INTRODUCTION

Tissue conditioners have been used in clinical routine to improve the condition of abused denture-bearing tissues\(^1\). However, such materials degenerate with time and are susceptible to colonization by microorganisms\(^2\). Microorganisms on denture materials may cause additional diseases in elderly persons, such as a denture stomatitis and pneumonia\(^3,4\). These problems are believed to originate from the change of the surface roughness after application and the difficulty in mechanical and chemical cleaning\(^5\). Many studies have been performed to prevent the colonization by microorganisms. Some investigations showed that a surface coating of tissue conditioners could preserve the surface smoothness\(^6-9\). Some studies have reported the antimicrobial effects of agents incorporated in tissue conditioners\(^10-14\).

Photocatalysts have attracted considerable attention in the current environmental market\(^15-17\). In particular, titanium dioxide (TiO\(_2\)) is a stable photocatalyst. Radicals are generated in response to irradiation of TiO\(_2\) with ultraviolet light. These radicals oxidize organic substances into water and carbon dioxide. Calcium hydroxyapatite (HAP) is also an interesting biomaterial because of its capacity to adsorb organic substances\(^18,19\). An atomic-level composite of a photocatalytic material has been developed by modifying calcium hydroxyapatite with Ti (IV)\(^19\). This resulting material possesses both an intense photocatalytic ability and adsorption affinity.

Based on the encouraging outcome of the photocatalytic composite material, this study speculated that the incorporation of photocatalyst into a tissue conditioner might likewise generate radicals and effectively resolve denture plaque, resulting providing the tissue conditioner with self-cleaning ability.

The aim of this study was to evaluate the antimicrobial and antifungal effect of a tissue conditioner containing a photocatalyst. This material will make cleaning dentures easier for a wide spectrum of beneficiaries: elderly persons, institutionalized patients, caregivers, and healthcare professionals.

MATERIALS AND METHODS

The tissue conditioner used in this study was Shofu TISSUE CONDITIONER II which consists of a powder, poly ethyl methacrylate and a liquid comprising dibutyl sebacate and ethanol (Shofu Inc., Kyoto, Japan).

**Specimen preparation**

The photocatalyst used in this study was PHOTOHAP (HAP modified with Ti (IV), Taihei Chemical Industrial Co. Ltd., Osaka, Japan). The composition at the site of Ca (II) in the hydroxyapatite was changed to Ti (IV) in this material. This material is composed of white particles measuring 3.78 µm in diameter, with a specific surface area of 41.59 m\(^2\)/g.

A total of 0.96, 1.44 and 1.92 g of the photocatalyst powders were added into 8.64, 8.16 and 7.68 g of tissue conditioner powder and mixed with a blender. The photocatalyst-containing tissue conditioner mixtures were evaluated by the CFU technique. The CFU values of each microorganism for tissue conditioners containing a photocatalyst showed significant decrease following UV-irradiation. The improvement in antimicrobial/antifungal effects was concomitant with the increase of the mixing ratio and the irradiation time. Therefore, the results indicated that tissue conditioners containing a photocatalyst might have photocatalytic ability.
were then mixed with 8.00 mL of liquid, respectively, thus resulting in a concentration ranging in from 0 to 10, 15 and 20% according to the weight of the powder (photocatalyst/powder). The mixture was placed between diaphragms 1 mm thick, which were then sandwiched by glass plates. They were hollowed out using a 24 mm diameter cylindrical cork cutter and soaked in distilled water for 24 hours. Test specimens were placed into 6-well plastic dishes (MULTIWELL™, BECTON DICKINSON, San Jose, USA). Each dish was wrapped to maintain humid conditions. Wrapped test specimens were irradiated by ultraviolet light (352 nm, FL15BLB, Toshiba Co., Tokyo, Japan) for 2 or 4 hours per day. The irradiation was performed at a distance of 20 cm (0.4 mW/cm² under the wrap). Test specimens for the determination of antimicrobial ability on C. albicans were irradiated by ultraviolet light at a distance of 10 cm (1.0 mW/cm² under the wrap). This was repeated for 1, 3, 5, 7, and 14 days to examine the effects of the irradiation on tissue conditioner.

Photocatalytic ability determination

The photocatalytic ability was estimated based on the inactivation of the bacteria and yeast strains. The bacteria and yeast strains used in this study were Escherichia coli NBRC3972 (E.coli), Streptococcus mutans UA159 (S.mutans), Staphylococcus aureus FDA209P (S.aureus), and Candida albicans ATCC10231 (C.albicans). E. coli and S. aureus are relevant for hygiene. S. mutans is commonly found in the human oral cavity and contributes to dental decay[20]. C. albicans is also found in the human oral cavity and is a causative factor in denture-induced stomatitis[21,22].

E.coli cells were cultured on growth medium agar (10 g polypepton, 2 g yeast extract, 1 g MgSO₄·7H₂O, 1,000 mL distilled water, 15 g agar, pH 7.0) plates. E.coli cells were grown aerobically to the stationary phase and subcultured in growth medium to the mid-logarithmic growth phase in a test tube at 30°C.

S.mutans cells were cultured anaerobically on brain heart infusion broth (BHI; Bact™ Becton Dickinson, USA) agar plates. S.mutans cells were grown anaerobically to the stationary phase and subcultured in BHI to the mid-logarithmic growth phase in a test tube at 37°C.

S.aureus cells were cultured on Tryptone-yeast extract medium agar (L-broth; 5 g yeast extract, 10 g tryptone, 5 g NaCl, 1,000 mL distilled water, 15 g agar, pH 7.0) plates. S.aureus cells were grown aerobically to the stationary phase and subcultured in L-broth to the mid-logarithmic growth phase in a test tube at 37°C.

C.albicans cells were cultured on yeast extract peptone dextrose broth agar (YEPD; 10 g yeast extract, 20 g peptone, 20 g dextrose, 1,000 mL distilled water, 20 g agar, pH 7.0) plates. C.albicans cells were grown aerobically at the stationary phase and subcultured in YEPD to the mid-logarithmic growth phase in a test tube at 37°C.

Test specimens were sterilized by 0.025% benzalkonium chloride solution and then separately placed into 6-well plastic dishes. A volume of 300 µL of microorganism suspension, adjusted to each mid-logarithmic growth phase was dropped directly on each specimen. The dish was wrapped to maintain humid conditions. Wrapped test specimens were irradiated by ultraviolet light at a distance of 20 cm. Test specimens containing C.albicans were irradiated by ultraviolet light at a distance of 10 cm.

The test specimens were divided into three groups, specimens of each groups were irradiated for 0, 2 and 4 hours (irradiated group). Test specimens in the 0-hours group were not irradiated before the antimicrobial test. Medium (2,700 µL) was added to the test wells following irradiation and mixed with the irradiated suspension. The mixture was diluted in a 10-fold series to 10⁻¹. A diluted suspension of bacteria and yeast strains was placed onto each agar plate and incubated aerobically at 37°C for 24 hours. Viable cells were counted according to the number of colony-forming units (CFUs). The experiment was repeated three times.

Another set of test specimens remained in a dark box for 2 or 4 hours after inoculation (non-irradiated group), and then incubated in the same manner as the irradiated group.

Five specimens were used for each experiment, thus resulting in a total of 1,800 specimens.

Observation of the surface of specimens

The test specimens were made in the same manner of those for antimicrobial and antifungal tests to observe the surface appearance. The test specimens were irradiated in the same manner of those for antimicrobial and antifungal test. The test specimens were coated with gold (24 nm in thickness) and observed using a scanning electron microscope (JSM LV5000, JEOL Ltd., Tokyo, Japan).

Statistical analysis

All data were analyzed using one-way ANOVA and Scheffe’s multiple range test at a significance level of p<0.01.

RESULTS

The CFU values of each microorganism incubated with photocatalyst-free specimens in a dark box for 2 or 4 hours showed no significant difference from those of 0 hour. The CFU values of E.coli, S.mutans and C. albicans for photocatalyst-containing specimens did not show a significant reduction after 2 or 4 hours. There were slight reductions in the CFU values of S.mutans incubated on photocatalyst-containing specimens. However, there was no significant difference. The CFU values of S.aureus incubated with specimens containing 15- and 20 wt% of photocatalyst without irradiation for 4 hours were significantly lower than those at 0 hours (Fig.1).

The CFU values of each microgram cultured with the UV-irradiated specimens were significantly lower than those of the non-irradiated specimens. The reduction of CFU values were inversely correlated with
an increase in the mixing ratio of the photocatalyst. The CFU values of *E. coli* cultured with photocatalyst-free specimens showed a slight reduction after irradiation. CFU values of *E. coli* for specimens containing 15- and 20 wt% of photocatalyst showed a significant reduction after irradiation. The CFU values of *S. mutans* and *S. aureus* cultured on specimens containing 10-, 15- and 20 wt% of photocatalyst demonstrated a significant reduction after irradiation for 4 hours.

CFU values of *C. albicans* after irradiation at a distance of 20 cm showed an ambiguous reduction in a preliminary experiment. Therefore, the irradiation on specimens was carried out at a distance of 10 cm. The CFU values of *C. albicans* exposed to specimens containing 10-, 15- and 20 wt% of photocatalyst were significantly reduced after irradiation (Fig. 2).

The CFU values of *S. mutans* cultured with specimens containing 15- and 20 wt% of photocatalyst irradiated for 4 hours were significantly lower than those irradiated for 0 hours at 3, 5, 7 and 14 days after polymerization (Fig. 3(a)). The CFU values of *E. coli* cultured with specimens 15 and 20 wt% of photocatalyst and those of *S. aureus* cultured with specimens 20 wt% of photocatalyst irradiated for 4 hours were significantly lower than those irradiated for 0 hours at 3 days after polymerization (data not shown). The CFU values of *C. albicans* cultured with specimens of 10-, 15- and 20 wt% of photocatalyst and irradiated did not show any reduction at 3, 5, 7 and 14 days after polymerization (Fig. 3(b)).

The surfaces of photocatalyst-free specimens were smooth through the experimental period. Particles (0.4–4 nm in diameter) were distributed on the surface of specimens containing 10-, 15- and 20 wt% photocatalyst 1 day after polymerization (Fig. 4).

The surface of specimens containing 15 and 20 wt% of photocatalyst showed an irregular and rough appearance at 5, 7 and 14 days after polymerization. Such a change of the surface appearance was observed in both the irradiated specimens and the non-irradiated specimens (Fig. 5).
DISCUSSION

The findings of this study indicated that a tissue conditioner containing a photocatalyst obtained antimicrobial ability for *E. coli*, *S. mutans* and *S. aureus* and antifungal ability for *C. albicans*. The improvement in antimicrobial ability and antifungal ability was dependent upon the increasing mixture ratio of the photocatalyst and the increasing irradiation time in the irradiation group.

There was a difference in the antimicrobial ability among microorganisms. Blake *et al.* described that the efficiency of photocatalysts varies with the type of microorganism. They suggested that the efficiency of photocatalysts is influenced by the complexity and thickness of the cell wall. Kühn *et al.* reported that the reduction efficiencies decreased in order of *E. coli* > *P. aeruginosa* > *S. aureus* > *E. faecium* > *C. albicans*. They suggested that this order was mainly determined by the complexity and density of the cell wall. Ultraviolet irradiation of TiO$_2$ causes electrons and holes to be generated in the TiO$_2$. The holes react with H$_2$O and produce hydroxyl radicals. Radicals oxidize organic substances into water and carbon dioxide. The first step in the photocatalytic attack to microorganisms is the decomposition of cell walls. *E. coli*, *S. mutans* and *S. aureus* were easily inactivated in the current study, while *C. albicans* had a stout resistance to photocatalytic decomposition. The cell wall of bacteria is thin and slack. The cytoplasmic or plasma membrane of bacteria lies underneath the cell wall, to which bacterial DNA is attached. Therefore, the destruction of the cell membrane may exert a deleterious effect on cell survival. On the other hand, *C. albicans* is a eukaryotic organism having a thick and complex cell wall. This cell structure allowed *C. albicans* to resist the effects of the photocatalyst.

In contrast to the report by Kühn, *S. aureus* were inactivated more easily than *E. coli* in this study. *S. mutans* and *S. aureus* were inactivated even under non-irradiated conditions. In particular, The CFU values of *C. albicans* exposed to specimens containing 10-, 15- and 20 wt% of photocatalyst were also significantly reduced after irradiation (*p* < 0.01).

Fig. 2 CFU values in irradiated conditions. The CFU values of *E. coli* cultured with photocatalyst-free specimens showed a slight reduction after irradiation. The CFU values of *E. coli* for specimens containing 15- and 20 wt% of photocatalyst showed a significant reduction after irradiation. The CFU values of *S. mutans* and *S. aureus* cultured on specimens containing 10-, 15- and 20 wt% of photocatalyst demonstrated a significant reduction after irradiation for 4 hours. The CFU values of *C. albicans* exposed to specimens containing 10-, 15- and 20 wt% of photocatalyst were also significantly reduced after irradiation (*p* < 0.01).
*S. mutans* and *S. aureus* are Gram-positive cells, having thicker and denser cell walls than Gram-negative cells, but they were inactivated more easily than *E. coli*. The inactivation of *S. mutans* and *S. aureus* under non-irradiated conditions were not induced by the photocatalytic effect, but due to the adsorption affinity of hydroxyapatite. Bacterial adhesion to biomaterials is dependent on their surface hydrophobicity and surface electric properties. Sonohara et al. measured electrophoretic mobilities of *E. coli* and *S. aureus* to investigate the surface electrical properties and demonstrated that *E. coli* had a more negatively charged and rigid surface than *S. aureus*. *S. aureus* and *S. mutans* were considered to have surface properties prone to adhere to the hydroxyapatite. In this study, *S. aureus* were more easily inactivated than *S. mutans* under non-irradiated conditions. The factor responsible for such a difference in adsorption affinity is unclear, but may have been due to differences in the components of the cell wall.

While *C. albicans* incubated on photocatalyst-free specimens proliferated, that incubated on specimens containing a photocatalyst did not proliferate under non-irradiated conditions. This result indicated that *C. albicans* was adsorbed by hydroxyapatite. *C. albicans* was resistant to photocatalytic inactivation. On the other hand, *E. coli* cultured with photocatalyst-free specimens was inactivated by ultraviolet irradiation. *E. coli* was therefore considered to be inactivated by ultraviolet irradiation, and then subsequently suffered from the photocatalytic attack. Therefore, there were three major mechanisms by which microorganisms were inactivated:

**Fig. 3(a)** The CFU values of *S. mutans* cultured with specimens at 1-, 3-, 5-, 7- and 14 days after polymerization. The CFU values for specimens containing 15- and 20 wt% of photocatalyst irradiated for 4 hours were significantly lower than those irradiated for 0 hours at 3, 5, 7 and 14 days after polymerization.
by ultraviolet irradiation, absorption by hydroxyapatite, and photocatalytic decomposition. Further experiments will need to be carried out to evaluate the inactivation efficiency of the treatment. However, *E. coli* was sensitive to ultraviolet light, while *C. albicans* showed strong resistance to ultraviolet light, absorption by hydroxyapatite and photocatalytic decomposition. The aim of this study was to evaluate the photocatalytic effect, therefore, *E. coli*, *S. mutans* and *S. aureus* were irradiated at a distance of 20 cm, and *C. albicans* was irradiated at a distance of 10 cm. Therefore, there appear to be major differences in the sensitivity of different microorganisms to these treatments.

The tissue conditioner containing a photocatalyst in this study was demonstrated to adsorb microorganism in the dark and to damage them by irradiation.

Tissue conditioners are soft, resilient materials. Mechanical and chemical methods to clean dentures may cause some problems such as deformation and degradation of tissue conditioners. Microorganisms are easily colonized on the rough surface of degraded tissue conditioners. Photocatalytic cleaning is performed without mechanical force and chemical agents; therefore, it was thought to be effective and valid method.

However, this material had a short duration of antimicrobial and antifungal ability. The change in the surface appearance was observed in the specimens containing 15- and 20 wt% of photocatalyst. A TiO₂ photocatalyst activated by UV can only decompose protein in contact with the TiO₂. The rough surface may block the UV light and thus prevent the light from reaching the photocatalyst. The amount of liquid to poly

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**Fig. 3(b)** The CFU values of *C. albicans* cultured with specimens at 1, 3, 5, 7, and 14 days after polymerization. The CFU values for specimens containing a photocatalyst did not show any reduction at 3, 5, 7 and 14 days after polymerization.
ethyl methacrylate was large in the photocatalyst containing specimens. As a result, a large amount of plasticizer and alcohol might have lost. This phenomenon led to both the occurrence of a rough surface and a reduction in the antimicrobial and antifungal ability. In particular, the inactivation of *C. albicans*, which is a eukaryotic organism and has a complex cell wall, was not show at 3-, 5-, 7- or 14 days after polymerization because of strong resistance to decomposition. The change in the surface appearance of specimens containing 15 and 20 wt% of photocatalyst at 5, 7 and 14 days after polymerization suggested that there was a change in mechanical properties. Therefore, prior to using this material clinically, examination of its mechanical properties will need to be carried out.

Tissue conditioners are used for treating inflamed, distorted tissues. Therefore, this material must to be easy to keep clean without deformation. Photocatalytic cleaning is thought to be a simple and easy method for elderly persons, institutionalized patients, caregivers and healthcare professionals. The use of this material for clinical practice will require further investigations in order to: (1) determine the effective intensity of ultraviolet irradiation needed to increase antimicrobial and antifungal ability; and (2) to maintain the antimicrobial and antifungal ability for an extended period of time.

**CONCLUSION**

The results of this study are:

1. Tissue conditioner containing a photocatalyst
obtained an antimicrobial activity for *E. coli*, *S. mutans* and *S. aureus* and antifungal activity for *C. albicans*.

(2) There was a difference in the antimicrobial ability and antifungal ability among microorganisms. This was thought to be due to the structure and components of the cell walls.

(3) Tissue conditioner had a short duration of antimicrobial and antifungal ability.

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