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ORIGINAL ARTICLE

# Effectiveness of 405-nm blue LED light for degradation of *Candida* biofilms formed on PMMA denture base resin

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## Abstract

This study investigated (i) the degradation effect of 405-nm blue light-emitting diode (LED) light irradiation on *Candida albicans* and *C. glabrata* biofilms formed on denture base resin and (ii) the effects of 405-nm blue LED light irradiation on the mechanical and surface characteristics of the resin. Polymethyl methacrylate denture base resin discs were prepared, and *C. albicans* or *C. glabrata* biofilms formed on the denture base resin discs. Each biofilm was irradiated with 405-nm blue LED light under a constant output power (280 mW/cm$^2$) for different times in a moisture chamber with 100% relative humidity. Postirradiation, each biofilm was analyzed using a colony-forming unit assay, fluorescence microscopy, and scanning electron microscopy (SEM). Parallelepiped specimens of acrylic resin were prepared, and changes in their flexural strength (FS), flexural modulus (FM), and surface roughness ($R_a$) preirradiation and postirradiation with 405-nm blue LED light were evaluated. Irradiation for 30 min completely inhibited colony formation in both *Candida* species. Fluorescence microscopy showed that almost all *Candida* cells were killed because of irradiation. SEM images showed various cell damage patterns, such as wrinkles, shrinkage, and cell surface damage. An increase in FS was noted postirradiation, but no significant changes were observed in FM and $R_a$ preirradiation and postirradiation. In conclusion, irradiation with 405-nm blue LED light induces degradation of *C. albicans* and *C. glabrata* biofilms on denture base resin, even in the absence of photosensitizers, without resin surface deterioration.

**Keywords** 405-nm LED · *Candida* · Biofilm · Denture stomatitis · Fungistatic

## Introduction

*Candida albicans*, a principal causative microorganism of denture stomatitis (DS) [1, 2], is a fungus most frequently isolated from denture plaque. *C. glabrata*, another *Candida* species, is the second-most predominant fungus isolated from denture plaque and can also cause DS regardless of the presence of *C. albicans* [3, 4]. The initial, critical steps in denture plaque development are (i) adhesion of microorganisms to denture surfaces and (ii) subsequent biofilm formation [5], especially *Candida* species, which adhere easily to fitting surface of dentures [6, 7] and form a large biomass as early as 12–30 h postadhesion [8–10].

One of the most effective methods of preventing DS is daily efficient cleaning of dentures to inhibit these fungi from adhering to denture surfaces. The most commonly recommended method for cleaning dentures is a combination of brushing and immersion in a chemical cleanser [11]. However, it is difficult for patients with motor incoordination to properly brush dentures [12]; therefore, a high amount of plaque can remain on denture surfaces. In these cases, long-term immersion in a chemical cleanser is necessary for residual plaque removal.

Although chemical cleansers can reduce microbial viability and biomass volume on denture surfaces, viable microorganisms still remain, which might cause DS [13, 14]. In addition, long-term immersion in chemical cleansers might adversely alter the physical and mechanical properties of artificial denture teeth, base materials, and the metal alloy [2, 15]. Therefore, a new cleansing method of removing denture plaque that cannot be removed by conventional procedures should be developed.

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Antimicrobial photodynamic therapy (aPDT) is a technique that combines visible light of a suitable wavelength with a photosensitizer in order to produce reactive oxygen species (ROS), which damage targeted microorganisms [16]. This widely studied technique is currently considered a possible way of disinfecting denture surfaces. In fact, studies have reported that the combination of light of different wavelengths and photosensitizers reduces *Candida* biofilms on dentures [17–19]. However, this technique needs a preirradiation step, in which the photosensitizer reacts with microorganisms [17–19]. In addition, light of specific wavelengths requires repeated irradiation in order to significantly reduce *Candida* biofilms on denture compared to single irradiation [19]. However, the effects of irradiation and photosensitizers on denture base resin have not yet been clarified.

Blue light-emitting diode (LED) light of 405-nm wavelength has been proven to have bactericidal/fungicidal effects, even without any photosensitizers [20–22]. The same studies have also reported that 405-nm blue LED light irradiation inhibits *Porphyromonas gingivalis*, *Prevotella intermedia*, *Staphylococcus aureus*, and *C. albicans* growth [20–22]. These findings strongly suggest that 405-nm blue LED light can inhibit *Candida* biofilm formation on dentures and can also disinfect denture surfaces quicker than conventional aPDT because of needing no photosensitizers. However, to date, it is unclear whether 405-nm blue LED light can inhibit *Candida* biofilm formation on denture base resin.

This study aimed to investigate the degradation effect of 405-nm blue LED light irradiation on *C. albicans* and *C. glabrata* biofilms formed on denture base resin and the effects of 405-nm blue LED light irradiation on the mechanical and surface characteristics of the resin.

## Materials and methods

### Specimen preparation and biofilm production

A set of 80 polymethyl methacrylate (PMMA) denture base resin discs (diameter: 10 mm; thickness: 2 mm; ACRON, GC, Tokyo, Japan) was prepared by sectioning cylindrical PMMA specimens (diameter: 10 mm; length: 80 mm) into discs [23]. The upper and lower surfaces of each denture base resin disc were polished with 320-grit abrasive paper under dry conditions. The surface roughness ($R_a$) of each denture base resin disc was determined by a profilometer (Surfcom Flex, Seimitsu, Tokyo, Japan); the mean value of two measurements was 1.12 ± 0.15 μm. All denture base resin discs were sterilized with ethylene oxide gas (EOG), stored in a sterilization chamber at 40 °C for 24 h to remove residual EOG, and then immediately used for investigation.

Each denture base resin disc was placed in a well of a 24-well plate with 500 μL of artificial saliva; 1.25 mM $Ca(NO_3)_2$ $4H_2O$, 0.90 mM $KH_2PO_4$, 129.91 mM KCl, 59.93 mM Tris buffer, and 2.2 g/L porcine gastric mucin (pH 7.4) [23]. The plate was incubated on a shaker at 75 rpm at 37 °C for 60 min and then washed twice with 1 mL of phosphate-buffered saline (PBS; pH 7.2). *C. albicans* (ATCC18804) and *C. glabrata* (ATCC90030) were grown in tryptic soy broth supplemented with 5% dextrose (TSBD; Becton, Dickinson and Company, NJ, USA) on a shaker at 75 rpm at 30 °C for 5 h. The yeast-like cells were standardized at $10^7$ cells/mL in TSBD medium using a counting chamber (One Cell Counter, Biochemical Science, Tokyo, Japan). For biofilm formation, we added 1 mL of the *Candida* cell suspension to each well and then the plate was incubated on a shaker at 75 rpm at 37 °C for 24 h. Finally, non-adherent cells were removed by washing the specimens twice with PBS.

### Irradiation conditions

The specimens were irradiated using an irradiation device equipped with a 405-nm blue LED (Osada Electric Co. Ltd., Tokyo, Japan). A spectrophotometer (Anritsu Keiki Co. Ltd., Tokyo, Japan) was used to confirm wavelength emission from the blue LED (Fig. 1). It covered the wavelength range from 380 to 440 nm, with maximum emission at 405 nm. The output power was measured thrice with a power meter equipped with a photodiode sensor (PD-300-UV, Ophir Optronics Ltd., Israel) and set at a constant value of 280 mW/cm$^2$. The diameter of irradiation tip was 0.32 cm with the beam divergence of 2.37 rad, and only the top surface (0.79 cm$^2$) of each specimen was irradiated at a distance of 1 cm. The specimen was recovered and placed in the center of a light-blocking cylindrical tube (1.5 cm in diameter and 1.2 cm in length) in a moisture chamber at 100% relative humidity. For the colony-forming unit (CFU) assay, denture base resin discs with either *C. albicans* or *C. glabrata* biofilms were irradiated with 405-nm blue LED light in a moisture

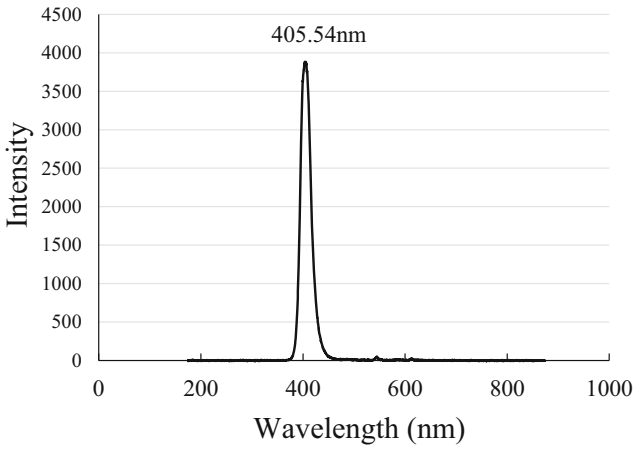


**Fig. 1** Spectrophotometry confirming a spectrum of blue LED light with a 405-nm peak wavelength LED, light-emitting diode

chamber at 100% relative humidity and at various irradiation times: 0 min (control), 5 min (47.4 J/cm$^2$), 10 min (94.8 J/cm$^2$), 20 min (189.6 J/cm$^2$), and 30 min (284.4 J/cm$^2$). On the basis of the CFU assay result, the denture base resin discs irradiated for 0 min (control) and 20 min (189.6 J/cm$^2$) were selected and further examined by fluorescence microscopy and scanning electron microscopy (SEM). Discs with biofilms were used randomly in each group.

### CFU assay

Four discs per each of the five groups were used for the CFU assay in the subgroups of *C. albicans* and *C. glabrata*. Each irradiated denture base resin disc was placed in a well of a 24-well plate, and 1 mL of PBS was added to each well. The *Candida* biofilm was scraped from each denture base resin disc with a cell scraper (Iwaki Co., Tokyo, Japan), and suspensions of dissociated cells were created by pipetting up and down [23]. Next, the fungal suspensions were serially diluted with PBS, and 100 μL of each suspension was inoculated on Sabouraud glucose agar plates. After aerobic incubation at 37 °C for 48 h, the CFUs were counted. As a preliminary calibration, one examiner (first author) accomplished the calibration procedures to confirm the consistency in the amount of biofilm on the basis of evaluation by means of SEM observation (data not shown). Thereafter, the same examiner performed all the final experiments.

### Fluorescence microscopy

Denture base resin discs of control and 20 min (189.6 J/cm$^2$) group (ten denture base resin discs from each group) were each placed in a well of a 24-well plate, and 500 μL of physiological saline (Otsuka Pharmaceutical Co., Tokyo, Japan) was added to each well. The biofilms on the denture base resin discs were then stained using the Live/Dead® FungaLight™ Yeast Viability Kit (Thermo Fisher Scientific, Waltham, MA, USA), which comprises SYTO9 and propidium iodide (PI). Briefly, 1 μL each of SYTO9 and PI was added to each well, and the denture base resin discs were incubated for 20 min in the dark at 30 °C. Then, the stained denture base resin discs were observed using a fluorescence microscope (BZ-X710; Keyence, Osaka, Japan).

### Scanning electron microscopy

Denture base resin discs of control and 20 min (189.6 J/cm$^2$) group (ten denture base resin discs from each group) were fixed with 2.5% glutaraldehyde at 4 °C overnight. Next, each denture base resin disc was dehydrated with graded concentrations of ethanol (50%, 60%, 70%, 80%, 90%, and absolute ethanol), transferred to liquidized t-butyl alcohol, and stored in a freezer until the butyl alcohol froze. Then, the specimens were transferred into a freeze-drying apparatus (ID-2; Eiko Engineering, Tokyo, Japan), where the frozen t-butyl alcohol was completely sublimated. Finally, the specimens were mounted on aluminum stubs, sputter coated with gold in an ion sputter coater (SC-701AT; Sanyu Denshi, Tokyo, Japan), and assessed using SEM (JCM-6000 NeoScope™; Jeol Ltd., Tokyo, Japan).

### Postirradiation characterization of denture base resin

For this investigation, we prepared 15 parallelepiped specimens (64 × 10 × 3.3 mm$^3$) using ACRON. The specimens were polished with 1000-grit abrasive paper under running water using a polishing device (ML-150P; Maruto Instrument Co., Ltd., Tokyo, Japan). Next, the specimens were irradiated all over the single-sided surface with 405-nm blue LED light under a constant output power of 280 mW/cm$^2$ for various irradiation times; 0 h (control; $n = 5$), 84 h (84,672 J/cm$^2$; $n = 5$), and 168 h (169,344 J/cm$^2$; $n = 5$). When a denture is irradiated for 30 min/day, irradiation times of 84 and 168 h are equivalent to exposure for a half year and 1 year, respectively. After irradiation for specified times, all specimens were stored at room temperature in total darkness for a month. Before examination, $R_a$ of all specimens was determined. Then, all the specimens were stored in deionized water at 37 °C for 50 h. Next, flexural strength (FS) and flexural modulus (FM) were calculated using a three-point bending test, in accordance with the International Organization for Standardization (ISO) 1567, with a universal testing machine (AGS-X; Shimadzu Corp., Kyoto, Japan) at a crosshead speed of 1 mm/min. The FM was calculated from the linear part of the load–time curve up to the proportional limit obtained by the three-point bending test.

### Statistical analysis

The CFU assay results were analyzed using the Kruskal–Wallis test. Significant differences among the groups were confirmed using the Mann–Whitney *U* test and Bonferroni correction. Results of the FS, FM, and $R_a$ of each specimen were subjected to one-way analysis of variance, followed by Tukey's honestly significant difference multiple comparison test. The significance level was set to 0.05. All analyses were performed using SPSS ver. 21.0 for Windows (IBM, NY, USA).

## Results

### CFU assay

Results of the CFU assay showed a significant decrease in the number of colonies of both *C. albicans* and *C. glabrata* with an increase in irradiation time. Interestingly, this inhibitory

effect was more prominent in *C. albicans* compared with *C. glabrata*. That is, colony formation in *C. albicans* was inhibited by ~ 46%, 96%, 99%, and 100% at irradiation times of 5 min (47.4 J/cm$^2$), 10 min (94.8 J/cm$^2$), 20 min (189.6 J/cm$^2$), and 30 min (284.4 J/cm$^2$), respectively. In contrast, colony formation in *C. glabrata* was inhibited by ~ 5%, 44%, > 99%, and 100% at irradiation times of 5 min (47.4 J/cm$^2$), 10 min (94.8 J/cm$^2$), 20 min (189.6 J/cm$^2$), and 30 min (284.4 J/cm$^2$), respectively (Fig. 2).

### Fluorescence microscopy

Results of the fluorescence microscopy revealed the viability of each fungal cell inside the biofilms (Fig. 3). Green fluorescence indicated living cells, and red fluorescence indicated dead cells. The vast majority of each fungus in group 1 appeared green, whereas irradiation for 20 min (189.6 J/cm$^2$) induced high mortality in both *C. albicans* and *C. glabrata* cells inside the biofilms, as seen by the increased red color. Few *C. albicans* cells were alive (green), whereas more *C. glabrata* cells were alive.

### Scanning electron microscopy

SEM images of *C. albicans* and *C. glabrata* irradiated for 20 min (189.6 J/cm$^2$) showed various cell damage patterns (Fig. 4). A large number of wrinkled or shrunken cells were noted in *C. albicans*, and cell surface damage was observed in *C. glabrata*. Group 1 showed no damage.

### Postirradiation characterization of denture base resin

Figure 5 shows the changes in FS, FM, and $R_a$ of the specimens preirradiation and postirradiation. The FS was significantly higher in all irradiation groups (> 105.4 MPa) compared with group 1 ($p < 0.05$), with no significant differences between groups. The FM in all irradiation groups ranged from 2.80 to 2.93, with no significant differences between groups ($p > 0.05$). The $R_a$ of the specimens from all groups ranged from 0.19 to 0.20 μm, with no significant differences between groups ($p > 0.05$).

## Discussion

*Candida* biofilms formed on the surface of PMMA denture base resin can be degraded by irradiation with 405-nm blue LED light, even without photosensitizers. Fluorescence microscopy observations showed that this degradation effect might be due to death of *Candida* cells caused by the irradiation. PI penetrates dead *Candida* cells through the damaged cell wall or cell membrane and yields red fluorescence when it binds to DNA [24, 25], suggesting that irradiation with the 405-nm blue LED light damages the *Candida* cell wall or cell membrane.

SEM images of irradiated *Candida* cells showed several morphological changes, such as cell body shrinkage, particularly at the hypha terminus, in *C. albicans* and depressions on *C. glabrata* cell surfaces. The cell wall and cell membrane play an important role in cell viability, morphogenesis, response to environmental stressors, pathogenesis, etc.; therefore, these morphological changes indicate a loss of cell function integrity [26]. The loss of cell function integrity, in turn, leads to cell homeostasis breakdown, resulting in cell death.

We can speculate the mechanisms underlying the fungicidal effects of 405-nm blue LED light irradiation on *Candida* species on the basis of previous reports. Plavskii et al. (2018) reported that *C. albicans* endogenously produces porphyrins, which might act as potent photosensitizers in aPDT under light irradiation, resulting in ROS generation (the major mechanism underlying aPDT efficacy) [27]. This ROS generation

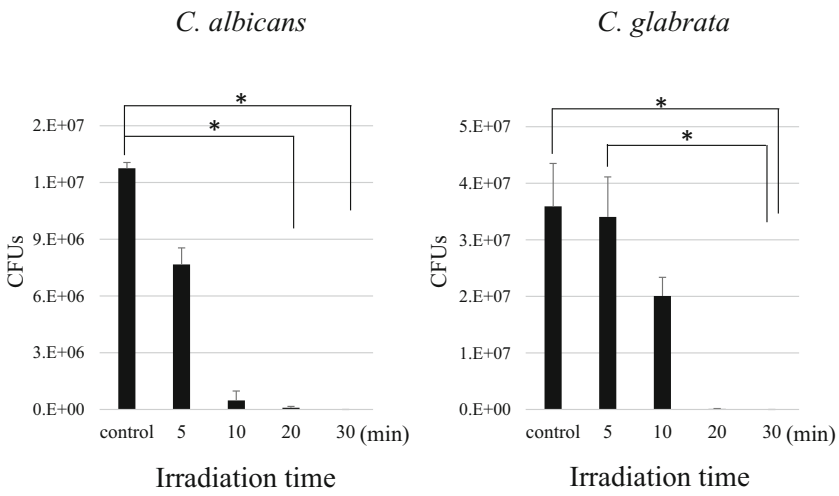


**Fig. 2** *C. albicans* and *C. glabrata* biofilm quantification by the CFU assay. Black bars represent the mean colony count for each *Candida* species ($n = 4$ in each group). The asterisk (*) indicates a significant difference between groups ($p < 0.05$)

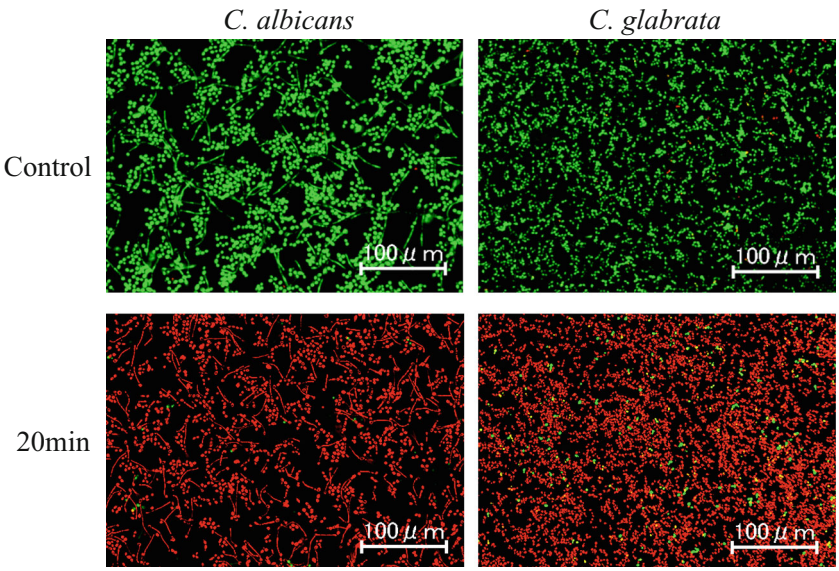


**Fig. 3** Biofilm on denture base resin disc surface viewed to obtain a representative sample from each group using fluorescence microscopy. Green fluorescence: living cells; red fluorescence: dead cells. The vast majority of each fungus in group 1 appeared green, whereas irradiation for 20 min induced high mortality in both *C. albicans* and *C. glabrata* cells inside the biofilms (increased red color). Few *C. albicans* cells were alive (green), whereas more *C. glabrata* cells were alive

could, in turn, damage biological macromolecules, such as proteins and lipids, leading to the death of *Candida* cells [28].

*Candida* biofilms on denture base resin discs tend to decrease with an increase in irradiation time, but in this study, irradiation for 30 min (284.4 J/cm$^2$) completely degraded the biofilms of both *C. albicans* and *C. glabrata*. This result showed that 405-nm blue LED light can exert a degradation effect even on the deepest layer (i.e., the part closest to the denture base resin disc surface) of a biofilm comprising multiple cell layers. Although the mechanism underlying this phenomenon is unclear, one possibility is that the irradiation induces the destruction of cell–cell or cell–matrix adhesion, which is important in forming biofilms [29], through cell wall dysfunction. Continuous irradiation might cause this destruction as deep as the denture base resin disc surface, eventually resulting in total degradation of the biofilm.

With regard to the effect of temperature rise on *Candida* by irradiation, it has been reported that irradiation with 405-nm blue light for more than 10 min does not generate sufficient heat to cause damage to living microorganisms [20]. Therefore, the present study suggests that irradiation with 405-nm blue LED light cannot generate sufficient heat to cause damage to *Candida*. However, this effect of temperature rise remains to be investigated.

Interestingly, in this study, irradiation exerted a more prominent effect on *C. albicans* compared with *C. glabrata*: colony formation in *C. albicans* was significantly inhibited by irradiation for even 5 min (47.4 J/cm$^2$). This is unlikely to be

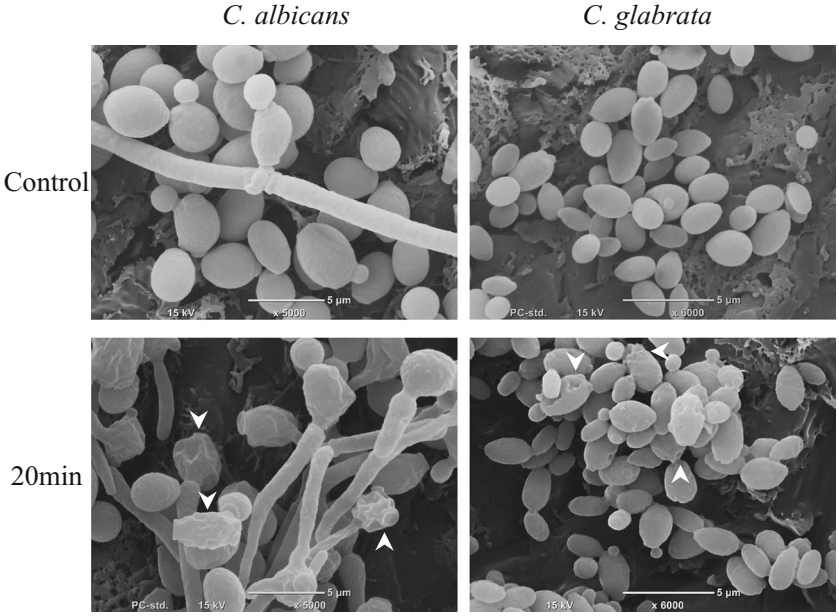


**Fig. 4** SEM images of *C. albicans* and *C. glabrata* biofilms formed on representative denture base resin disc surfaces from each group. White arrows indicate cell body shrinkage, particularly at the hypha terminus, in *C. albicans* and depressions on *C. glabrata* cell surfaces, which were not seen in group 1. SEM, scanning electron microscopy

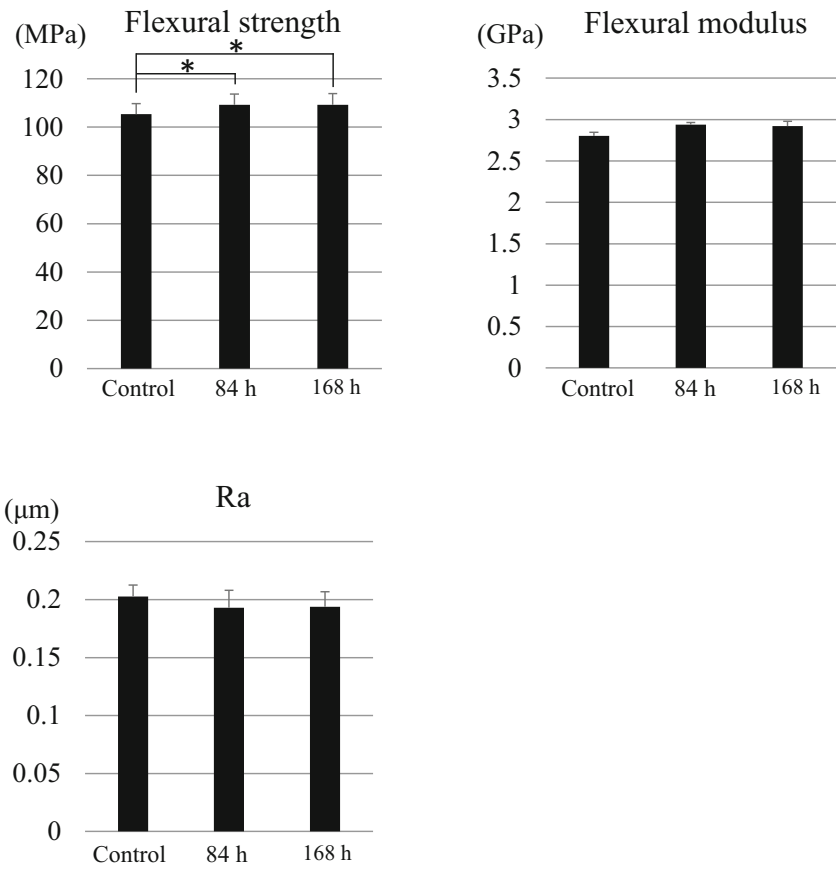


**Fig. 5** Mechanical and surface characteristics of denture base resin preirradiation and postirradiation with 405-nm blue LED light. Black bars represent the means and standard deviations (SDs) of the FS, FM, and $R_a$ of the specimens ($n = 5$ in each group). The asterisk (*) indicates a significant difference between groups ($p < 0.05$). LED, light-emitting diode; FS, flexural strength; FM, flexural modulus; $R_a$, surface roughness

because of the difference in biofilm thickness between the two *Candida* species, because *C. glabrata* biofilms tend to grow slower compared with *C. albicans* [30]. Although a complete explanation of this finding is outside the scope of this study, we feel the difference might be due to species-specific differences; indeed, SEM images of irradiated *C. albicans* and *C. glabrata* also showed different images (*C. albicans* showed shrunken cells, whereas *C. glabrata* showed depressions on cell surfaces). Abegg et al. (2010) reported that different microorganisms respond differently to ROS and use different mechanisms to detoxify ROS [28], suggesting that ROS produced by irradiation with 405-nm blue LED light might cause different reactions in *C. albicans* and *C. glabrata*.

According to ISO 1567, the minimum FS of heat-polymerizing acrylic resin should be > 65 MPa and the minimum FM should be > 2 GPa. In this study, the FS was > 105.4 MPa and the FM was > 2.80 GPa, even after irradiation for as long as 168 h; this result complied with the ISO 1567 requirement regarding both parameters.

PMMA is a polymer that degrades because of photoirradiation, and its degradation mechanism is main-chain scission [31]. The threshold wavelength of main-chain scission is < 320 nm [31], so irradiation with 405-nm blue LED light does not cause main-chain scission in PMMA. Interestingly, however, in this study, the FS was significantly higher in all irradiation groups compared with group 1. The reason the FS increased postirradiation could be that irradiation changed the degree of polymerization in PMMA. Further studies are required in order to elucidate this phenomenon. There were no significant changes in the FM and $R_a$ preirradiation and postirradiation. Surface degradation increases $R_a$ [32], indicating that irradiation induces no resin surface deterioration.

Photoirradiation from limited directions creates dark-field sections that do not receive light [33, 34], suggesting that irradiation with 405-nm blue LED light from only one direction cannot degrade biofilms on every surface of a denture. Therefore, irradiation from multiple directions or a combination with conventional denture-cleaning methods might be required in any clinical application of 405-nm blue LED light for disinfection of denture surfaces.

Denture plaque is composed of many types of bacteria and yeasts as well as *Candida* [35]. These microorganisms may contribute to not only DS, oral malodor, caries, and periodontitis but also bacterial endocarditis, aspiration pneumonia, gastrointestinal infection, and chronic obstructive pulmonary disease [36]. Therefore, further investigations are needed in order to verify the inhibitory effect of 405-nm blue LED light on the other microorganisms contained in denture plaque.

## Conclusions

Taken together, the results of this study strongly suggested that 405-nm blue LED light can be very useful for disinfection of denture surfaces. Irradiation with 405-nm blue LED light effectively degrades *C. albicans* and *C. glabrata* biofilms on denture base resin. In addition, irradiation with 405-nm blue LED light does not cause surface deterioration of denture base resin. In clinical application, irradiation from multiple directions or a combination with conventional denture-cleaning methods might be also required for effective disinfection of denture surface.

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