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RESEARCH

Antimicrobial surface processing of polymethyl methacrylate denture base resin using a novel silica-based coating technology

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Abstract

Objectives This study investigated the surface characteristics of denture base resin coatings prepared using a novel silica-based film containing hinokitiol and assessed the effect of this coating on *Candida albicans* adhesion and growth.

Methods Silica-based coating solutions (control solution; CS) and CS containing hinokitiol (CS-H) were prepared. *C. albicans* biofilm formed on denture base specimens coated with each solution and these uncoated specimens (control) were analyzed using colony-forming unit (CFU) assay, fluorescence microscopy, and scanning electron microscopy (SEM). Specimen surfaces were analyzed by measuring the surface roughness and wettability and with Fourier-transform infrared (FT-IR) and proton nuclear magnetic resonance (^{1}H NMR). Stability of coated specimens was assessed via immersion in water for 1 week for each group (control-1w, CS-1w, and CS-H-1w) followed by CFU assay, measurement of surface roughness and wettability, and FT-IR.

Results CS-H and CS-H-1w contained significantly lower CFUs than those present in the control and control-1w, which was also confirmed via SEM. Fluorescence microscopy from the CS-H group identified several dead cells. The values of surface roughness from coating groups were significantly less than those from the control and control-1w. The surface wettability from all coating groups exhibited high hydrophobicity. FT-IR analyses demonstrated that specimens were successfully coated, and ^{1}H NMR analyses showed that hinokitiol was incorporated inside CS-H.

Conclusions A silica-based denture coating that incorporates hinokitiol inhibits *C. albicans* growth on denture.

Clinical relevance We provide a novel antifungal denture coating which can be helpful for the treatment of denture stomatitis.

Keywords Silica-resin coating · Hinokitiol · *C. albicans* · Denture · Denture stomatitis

Introduction

The fungus *Candida albicans* is a common component of denture plaque that adheres to denture surfaces [1, 2], and a pathogenic overgrowth of *C. albicans* is a main factor in denture stomatitis [1–3]. Thus, the removal of *C. albicans* from denture surfaces has been assumed to be effective for the treatment and prevention of denture stomatitis [1, 2]. *C. albicans* can switch from the yeast to hyphal growth form in response to environmental cues, and this ability is considered the key element of virulence [4]. *C. albicans* yeast easily adheres to the fitting surfaces of dentures and develops into the hyphal form by thigmotropism [5, 6]. The hyphae invade microcracks and irregularities on the denture surface to form deeply embedded biofilms [3, 5, 7, 8]. Although antifungal therapy and mechanical/chemical cleaning have been investigated for disinfecting and removing *C. albicans*

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on denture surface, these methods cannot reach biofilms embedded in cracks and irregularities [3, 7, 8]. Therefore, a new approach is required to reduce the adhesion of *C. albicans* and inhibit formation of the associated biofilm on the denture surface.

It has been reported that the denture surface modification such as a coating is one of the ways to prevent *C. albicans* adhesion and growth on dentures [9, 10]. Several studies showed some denture coatings have the potential to contribute to prevent the fungus adhesion and growth because the surface roughness, surface energies, and surface hydrophobicity were modified [9, 10]. On the other hands, the methods incorporated antimicrobial/antifungal agents within the denture base resin have been also reported to inhibit *Candida* growth [11]. Although the use mechanical denture cleansing methods such as brushing becomes easy to peel coatings, these techniques can be applied to dentures repeatedly unlike the methods incorporated antimicrobial/antifungal agents within the denture base resin. Therefore, to develop the denture coating, incorporated antimicrobial/antifungal agents can be effective to inhibit *Candida* adhesion and growth.

Silica, also known as silicon dioxide (SiO_2), is the most common inorganic material on earth and has been used as a coating material to protect various reactive materials from environmental degradation because it is chemically stable and harmless to organisms [12, 13]. Silica coating forms a glass film with strong siloxane bonding of $Si-O-Si$ and is hard and brittle. Thus, this coating is not suitable as a denture base resin because it may easily peel off from a flexible substrate. However, a recent report described that a novel silica-based coating technique [14] does not form a rigid silica glass with four siloxane bonds in the SiO_2 composition but rather forms a silica glass containing numerous alkyl groups that remain inside a loose three-dimensional glass network made of siloxane bonds. This film, which was formed on the surface of cellulose fibers by newly formed covalent bonds between hydroxy groups (OH groups) of cellulose and methyltrimethoxysilane (MTMS), was flexible and did not peel off when paper coated with the film was folded or crumpled [14]. In addition, the MTMS alkyl groups, which do not need to be specific, may create spaces within the glass network, suggesting that various types of antifungal substances can be incorporated into this network.

Hinokitiol is a natural antibacterial and antifungal compound isolated from trees in the cypress family, such as hiba [15] that has antimicrobial activity against several antibiotic-resistant pathogens [16–18]. We considered that the small molecular size of hinokitiol would enable it to be incorporated into spaces inside the three-dimensional glass network within the silica-based coating [19]. The resulting silica-based coating incorporating hinokitiol could then be used to cover cracks and irregularities on denture and thereby inhibit the adhesion and growth of *C. albicans* via antifungal activity.

Therefore, this study investigated the surface characteristics of denture base resin coatings prepared with a novel silica-based film containing hinokitiol and the effect of this on *C. albicans* adhesion and growth. The null hypothesis was that the novel silica-based coating including hinokitiol on denture base resin had no significant effect on antifungal activity.

Materials and methods

Specimen preparation

The coating agent was a solution of oligomers (with a degree of polymerization of approximately three) of methyltrimethoxysilane (MTMS; $CH_3Si(OCH_3)_3$), poly(methylphenylsiloxane) (PMPS; $[Si(CH_3)(C_6H_5)O]n$), and isopropyl alcohol (IPA). After adding a small amount of tetraisopropyl titanate (TPT, $[(CH_3)_2CHO]_4Ti$), the solution was mixed for 1 h at room temperature. TPT triggers the hydrolysis reaction between an oligomer of an alkoxysilane in the solution and OH group which presents on the denture or atmosphere. A solution with a weight ratio of MTMS/PMPS/IPA/TPT = 36.5:13.35:50:0.15 was the control solution (CS). Hinokitiol solution (Aomori Hiba oil containing 0.2% hinokitiol; CHOETSU KAKEN Co., Ltd., Yokohama, Japan) was mixed with CS using a vortexer (CS-H); the CS-to-hinokitiol solution ratio was 8:1 (vol%).

A total of 135 square-shaped specimens (10 × 10 × 2 mm) were prepared from polymethyl methacrylate (PMMA) denture base resin (ACRON, GC, Tokyo, Japan) by sectioning cuboid samples (10 × 10 × 80 mm) which were made using heat polymerizing [11, 20]. The upper and lower surfaces of each denture base resin specimen were polished with 320-grit abrasive paper under dry conditions to simulate the very rough fitting surface of dentures [20]. After ultrasonic cleaning using ultrapure water, all specimens were immersed in water at 37℃ for 24 h to reduce the residual monomer [21] and sterilized under ethylene oxide gas. The specimens were then treated with 100% ethanol for a few seconds and were immersed in each coating solution (CS and CS-H) for 5 min. After wiping the extra solution, surfaces were dried and cured for 30 min at 37℃ by evaporating a volatile isopropyl alcohol in the coating solution, and then immediately used for testing; specimens without these coatings were termed control. In addition, to assess the stability of the coating under water, specimens from each group were immersed in ultrapure water for 1 week (control-1w, CS-1w, and CS-H-1w, respectively) at room temperature. Data for these groups were compared before and after the 1-week immersion in

water via CFU assay, measurement of surface roughness and wettability, and FT-IR spectroscopy.

Candida growth conditions

C. albicans (ATCC 18,804) was inoculated from frozen stock onto Sabouraud dextrose agar, and a resulting colony was transferred into tryptic soy broth supplemented with 5% dextrose (TSBD; Becton, Dickinson and Company, NJ, USA). The cell suspension was cultured aerobically at 75 rpm at 30 °C for 5 h and then centrifuged. The supernatant was replaced with the yeast nitrogen base (YNB) medium, and a standard suspension of *C. albicans* (10^4 cells/mL) was prepared. Denture specimens were placed into the wells of a 24-well plate, and artificial saliva (500 µL) [9] was added to each well. Plates were incubated at 37 °C with shaking at 60 rpm for 60 min; a pellicle was observed to form on all specimens. Each specimen was washed twice with 1 mL of phosphate-buffered saline (PBS). The standard cell suspension (1 mL) was added to each well, and all plates were then aerobically incubated at 37 °C for 1.5 h (adhesion phase) [9]. Specimens were washed twice with PBS and then transferred to a well of a new 24-well plate containing fresh YNB medium. To form *C. albicans* biofilm, the well plates were allowed to incubate for 24 h at 37 °C under aerobic conditions. Specimens were washed twice with 1 mL PBS to remove loosely attached cells before all biofilm analyses.

CFU assay

Specimens from the control, CS, CS-H, control-1w, CS-1w, and CS-H-1w groups were each placed into wells in a 24-well plate, and 1 mL of PBS is added per well. The *Candida* biofilm was then scraped off each specimen using a cell scraper and suspended via repeated pipetting [9, 11]. These suspensions were serially diluted with PBS and then inoculated onto Sabouraud glucose agar plates. Plates were incubated for 48 h at 37 °C, and the total CFUs were enumerated. Six specimens from each group (total 36 specimens) were used for this assay.

Fluorescence microscopy

The Live/Dead® BacLight™ Bacterial Viability Kit (Thermo Fisher Scientific) was used to label live and dead bacteria with two nucleic acid dyes SYTO 9 and propidium iodide (PI, respectively. The specimens with *Candida* biofilm from the control, CS, and CS-H groups were transferred to wells of a fresh 24-well plate containing 500 µL of physiological saline. SYTO9 (1 µL) and PI (1 µL) were added to each well, and the biofilm on each specimen was stained for 20 min in the dark at 30 °C. *Candida* biofilm on the specimens was assessed using a fluorescence microscope (BZ-X710; Keyence, Osaka, Japan). Six specimens from each group (total 18 specimens) were used for this assay.

Scanning electron microscopy (SEM)

Specimens with *Candida* biofilm from the control, SC, and CS-H groups were placed in fixative (2.5% glutaraldehyde) at 4 °C overnight and then dehydrated in increasing concentrations of ethanol (50%, 60%, 70%, 80%, 90%, and absolute ethanol). Each specimen was immersed in t-butyl alcohol and frozen in a freezer. Samples were completely dried after the frozen alcohol had sublimated in a freeze-drying apparatus (ID-2; Eiko Engineering, Tokyo, Japan). Finally, the specimens were sputtered with gold using an ion sputter coater (SC-701AT; Sanyu Denshi, Tokyo, Japan) and assessed using SEM (JCM-6000 NeoScope™; Jeol Ltd., Tokyo, Japan). Six specimens from each group (total 18 specimens) were used for this assay.

Surface roughness and wettability

The surface roughness (Ra) and water contact angle were measured to characterize the surfaces of each group using three specimens of each group (total 36 specimens). In addition, specimen surfaces were observed using a phase-contrast microscope (BZ-X710; Keyence, Osaka, Japan). Surface roughness was measured using a profilometer (Surfcom Flex, Seimitsu, Tokyo, Japan), and surface wettability was determined by measuring the water contact angle. Drops of purified water on the specimen surfaces were measured with a contact angle meter (P50, Meiwafosis Co., Ltd., Tokyo, Japan). Images of the drops on the surfaces were recorded using a CCD camera.

Fourier-transform infrared (FT-IR) spectroscopy analysis

FT-IR spectroscopy analysis was performed by measuring the surface of PMMA specimens prepared for the biofilm analysis using an attenuated total reflectance (ATR) system. The FT-IR spectra were measured with an FT/IR-4100ST (Nihon Bunko Co. Ltd., Tokyo, Japan) equipped with ATR spectroscopic unit (PRO670H-S, Nihon Bunko Co. Ltd., Tokyo, Japan). The wavenumber range of the FT-IR spectra was 600–4000 cm^{-1}, and the resolution was 4 cm^{-1}. Each spectrum was determined from an average of 16 scans. All measurements were performed at room temperature. Three specimens from each group (total 18 specimens) were used for this assay.

Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy analysis

The presence of hinokitiol was determined via ^{1}H NMR spectroscopy analyses using acetone-d_6 as a solvent of NMR measurements. Samples for the ^{1}H NMR spectroscopy analysis were prepared by removing SiO_2 powder from the surface of PMMA specimen and suspending this in 700 µL of acetone-d_6. Each sample was placed in a 5-mm NMR tube and the ^{1}H spectra recorded on a JEOL JMTC-400/54/JJ/YH spectrometer (1H: 400 MHz, JEOL Ltd., Japan). Three specimens from each group (total 9 specimens) were used for this assay.

Statistical analysis

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 surface roughness and wettability were assessed using one-way analysis of variance followed by Tukey's honestly significant difference multiple comparison test. Student's *t* tests were performed for results from specimens before and after 1-week immersion; the significance level was set to 0.05. All analyses were performed using SPSS ver. 24.0 for Windows (IBM, NY, USA).

Results

We found that specimens from CS-H and CS-H-1w groups contained significantly lower quantities of biofilm (CFU assay) than that in the control and control-1w groups, respectively ($p < 0.05$, Fig. 1a, b); however, no significant differences were observed between the other groups. Following 1-week immersion in water, specimens from control and CS groups had significantly lower biofilm content than that from control-1w and CS-1w, respectively ($p < 0.05$, Fig. 1c), while there were no significant differences between the CS-H and CS-H-1w groups. Fluorescence microscopy was used to assess the quantities and viability of the *Candida* biofilm on each specimen with live cells fluorescing green with SYTO9 and dead cells fluoresced red with PI. The quantities of the biofilm visually indicated a greater amount of biofilm in the control group than that in the CS and CS-H groups (Fig. 2a). Most *Candida* cells in the control and CS groups appeared green, whereas several cells in CS-H group fluoresced red, indicating that CS-H coating had induced a degree of cell death (Fig. 2a). Low-magnification SEM images (×500) showed the microstructure of the *Candida* biofilm (Fig. 2b). A complex three-dimensional structure with long hyphae arranged in a multilayered network was observed in the control group. Although the image in the CS group showed the biofilm development, this lacked a complex three-dimensional structure and there were fewer hyphae than those in the control group. *Candida* adhesion and growth were poorly represented on the surface of the specimen from the CS-H group. As shown in high-magnification SEM images (×2000), there was no notable damage or change to *Candida* cell surfaces in any of the groups (Fig. 2b).

The values of surface roughness from CS and CS-H groups were significantly less than those from the control group ($p < 0.05$), and there were no significant differences between the CS and CS-H groups (Table 1). This outcome did not change even when each specimen was immersed in water for 1 week (CS-1w, CS-H-1w, and control-1w). There were no significant differences between the groups in surface roughness before and after the 1-week immersion in water. The microscopic images of representative specimen show smooth surfaces for all coating groups; however, many scratches are observed on surfaces from the control and control-1w groups (Fig. 3). The mean water contact angles from the control, CS, and CS-H groups were more than 90°, and all specimens exhibited high hydrophobicity; there were no significant differences among the groups. However, specimens from the CS-H-1w group had significantly higher water contact angles (the highest hydrophobicity) than those from CS-1w group ($p < 0.05$), with no significant differences between the others. The water contact angles before and after 1-week immersion in water in the control-1w group were significantly different from those in the control group (with a decrease in hydrophobicity in the control-1w group) ($p < 0.05$), while there were no significant differences between the other groups. The surface-drop images of specimens from the control, CS, and CS-H groups showed low wettability (Fig. 4). The specimens from the control-1w and CS-1w groups were more wettable than those from the control and CS groups, respectively. However, the specimens from the CS-H-1w group were as wettable as those from CS-H, indicating that the specimens from the CS-H-1w group maintained a high hydrophobicity.

The microscopic structure of the SiO_2 thin-layer samples that were synthesized from CS and CS-H solutions was investigated using FT-IR spectroscopy analyses at room temperature. The FT-IR spectra of the SiO_2 thin-layer samples between 700 and 4000 cm^{-1} are shown in Fig. 5. The absorption peaks for C-H rocking (752 cm^{-1}), C-O stretching (1143 and 1238 cm^{-1}), C-H bending (1434 cm^{-1}), C=O stretching (1723 cm^{-1}), and C-O stretching (2849–3012 cm^{-1}), which were attributed to the PMMA molecule, were observed in the spectra of a PMMA specimen. However, in addition to the PMMA-assigned peaks, the spectrum of the CS sample contains Si–C stretching (773 cm^{-1}), O–H (Si–OH) stretching (920 cm^{-1}), Si–O-Si stretching (1010 cm^{-1}), and C-H (Si-CH_3) bending (1267 cm^{-1}) peaks. This indicates

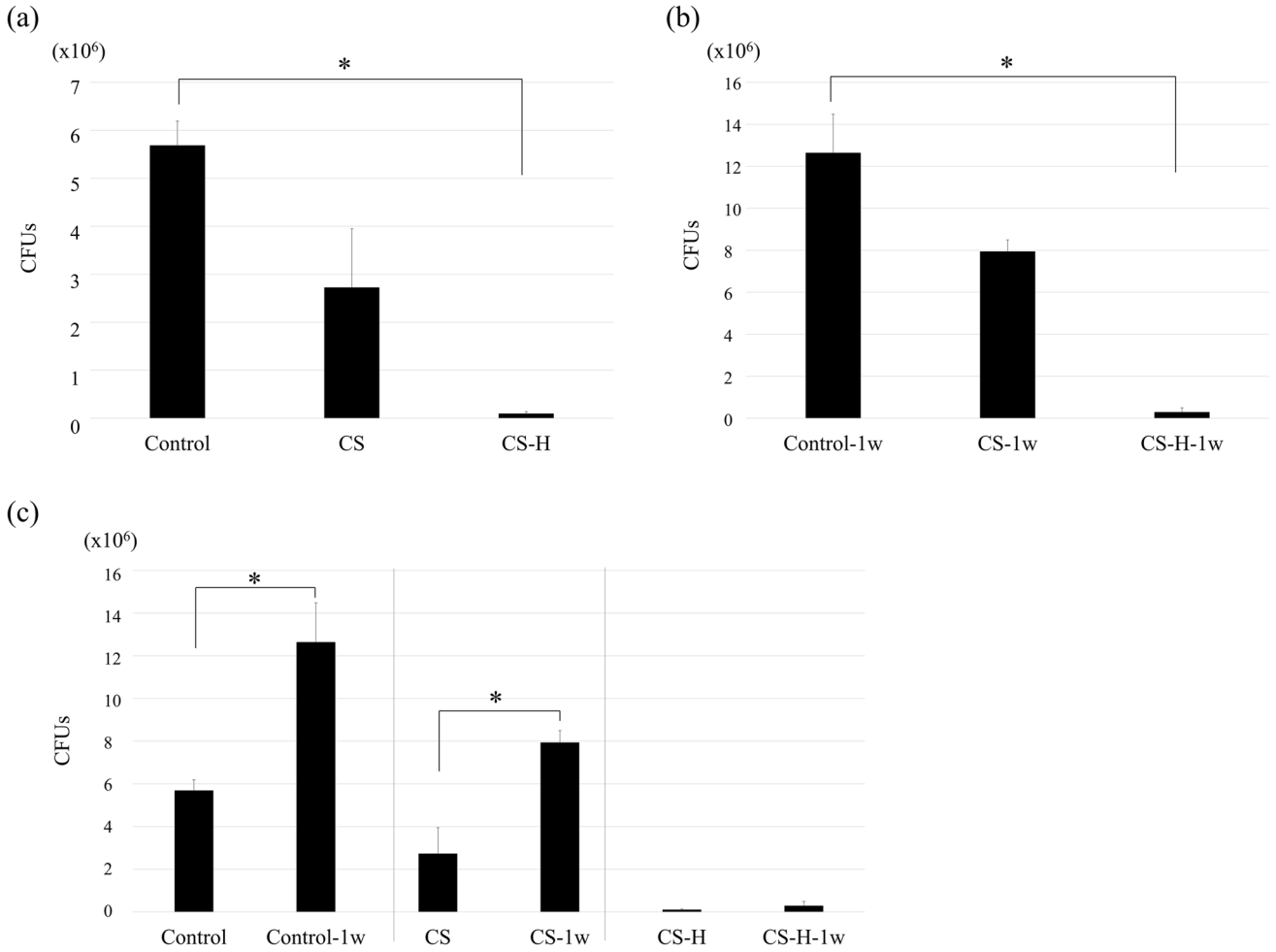

Fig. 1 *C. albicans* biofilm quantification via CFU assay. **a** Comparison among all experiment groups before 1-week immersion in water. **b** Comparison among all experiment groups after 1-week immersion in water. **c** Comparison before and after the 1-week immersion in water in each experiment group. Black bars represent the mean CFU ($n=6$ in each group). The asterisk (*) indicates a significant difference between groups ($p<0.05$)

that a certain amount of a glass layer is present on the surface of the PMMA specimen. Moreover, in addition to the peaks assigned to the PMMA and glass layer, the spectrum of the CS-H sample contains O–H stretching (at around 3400 cm^{-1}), indicating that the Si–OH group of silica-based coating and/or the OH group of hinokitiol are present in the glass layer of the CS-H sample. We also measured the FT-IR spectra of the control-1w, CS-1w, and CS-H-1w samples. However, there was no large difference between the dry and wet samples. Therefore, we concluded that the structure of the thin-layer samples immersed for 1 week in water is macroscopically identical to the dry one.

In addition, the presence of hinokitiol in the glass thin layer was determined using ^{1}H NMR spectroscopy analyses (Fig. 6). In the ^{1}H NMR spectrum of hinokitiol, aromatic proton signals appeared between 6.98 and 7.46 ppm. The ^{1}H NMR spectrum of the CS sample showed a peak at 0.1 ppm that can be assigned to Si-CH_3. However, the ^{1}H NMR spectrum of CS-H sample showed three peaks between 0 and 0.2 ppm (Si-CH_3) and at 7.30 and 7.67 ppm. The latter peaks could be assigned to aromatic proton signals from hinokitiol when considering these results together with the FT-IR analyses, although the peak position of hinokitiol in the CS-H sample had shifted.

Discussion

Our results show that less biofilm was formed on specimens coated with CS-H than those of the control group indicating that the denture base resin coated with CS-H can reduce adhesion or growth of *C. albicans*. We believe that there are two reasons for this observation. First, CS-H exerts an excellent antifungal and growth inhibitory effect on *C.*

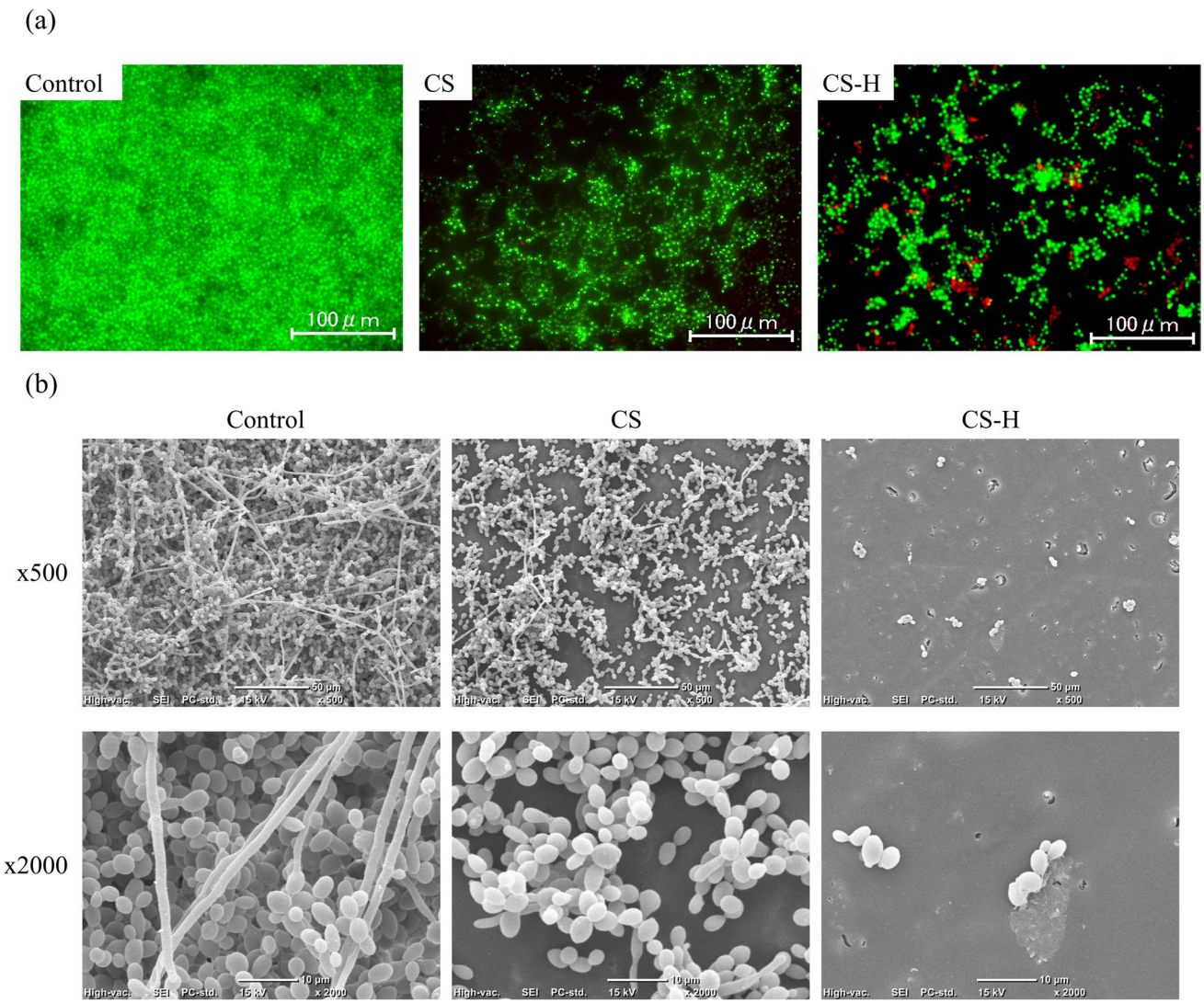

Fig. 2 Fluorescence microscopy and SEM images of *C. albicans* biofilm formed on representative denture base specimen from each group. **a** Fluorescence microscopy images. Live cells are green and dead cells are red. Most of the cells in both the control and CS groups fluoresced green, whereas several cells in the CS-H group fluoresced red, indicating that CS-H coating induced some cell death. **b** SEM images. Low-magnification image (×500) from control group shows a complex three-dimensional structure with long hyphae arranged in a multilayered network, while adhesion and growth of *C. albicans* are almost absent in the CS-H group. High-magnification images (×2000) show no notable damage and change on the cell surfaces from all groups

Table 1 Surface roughness and water contact angle

	Control	CS	CS-H
Roughness (μm)	1.14 (0.03)[a]	0.56 (0.01)[b]	0.70 (0.03)[b]
Water contact angle (°)	99.86 (0.76)	92.31 (2.91)	95.60 (3.77)
	Control-1w	CS-1w	CS-H-1w
Roughness (μm)	1.03 (0.08)[a]	0.65 (0.04)[b]	0.80 (0.05)[b]
Water contact angle (°)	89.60 (0.52)[ab]	86.66 (2.98)[a]	93.29 (1.86)[b]

Different lowercase letters in the same row represent statistically significant results ($n=3$, $p<0.05$). Regarding the comparison before and after 1-week immersion in water, the mean values in water contact angle from control-1w group were significantly lower than those from control group ($p<0.05$), with no significant differences between the other groups in surface roughness and water contact angle

albicans. The fluorescence microscopy images show that the CS-H samples contained several dead cells and were lower in biofilm content than that in the CS and control groups. The SEM of CS-H shows less *Candida* adhesion than that in the CS and control groups and supports this result. The antifungal and growth inhibitory effect of CS-H is probably associated with hinokitiol. Hinokitiol has been reported to inhibit the growth and virulence factors of *Candida* species by regulating various factors associated with hyphal formation [17] and has antifungal activity caused by interference with iron homeostasis [22]. In this study, we used FT-IR analysis to demonstrate the presence of the Si peak in denture base specimens and confirm that these were coated with

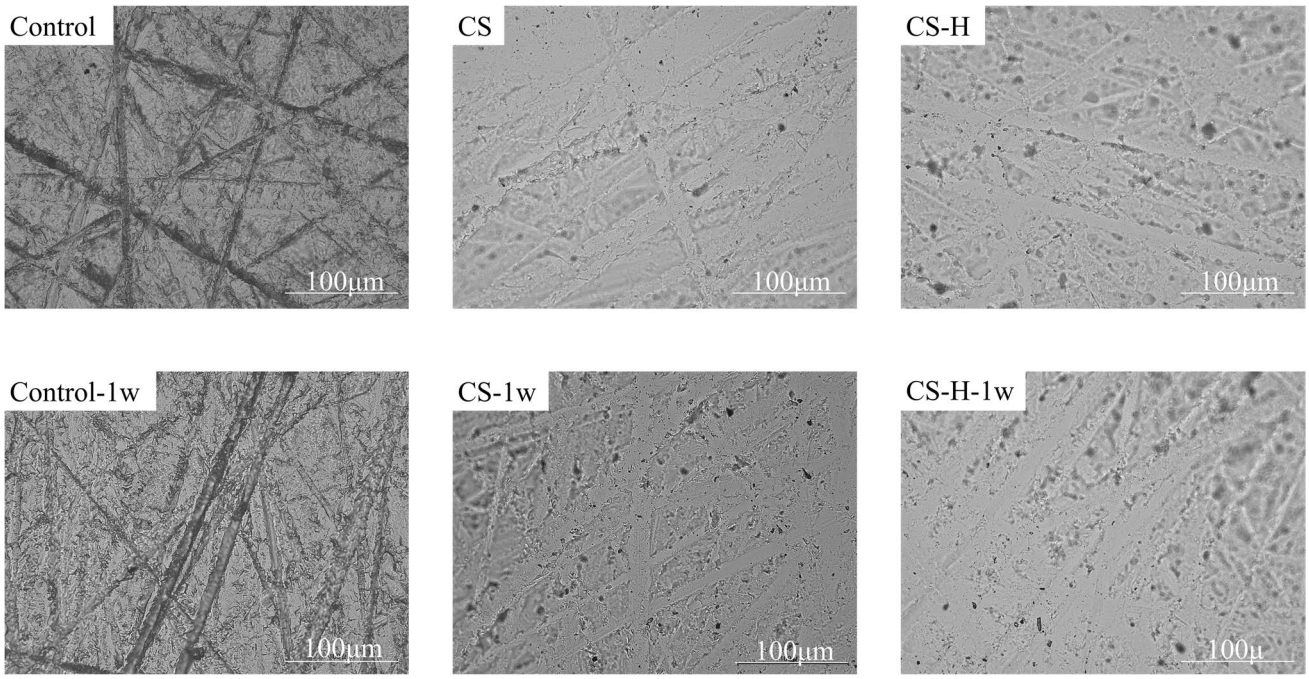

Fig. 3 Microscopic images of a representative denture base specimen from each group. The surfaces from all coating groups are smooth; however, many scratches are observed on surfaces from control and control-1w groups

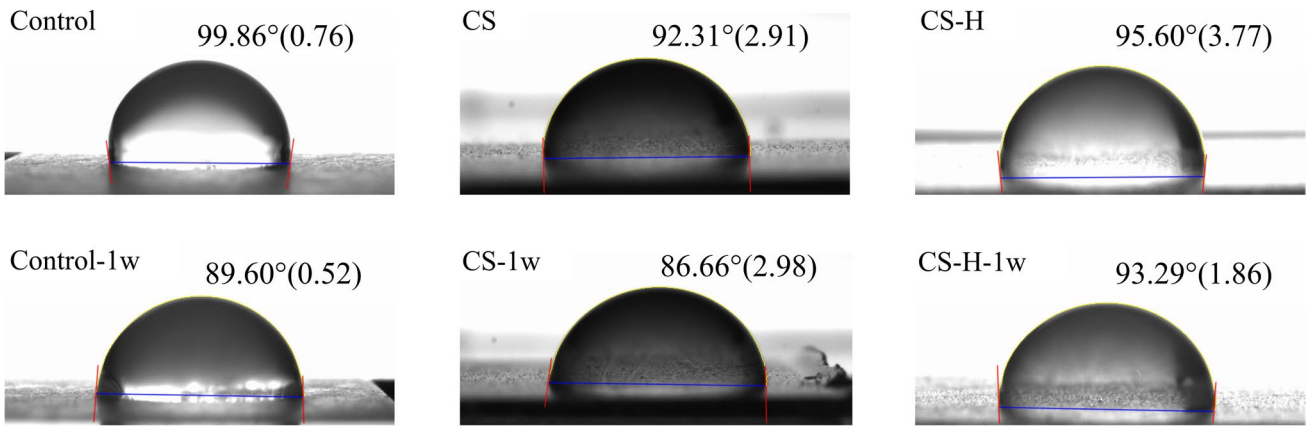

Fig. 4 Surface-drop images of a representative denture base specimen from each group. The surfaces from the control, CS, and CS-H groups exhibit high hydrophobicity, while CS-H-1w maintains high hydrophobicity

CS or CS-H. In addition, ^{1}H NMR analysis demonstrated that CS-H contained hinokitiol. Therefore, we suggest that SC-H has antifungal activity and can inhibit the growth of *C. albicans*.

It has been reported that the factors, surface roughness (Ra) and hydrophobicity of denture surface, contribute to *Candida* adhesion to dentures [23], so surface roughness may be associated with adhesion and growth of *C. albicans*. The values of surface roughness in CS and CS-H groups were significantly lower than those in the control group. Previous reports indicate that surface roughness above an Ra of 0.2 μm has an impact on biofilm formation and bacteria adhesion [24–26], and in this study, the values of Ra from all groups were greater than 0.56 μm. We think the coating might mimic the surface cracks or roughness of dentures because it is so thin. In addition, an increase in surface roughness above an Ra of 0.2 μm is well known to facilitate biofilm formation on various materials [26, 27]. Although there were no significant differences, the specimens coated with CS tended to have less biofilm quantities in the CFU, fluorescence microscopy, and SEM analysis than those from the control group. This suggests that the differences

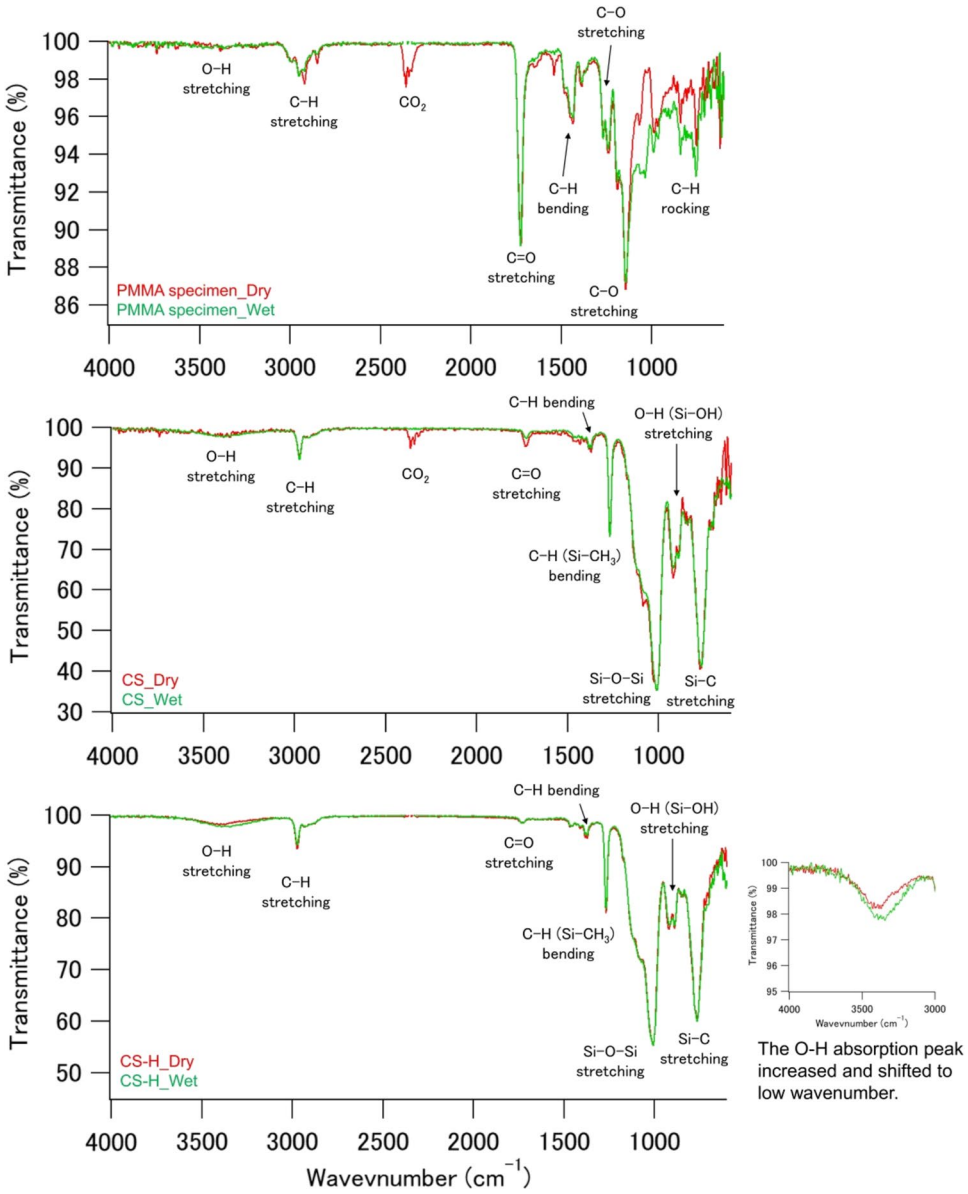

Fig. 5 FT-IR spectra of control (PMMA specimen _Dry), control-1w (PMMA specimen _Wet), CS (CS_Dry), CS-1w (CS_Wet), CS-H (CS-H_Dry), and CS-H-1w (CS-H_Wet). The inset is an enlarged profile of the O–H stretching absorption peak

of surface roughness between these groups may impact the adhesion or growth of *C. albicans* on denture base resin.

Specimens immersed for 1 week in water had significantly lower quantities of biofilm in the CS-H-1w group than those in the control-1w group. In addition, the specimens from the CS-1w group tended to show lower quantities of biofilm than those from the control group. These results showed the same tendency as those from specimens without immersion (control, CS, and CS-H groups); i.e., antifungal activity was maintained by CS-H for 1 week, while the surface roughness of specimens was maintained by both CS and CS-H. Indeed, the immersion in water for 1 week had little effect on the surface roughness in each group. In addition, the FT-IR analysis also showed the Si peak in specimens from both the CS-1w and CS-H-1w groups, indicating the persistence of the coatings on denture base specimens during immersion. However, the biofilm quantities recovered from the specimens in the CS-1w and control-1w groups were significantly greater than those from the CS and control groups, respectively, although there were no significant differences between the CS-H and CS-H-1w groups. This indicates that hinokitiol helps maintain the quality of the coating after immersion in water for 1 week. We believe that this may be due to the differences in the surface wettability. The values of the water contact angles from the CS and control groups decreased but stayed approximately the same in the CS-H group stayed after immersion in water for 1 week. Reversible initial adhesion phase of oral bacteria and fungus depends on the hydrophobic interactions [14, 28–31], and the wettability of the denture surface is an important factor for the

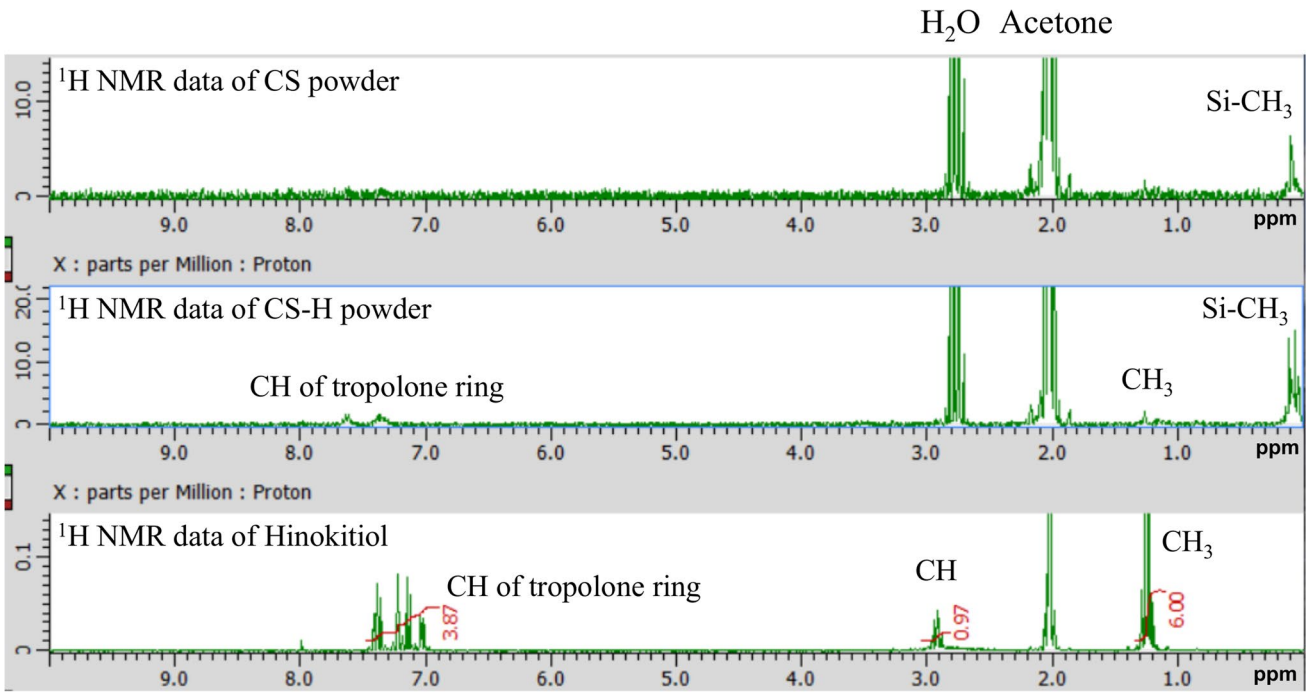

Fig. 6. ^{1}H NMR spectra of CS powder, CS-H powder, and hinokitiol

initial adhesion of *C. albicans* [32, 33]. Therefore, several studies have reported that one method for preventing adhesion of oral bacteria and fungus to dentures is to alter the hydrophobic or hydrophilic surfaces to higher than that of conventional surfaces [14, 29, 31]. Although the relationship between the wettability of specimens from control-1w and CS-1w groups and the adhesion of *C. albicans* to the specimens cannot be completely explained within the design of this study, the decrease in hydrophobicity of specimens from both groups may promote the hydrophobic interactions with *C. albicans*. Thus, the surfaces of specimens from control-1w and CS-1w groups may affect the wettability to promote the adhesion of *C. albicans*.

The decrease in hydrophobicity in the control-1w and CS-1w groups may be due to water absorption of denture base resin and the CS coating. Acrylic resin dentures are known to absorb water [34, 35]. The CS coating is a silica-based glass consisting of a loose three-dimensional glass network that contains a large degree of available space inside the network [14], and as a result of the long-term immersion in water, water molecules could be incorporated into these spaces. The water absorption of materials influences the surface free energy (SFE), which is indicative of the surface wettability [36, 37]. In general, the SFE of polymeric materials increases when these materials absorb water because the SFE of water is higher than that of polymeric materials [38]. Thus, the increase in SFE indicates the decrease in hydrophobicity [36–38]. Therefore, the specimens from control-1w and CS-1w groups may demonstrate a decrease in hydrophobicity due to their water absorption.

The ^{1}H NMR analysis showed the peak associated with hinokitiol on the specimens from the CS-H groups. However, we did not obtain any data to indicate how hinokitiol was incorporated into the CS. Spaces within the loose three-dimensional glass network of CS may be useful for incorporating hinokitiol. Further experiments are needed to clarify these mechanisms.

The silica glass coating has been shown to react with OH bonds that were originally present on the surfaces of materials to form a covalent bond, resulting in the strong adhesion of the coating to the materials [14]. Although the CS and CS-H coating may adhere to OH bonds on surfaces of denture base resin, the adherence strength and mechanical stability of this remain unclear. Therefore, these properties of the coatings require further investigation prior to any clinical application.

Our coating might be applied on new dentures or when constant maintenance of dentures is performed. By using a brush, we can also coat on only the fitting surface of dentures where microorganisms easily adhere. The technique is so easy because we just coat the solution on the denture and dry it at room temperature. Therefore, we aim to realize self-coating by patients in the future. In addition, the coating thickness has been reported to be 3 μm [14]. It has been reported that the physiological tooth mobility is about 200 μm [39], suggesting that the thickness, namely 3 μm

might not be too thick as a denture coating. However, the biological safety and mechanical stability of the coating need to be investigated before any clinical application.

Conclusion

We demonstrated that a silica-based denture coating that incorporates hinokitiol inhibits *C. albicans* growth on denture. Our data also showed antifungal activity of the coating was maintained in water for 1 week. The biological safety of the coating needs to be investigated before any clinical application. Additionally, further experiments are needed to analyze the mechanical stability of the coating. This technology has the potential to contribute to not only the prevention of mucosal diseases such as *Candida* stomatitis but also aspiration pneumonia.

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Author contribution Chiaki Tsutsumi-Arai wrote the main manuscript text and prepared Figs. 1, 2, 3 and 4. Kazuhiro Akutsu-Suyama prepared Figs. 5 and 6. All authors reviewed the manuscript.

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Declarations

Ethics approval This study has no need for prior approval by an ethics committee.

Conflict of interest The authors declare no competing interests.

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