antifungal therapy can be helpful in eliminating the source of the infectious agent. Generally, tissue treatment involves topical application of Nystatin (Sandoz Phamaceuticals, East Hanover, NJ), but patient complication is often poor because the unpleasant taste causes nausea and vomiting. These side effects somehow decrease its therapeutic value.[12]

Proliferation of *Candida albicans* is primarily within the plaque on the fitting surface of the denture rather than on the inflamed mucosa. Consequently, the treatment of the denture is equally important as treatment of the tissue.[11,12] With regard to the treatment of dentures, cleansing and disinfection should be efficiently carried-out as the organisms can penetrate into the voids of the acrylic resin and grow in them, from which they can continue to infect and reinfect bearing tissues.[12] Often, approximately 1mm of internal denture base acrylic is removed, and a soft liner is placed. This liner must be changed frequently as it also becomes penetrated by the organism.

Most commercially available denture cleansers aim to remove hard and soft deposits and bacterial plaque either by chemical or mechanical ways.[13] There are a large number of solutions, pastes and powders with a variety of claims for their relative efficacies. It was reported, however, that some of them caused problem such as discoloration, the generation of porosity and surface roughness of the denture base resin surface.[14]

A potential alternative may be provided by substrates made of light- guiding materials, coated with specific semiconductors and stimulated by indirect mild ultraviolet A (UVA) light (320-400 nm). This method shows oxidative and disinfectant activity. The semiconducting materials about which most information is available is titanium dioxide (TiO_2). It is well known that the TiO_2 in anatase is capable of oxidizing and the decomposing various kinds of compounds.[15,16] The recent review article provides a comprehensive report of the mechanisms involved and the potential fields of application.[17] There is now a wide agreement regarding the mechanism: in TiO_2, an electron is transferred from the valance band to the conduction band by absorption of a photon, and the resulting electron hole pair reacts with molecules on the surface of the semiconductor. Various reactive oxygen radicals caused by reactions of the hole have been identified in aqueous

solution, mainly the OH radical.[18-20] The free electron simultaneously created reacts with dissolved oxygen, to produce among other things hydrogen peroxide.[21,22] These reactive species can oxidize organic material up to complete mineralization, depending on the experimental conditions.[17] Overall, the organic molecules react with dissolved oxygen to produce CO_2 and H_2O.

Photocatalytic materials such as titanium dioxide(TiO_2) have been studied for more than 20 years on their capacity to degrade organic contaminants from air and water. Because of its unique photoinduced characteristics, it has been widely studied for applications such as auto cleaning agent, deodorant, antibacterial, and for purification of water and air.[23,24]

In 1985, Matsunaga et al. first reported the antibacterial effect of TiO_2 photocatalytic action: bacteria cultures in contact with UV-irradiated TiO_2-Pt thin film, during 60 to 120 min, had a significant reduction in number of cultivable cells.[25] Since this report, the photocatalytic property has been widely studied in variety of microorganisms such as viruses, bacteria, fungi, algae, and also in cancer cells.[26,27]

The purpose of this study was to evaluate the applicability of photocatalytic reaction to eliminate *Candida albicans* from acrylic resin denture base, and to investigate the antifungal effect with various UVA illumination time.

MATERIAL AND METHODS

1. Photocatalyst preparation and characterization

LT (Low Temperature) photocatalyst used in this study was made from alkoxide (titanium isopropoxide, Aldrich Co., 99.9%) and nitric acid. Titanium precursor was dropped to distilled water little by little and agitated with inorganic acid at room temperature and pressure. Final solution was made by hydrothermal method for 6 hours. It was reported that anatase crystalline phase could be made by hydrothermal method from hydro titanium.[28]

The crystalline structure, particle size, and specific surface area of the LT were tested. Powder acquired from photocatalyst sol after drying at 100 ℃ was tested with X-Ray diffraction (XRD, Rigaku D/MAX-1200, Japan), transmission electron microscopy (TEM, JEOL, JEM-2000FX Ⅱ, Korea Basic Science Institute/ Gwangju Branch), and N_2 adsorption method (ASAP 2010, Micromeritics, USA). Characteristics of the LT photocatalyst used in this study were compared with those of the commercial P-25 photocatalyst (Degussa, Germany).

2. Preparation of acrylic resin plates

A mold for acrylic resin plates, which was 35 × 35 × 3 mm, was prepared. A 3 mm layer of base plate wax (Plate wax rose; Degussa Dental, Vienna, Austria) was placed between 2 heat-resistant glass plates measuring 35 × 35 × 5 mm and flasked with dental stone (New Plastone; GC, Tokyo, Japan) according to the conventional procedures. As soon as the stone was set, flasks were separated, then the wax was removed.

Polymerization of acrylic resin materials (Vertex RS; Dentimex, Netherlands) was performed in strict compliance with the manufacturer' s instructions. The clamped flask with heat-polymerizing resin was immersed in boiling water for 20 minutes. Then the flask was bench cooled before deflasking.

After a completion of polymerization, each plate was retrieved from the mold and all six sides of the plates were mechanically polished with #320, #600, and 1000 grit SiC papers on the polishing machine (OMNILAP 2000 SBT Inc). The plates then were cleaned with compressed steam.

3. TiO_2 thin film coating and characterization

One of the resin plates was coated with the LT photocatalyst. The specimen was coated with TiO_2 (LT) and binder material (silane) using a dip-coater (1 mm/min speed) and was dried for 2 hours at 80℃.

The specimens divided into two classes according as whether they were coated or not ; 1) polished type, 2) TiO_2 coated type. Prior to testing, the polished specimens were stored in tap water at 22℃ for 48 hours.

For the characterization of TiO_2 film, field emission scanning electron microscope/ energy dispersive x-ray spectrometer (FE-SEM, XL 30 SFEG, Phillips Co., Netherlands / EDS, Link Analytical LTD. AN ISIS 310, England), UV spectrophotometer (UV-1601), XRD and contact angle analyzer (Phoenix 450) were used to confirm nano-coating of TiO_2, chemical composition on particle surface, photocatalytic activity with methylene blue, the crystallinity and hydrophilicity of TiO_2 film.

Specimens were observed under SEM at low magnification(× 2,000) and high magnification(× 100,000) each. XRD patterns were obtained at the angle of diffraction, 2θ=10-70 ° . The operating conditions of the X-ray source were 30 KV, 15 mA, target=Cu, and a scan speed of 2° / min. 35ml of methylene blue solution at 10 ppm of initial concentration was dropped on Petri dish, then the specimens of the experimental and control groups were put on the Petri dish and adsorbed the methylene blue for 30min. The amount of methylene blue degradation was measured for 2 hours with the UVA light on. Right and left contact angle was measured 5 times and the average was taken.

4. Microorganisms

Candida albicans KCTC 7629 was inoculated in a BHI (Bacto™ Brain Heart Infusion; BD, USA) agar medium and grown aerobically at 37℃ for 24h. One colony of the culture strain was taken and was inoculated into the 3ml of BHI broth.

After overnight culture, an accurate measurement of optical density of the cells was done by enzyme-linked immunosorbent assay (ELISA) at 450 nm. Then, the yeast was centrifuged, washed twice with phosphate-buffer saline (PBS, pH 7.2), and resuspended to required final concentration (10^5cfu/ml) by serial dilution with 1 × PBS.

5. Antifungal activity test

Each specimen was placed in a Petri dish. 1 ml of the fungal solution was pipetted onto the coated and polished specimens and the specimens were illuminated from 15cm above with two of UVA light (2 × 10W, 352 nm peak emission, Blacklight Blue; Sankyo Denki, Japan). Each 2 ㎕ of sample was plated on the BHI agar plate in duplicates at 10 min intervals for 2 h. The temperature increase of the samples was 8℃ (from 27 ℃ to 35℃) at most after 120 min of irradiation. All plates were incubated for 24 h at 37℃, and the colony-forming units (CFUs) were then counted.

controls: The effect of UVA light alone on the microorganisms was determined on uncoated (polished) specimen.

RESULTS

1. Characterization of TiO_2 photocatalysts

LT photocatalyst used in this study is composed of TiO_2 which has photocatalytic activity under room temperature. Fig. 1 shows XRD patterns of the LT particles dried under 100℃ and the commercial P-25 photocatalyst. LT showed the diffraction peak of anatase phase at 25° but showed

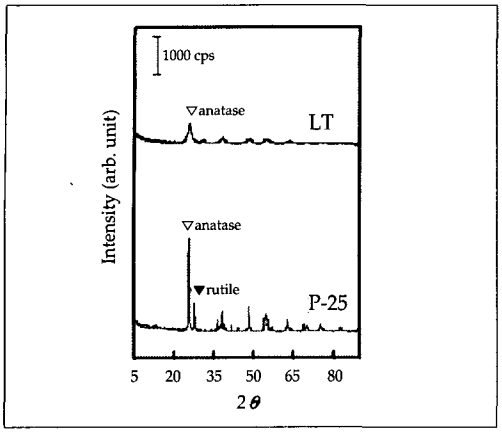


Fig. 1. XRD patterns of LT and P-25 photocatalysts.

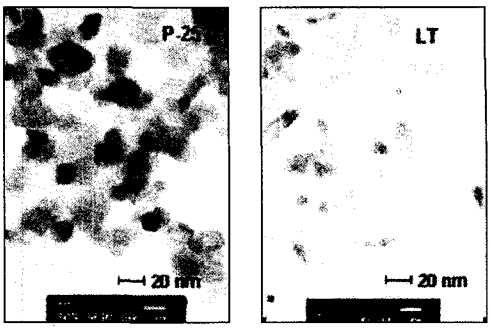


Fig. 2. TEM images of LT and P-25 photocatalysts.

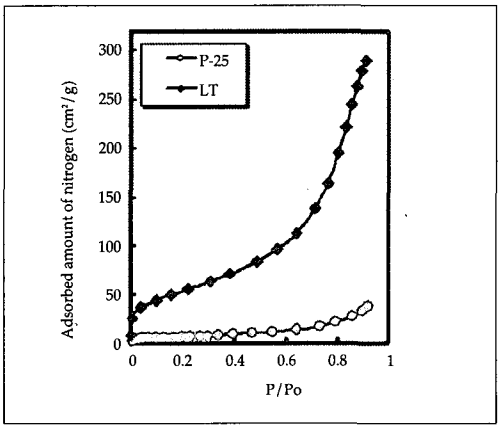


Fig. 3. Nitrogen adsorption isotherms of the LT powders dried at 100℃ and P-25 photocatalysts.

very little rutile phase. On the contrary, P-25 showed not only the peak of anatase phase but also that of rutile phase at 27, 36, 41°. The rutile content of the P-25 measured from the peak area was 20-25%, which seemed somewhat high.

Fig. 2 is TEM photographs which show the size and morphology of TiO_2 particles. P-25 particles were 20-50 nm in diameter and had irregular shapes. The shape of LT particle was like an embryo bud of rice which was 30 nm in long axis and 0-7 nm in short axis.

Fig. 3 shows N_2 adsorption isotherms to measure the surface areas of LT powder dried at 100℃ and P-25 photocatalysts. Surface area of LT was about 200㎡/g, which was four times larger than that of P-25 (about 50m^2/g).

2. Characterization of TiO_2 thin film supported on acrylic resin

XRD was used to detect the anatase and rutile crystallite phases of TiO_2 coated surface. XRD patterns of the polished and TiO_2-coated specimens are shown (Fig. 4). The diffraction peaks of polished type had a very low intensity indicating that the material was amorphous. In TiO_2-coated type, intensities of diffraction peaks were too weak to confirm their crystalline structure; Despite this, the observed peaks corresponded to the anatase phase. And these patterns showed a high dispersion of TiO_2.

To investigate the shape and size of the TiO_2 particles, the author examined the TiO_2-coated specimen with scanning electron microscopy. And general surface texture of the specimen was compared with the polished specimen. Fig. 5 shows SEM images of the specimens. TiO_2 thin film consist-

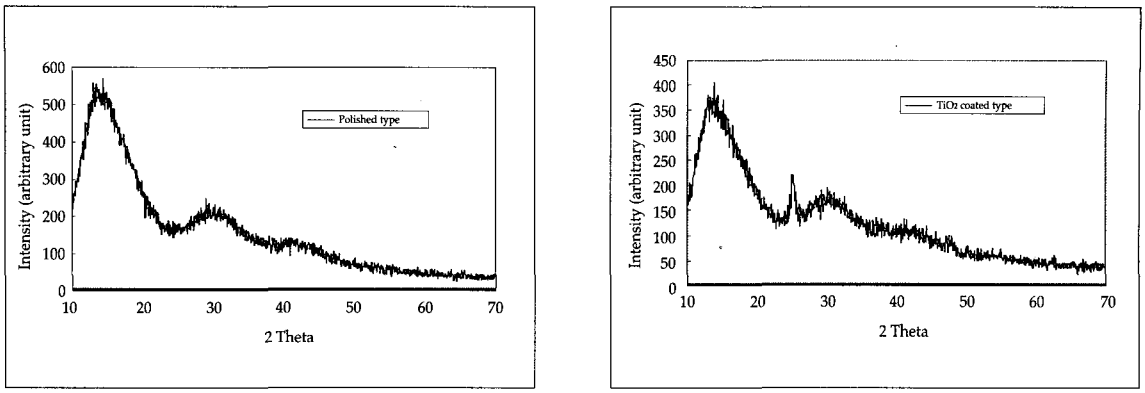


Fig. 4. XRD patterns of polished and TiO_2(LT)-coated acrylic resin specimens.

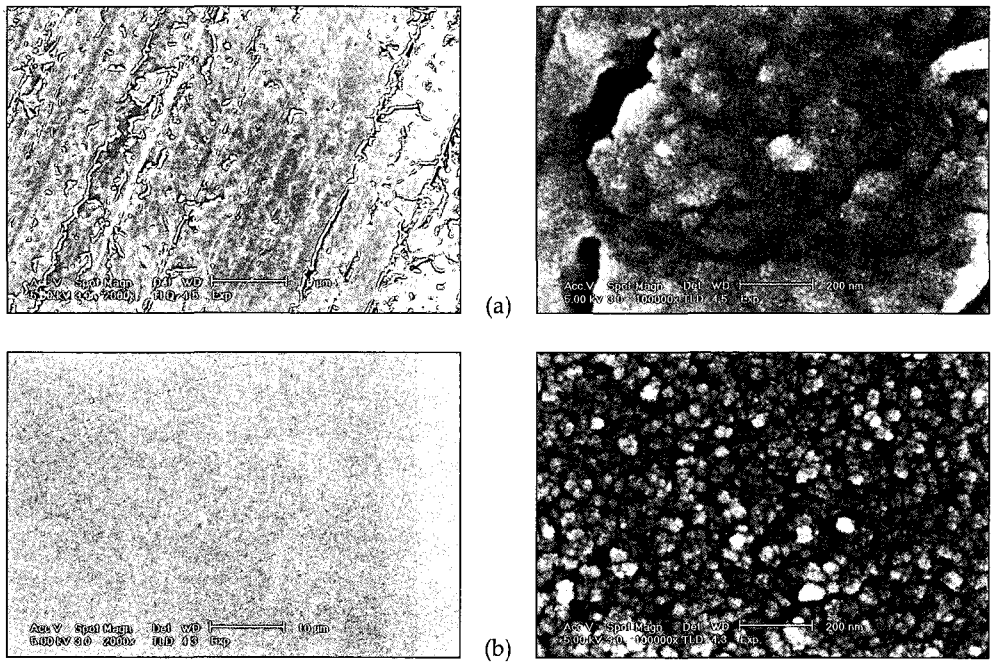


Fig. 5. SEM images of polished(a) and TiO_2(LT)-coated(b) acrylic resin specimens. (Upper: × 2000, Lower: × 100000)

ed of heterogeneous spherical particles with a very small size (below 20 nm). At low magnification, polished specimen had a rough surface and scratch due to polishing procedure. On the contrary, TiO_2-coated specimen had a relatively smooth surface.

Fig. 6 shows the photocatalytic degradation efficiency of methylene blue on polished and LT-coated acrylic resin specimens. Methylene blue is useful to evaluate photocatalytic activity since it is easy to detect the color change in accordance with the degree of degradation. More than 5% of methylene blue was degraded after 2 hours in LT-coated specimen. However, uncoated spec-

Table I. EDS data of specimens

Element	Wt%	At%
C	76.61	81.35
O	23.39	18.65
Total	100.00	100.00

<polished specimen>

Element	Wt%	At
C	7.55	14.75
O	39.70	58.25
Na	1.70	1.73
Si	0.69	0.58
Ti	50.36	24.68
Total	100.00	100.00

<TiO_2(LT)-coated specimen>

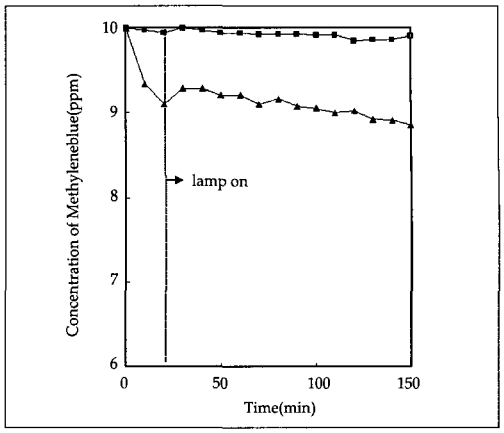
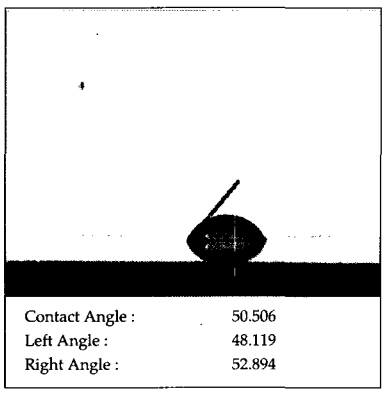
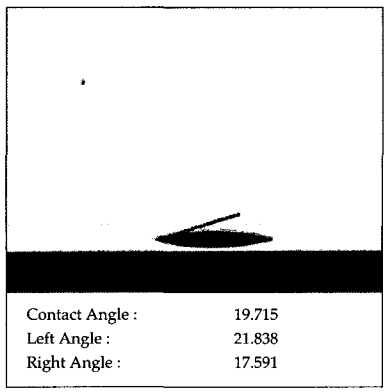


Fig. 6. Photocatalytic degradation efficiency of methylene blue on polished(■) and TiO_2 (LT)-coated(▲) acrylic resin specimens.



(a)



(b)

Fig. 7. Mean contact angle of polished(a) and TiO_2(LT)-coated(b) acrylic resin specimens.

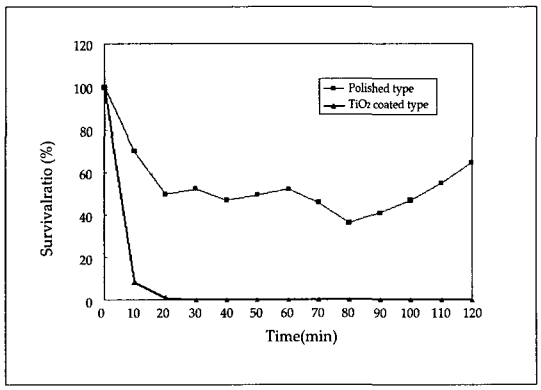


Fig. 8. Antifungal activity against *Candida albicans*.

imen showed almost no change after UV irradiation.

The hydrophilic property of photocatalyst is the phenomenon where H_2O molecule adsorb to the surface of a material to develop the highly hydrophilic membrane, therefore, water drops to disperse without drop formation. In this study, the uncoated specimen showed well-formed water drop. In LT-coated specimen, however, water dispersed to form aqueous membrane with its hydrophilic property.(Fig. 7) It means that it' s easy to get rid of contaminated materials.

3. Photocatalytic antifungal activity

Inactivation of *Candida albicans* by TiO_2 is shown in Fig. 8. The time course of viable cells when cell suspensions were irradiated with TiO_2 under Black Light fluorescent lamp was determined. The number of viable cells decreased rapidly and complete inactivation was achieved after 30 min. However, UV light only without TiO_2 had a little effect on cell survival and about 50% of the cells lost viability. In addition, complete killing was not achieved after 120 min of illumination. On the contrary, the increase of the viable cell number was observed at the final stage. It was due to the use of young cultures being in a state of two about to divide into four.

DISCUSSION

Bacterial and yeast plaque on dentures is thought to be an important etiologic factor in the pathogenesis of denture stomatitis. Since these fungi have been reported to colonize easily and penetrate denture materials, particularly tissue conditioners, and mechanical cleaning per se is insufficient to remove harbored *Candida* and harmful to soft-lining materials, chemical cleansing is suggested to be indispensable to denture plaque control. Therefore, many denture cleansers have been marketed for the removal or reduction of denture plaque. However, it has been pointed out that the chemical denture cleaning and sterilization often induced some problems such as color change, porosity and surface roughness on the denture base resin[14] as well as porosity and surface roughness on the tissue conditioner. Moreover, in the preliminary study, fungicidal effects of some cleansers tested were less effective with a soaking period of 30 minutes.[29]

The ability to reduce the colonized yeasts and/or fungal biofilm from acrylic resin may be particularly important in clinical term, since it is widely accepted that the biofilm organisms in vitro have a substantially reduced sensitivity to chemical agents such as antibiotics compared with the same organism in the dispersed form.[30]

The use of the photocatalytic materials for denture cleaner seems to be able to solve those problems. In this study, *C. albicans* with 10^5 cfu/ml deposited on TiO_2-coated acrylic resin specimen was illuminated for 2 h. The results of UVA-TiO_2 reaction showed that complete inactivation was achieved after 30 min.

However, the lethal mechanism of TiO_2 is not well understood yet. Some authors have proposed that the cell wall is the target of this effect. Sunada et al. showed endotoxin degradation in *E. coli*[31], and Pinching et al. suggested the peroxidation of lipids as the initial effect.[32] Some papers deal with the structural damage on the bacterial cell; Satio et al. reported by TEM analysis a complete destruction of *Streptococcus sobrinus* AHT cells, after 60-120 min of photocatalytic action. They suggested a change in cell membrane permeability.[33] *C. albicans* has a thick eukaryotic cell wall. So, *C. albicans* appears to be more resistant to cleansers than the bacteria.[34] It is assumed that primary step in photocatalytic decomposition is an attack by OH radicals on the cell wall, leading to punctures.

Since the effectiveness of heterogeneous catalysis also depends on the adsorption of reaction partners to the TiO_2,[35] it would be interesting to measure the surface charge and the hydrophobicity of the bacteria and surfaces, and to correlate these findings with the sensitiveness to photocatalystic oxidation.

LT photocatalyst used in this study is specific synthesis which is able to coated to the acrylic resin at room temperature. In XRD patterns,(Fig. 2) LT photocatalyst which has few rutile structure is expected to have higher activity than P-25, because TiO_2 in anatase phase generally has

been proved to have higher efficiency than in rutile phase.[36,37] Since degradation activity by the photocatalyst is shown on the surface of the TiO_2 particles, we can figure out that photocatalytic activity of LT with surface area four times larger than p-25 is maximized.

To date, little emphasis has been placed on the possibility of preventing plaque formation and accumulation on dentures. LT photocatalyst-coated acrylic resin specimen used in this study had a hydrophilicity.(Fig. 7) We expect the self cleaning effect duo to this hydrophilicity which suppress biofilm formation on the denture base. The larger the contact angle is, the more irregular the surface has as the uncoated specimen. Irregularities and porosities present on the denture surface play a major role in reducing the activity of denture cleaning agents and hence increased stain and plague retention.

It is confirmed by the results of SEM images.(Fig. 5) Uncoated specimen had irregularities, microcrack, and scratch made by polishing precedure. But, LT photocatalyst-coated specimen had a smooth surface, because the irregularities could be filled up with TiO_2 particles with a small size below 20 nm.

UVA light source with 352 nm peak emission was used in this study. The light used for photocatalyst is high energy-ultraviolet light whose mean wavelength is shorter than 400 nm, and it is included in sunlight or lighting in the residental space. In comparison to the whole intensity of light, however, usable amount of ultraviolet light is very small. In case of sunlight, ultraviolet light intensity during day when the light is the most intensive is about 1 mW/cm^2, which is just 1% of the whole sunlight. So, in the room with less light intensity, the technology to maximize the amount of usable ultraviolet light is necessary.

The photochemical reaction of TiO_2 comes from the reaction of hole and electron generated from the resorption of the photo-energy which corresponds to TiO_2 band gap energy (3.4 eV). The measures to lower the band gap energy to utilize long waved photo-energy should be investigated by slowing down the reunion rate of these electron pairs and doping method etc.

Generally, as in this study, photocatalyst adheres to the substrate by using condensing polymer of silicon alkoxide as a binder, or the photocatalyst nano-particles are fixed to the substrate by using fluorine resin as a binder which is relatively stable against photocatalytic reaction. A little amount of the silane binder can make fixed membrane with high hardness under high hardening temperature. The types and amount of the binder should be chosen in accordance with the substrate. The amount of the binder should be controlled according to the properties of matter that the operator is willing to use, because the increase of the membrane strength from the increased amount of the binder and the photocatalyst activity are in inverse proportion to each other. This study didn' t consider binding strength of the binder and TiO_2 sol to the specimen, and so additional investigations about this subject are required.

CONCLUSIONS

Within the parameters of the study design and materials tested, it was possible to disinfect acrylic resin denture surfaces coated with a specific semiconductor(TiO_2) and stimulated by UVA. The results reported above suggest that TiO_2 photocatalysis may be a viable process for inactivation of *C. albicans* and could be applicable to the clinical use as a cleansing method.

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