치의과학석사 학위논문

# Effect of Manganese Oxide Nanozyme-doped diatom microbubbler on Candida albicans removal from denture base resin

의치상 레진에서 Manganese Oxide Nanozyme-doped diatom microbubbler 의 캔디다 균 제거 효과에 대한 연구

2022 년 8 월


**서울대학교 대학원**

**치의과학과 치과보철학 전공**

**전 윤 호**

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지도교수 김 명 주

이 논문을 전윤호 석사학위논문으로 제출함

**2022 년 5 월**

서울대학교 대학원

치의과학과 치과보철학 전공

전 윤 호

전윤호의 석사학위논문을 인준함

**2022 년 6 월**

위 원 장 _____ (인)

부 위 원 장 _____ (인)

위 원 _____ (인)

**-ABSTRACT -**

# Effect of Manganese Oxide Nanozyme-doped diatom microbubbler on Candida albicans removal from denture base resin

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***Purpose*:**. In order to prevent denture stomatitis caused by Candida, which is often observed in patients using dentures, it is very important to remove Candida biofilm from the denture. However, common denture cleaners are not effective enough to remove the biofilm formed by Candida. Manganese oxide ($MnO_2$) nanozyme-doped diatom microbubbler (DM) can generate oxygen gas microbubbles by catalase-mimicking activity in hydrogen peroxide ($H_2O_2$) solution.

In this way, DM can invade and destroy the biofilm by the driving force of the continuously generated microbubbles. Therefore, this study compares biofilm removal effect of DM on the denture resin surface with the existing denture cleaner as a method of cleaning dentures.

***Materials and methods*:** Fine-sized diatom particles in the form of hollow cylinders doped with manganese dioxide nanosheets were fabricated. The shape and composition

were analyzed using a scanning electron microscope (SEM). Acrylic resin specimens with a diameter of 10 mm and a thickness of 2 mm were subjected to cold curing (Vertex Self-Curing, Vertex Dental, Soesterberg, The Netherlands), heat curing (Meliodent Heat Cure, Heraeus Kulzer GmbH, Wehrheim, Germany), milling (Pink PMMA BLOCK, large Dental Material, Shanhai, China) and 3D-printing (Denture Plus ARUM 5.0, ARUM Dentistry, Daejeon, Korea) were prepared using four fabrication techniques. Biofilm was formed by culturing Candida albicans on the prepared specimen at 37 degrees Celsius for 24 hours. The 4 types of denture base resin specimens were divided into phosphate buffered saline group (PBS group), Polident group (brand name, Polident group), 0.12% chlorhexidine group (0.12% Chlorhexidine gluconate group), 3% hydrogen peroxide group (3% Hydrogen peroxide group) and DM group (DM and hydrogen peroxide). Treatment methods were determined by previous studies and manufacturer's guidelines. 10 minutes for Phosphate-buffered saline group (PBS group, Martínez-Serna et al. 2021), 5 minutes for Polident group (brand name, Polident group, GlaxoSmithKline, Philadelphia, PA, USA, manufacturer's instructions), 10 minutes for Chlorhexidine group (0.12% Chlorhexidine gluconate group, Martínez-Serna et al. 2021), 10 minutes for Hydrogen peroxide group (3% Hydrogen peroxide group, Martínez-Serna et al. 2021), and 5 minutes for DM group (DM and 3% hydrogen peroxide, Seo et al. 2018) were applied.

The biofilm removal effect of each group was quantitatively analyzed by crystal violet assay and confirmed by scanning electron microscope (SEM) images. Viability was evaluated by observing the remaining Candida albicans after treatment with a confocal

laser scanning microscope (CLSM). Data from crystal violet assay were analyzed by Kruskal-Wallis test followed by Dunn's multiple comparison test ($\alpha = 0.05$).

***Results*:** Although the biofilm was not effectively removed in the 5-minute Polident group (brand name, Polident group) and Chlorhexidine group, chlorhexidine was able to kill the *C. albicans* effectively. Hydrogen peroxide group was able to remove the biofilm of the heat cured and 3D printing resin. The DM group (DM and 3% hydrogen peroxide) was the only one that significantly removed Candida albicans from all types of resin samples. Simultaneous DM and hydrogen peroxide co-treatment is an effective method to remove Candida albicans biofilms from various denture base resin surfaces.

***Conclusion*:**. The denture cleaning method using DM and hydrogen peroxide solution more effectively removed the Candida albicans biofilm on the denture base resin specimen compared to the existing chemical methods, and it could be an alternative that can solve the limitations of the existing denture cleaning method.

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**Key words**: denture cleansers, biofilms, candidia albicans, chlorhexidine, hydrogen peroxide, manganese oxide nanozyme-doped diatom microbubbler

**Student Number**: 2017-29488

# CONTENTS

# I. INTRODUCTION

Oral candidiasis is denture-related stomatitis, the most common human fungal infection (Gendreau and Loewy 2011). This oral disease is caused by the presence of Candida species (Skupien et al. 2013). Candida can penetrate the oral mucosal tissue, evade the host's defensive mechanisms, and has several virulence factors (Moyes et al. 2016). *Candida albicans* is a primary microbe for denture-related stomatitis (Skupien et al. 2013), and there is a greater number on the surface of the dentures covering the mucous membrane than the mucosa itself of the patient, indicating that the dentures can become reservoirs of infection (Davenport 1970; Fanello et al. 2006).

Polymethyl methacrylate (PMMA) denture base is porous and rough, so Candida can easily adhere and form a biofilm (Radford et al. 1999; Radford et al. 1998). The presence of biofilms on dentures has been associated with denture stomatitis, as well as with systemic conditions, especially in elderly patients (Felton et al. 2011). In order to prevent denture stomatitis caused by Candida and protect the health of patients, it is necessary to remove the biofilm formed on the denture by proper cleansing procedure (Işeri et al. 2011; Nalbant et al. 2008).

Dentures can be cleaned mechanically and chemically, as well as a combination of the two (Köroğlu et al. 2016). Mechanical methods generally use brushing with or without dentifrice (Nikawa et al. 1999). Since it is difficult for elderly or disabled patients to use the correct mechanical cleansing methods, it

is recommended to use the chemical methods (Alam et al. 2011; Kulak-Ozkan et al. 2002). Chemical denture cleansers such as hydrogen peroxides, chlorhexidine digluconate, and commercial effervescent denture cleansers have been conventionally used (Machado de Andrade et al. 2012; Martínez-Serna et al. 2021; Montagner et al. 2009; Vasconcelos et al. 2020). However, these denture cleansers are ineffective in removing mature biofilms, especially formed by Candida (Lucena-Ferreira et al. 2013; Paranhos et al. 2007). The biofilm formed by *C. albicans* attached to dentures has resistance to denture cleansers and antifungal drugs (Chandra et al. 2001; Sardi et al. 2013).

Diatom Microbubbler (DM), a recently developed material for use as an active cleansing agent, is a hollow cylinder-shaped diatom biosilica with manganese oxide ($MnO_2$) nanozyme sheets (Seo et al. 2018). DM generates oxygen gas bubbles by rapidly decomposing hydrogen peroxide ($H_2O_2$) with the catalase-mimicking activity of $MnO_2$ in $H_2O_2$. In a previous study, DM invaded and destroyed Escherichia coli biofilm with the driving force of continuously generated microbubbles, and hydrogen peroxide molecules diffused into the biofilm, effectively removing the biofilm (Seo et al. 2018).

The purpose of this research was to evaluate the feasibility of using DM as a novel denture cleanser. The effect of DM on the removal of *C. albicans* biofilms formed on cold-cured, heat-cured, milled, 3D-printed resin specimens was studied and compared to the effect of conventional denture cleaners. The null

hypothesis of this study is that the effectiveness of the denture cleansers tested would be similar.

# II. MATERIALS AND METHODS

## 1. Physicochemical characterization of the Diatom microbubblers (DMs) and preparation of denture base acrylic resin specimens

### 1. 1. Physicochemical characterization of the DMs

#### 1. 1. 1. Fabrication of the DMs

DMs were fabricated as described previously (Seo et al. 2018). To prepare amine-substituted diatom particles, 2 g of diatom particles were added to 60 mL of toluene in a three-necked round-bottom flask equipped with a thermometer, a reflux condenser and an $N_2$ gas tube. After that, 0.6 mL of distilled water was added to the mixture, and stirred for 2 hrs at room temperature. Then, 3.4 mL of (3-aminopropyl) triethoxysilane (APTES, Sigma-Aldrich, St. Louis, MO, USA) was added to the mixture and refluxed for 6 hrs at 60 °C. After cooling the

mixture, it was washed with toluene, 2-propanol, and distilled water three times. After drying in a vacuum desiccator for 2 days, 0.1 g of amine-substituted diatom particles were added to 1 mL of 50 mM potassium permanganate ($KMnO_4$, Sigma-Aldrich, St. Louis, MO, USA) solution and sonicated for 30 mins at room temperature. Finally, the samples were washed with distilled water and ethanol three times and dried in an oven for 1 day at 60 °C.

### 1. 1. 2. Physicochemical characterization of DMs

The scanning electron microscope (SEM) images for observing the morphology of DMs were obtained with Apreo S (Thermo Fisher Scientific, Waltham, MA, USA) operating at 10.0 kV. The element mapping of DMs was analyzed by Energy Dispersive Spectrometer (EDS) coupled with SEM at 20.0 kV acceleration voltage.

## 1. 2. Preparation of denture base acrylic resin specimens

Disk-shaped (Ø10×2mm) denture base acrylic resin specimens were prepared by using four fabricating techniques as follows: cold curing (Vertex Self-Curing, Vertex Dental, Soesterberg, Netherlands), heat curing (Meliodent Heat Cure, Heraeus Kulzer GmbH, Wehrheim, Germany), milling (Pink PMMA BLOCK, Huge Dental Material, Shanhai, China), and 3D-printing (Denture Plus ARUM

5.0, ARUM Dentistry, Daejeon, Korea). The specimens were designed with a CAD software program (Meshmixer, Autodesk, San Rafael, CA, USA) to prepare specimens of the same design and size regardless of the manufacturing techniques. Cold cured and heat cured resin specimens were fabricated by using a conventional flasking and pressure-pack technique. Milled resin specimens were fabricated with a milling machine (DEG-5X100, ARUM Dentistry, Daejeon, Korea). The 3D-printed resin specimens were fabricated by using a digital light processing 3D printer (ASIGA MAX UV; ASIGA, Alexandria, Australia) and post-polymerized with an ultraviolet light-polymerization unit (PURE PRO, U-Dent, Daegu, Korea) according to the manufacturer's instructions. And all the resin specimens were prepared in an unpolished state in order to resemble the state of denture in contact with gingiva. The prepared resin specimens were rinsed for 5 mins in an ultrasonic cleaner and immersed in distilled water for 24 hrs (Lee et al. 2022). All specimens were packaged and sterilized in a plasma sterilizer for 50 minutes. After that the specimens were sterilized under an ultraviolet light for 8 hrs per side and then stored in sterile bags (Martínez-Serna et al. 2021).

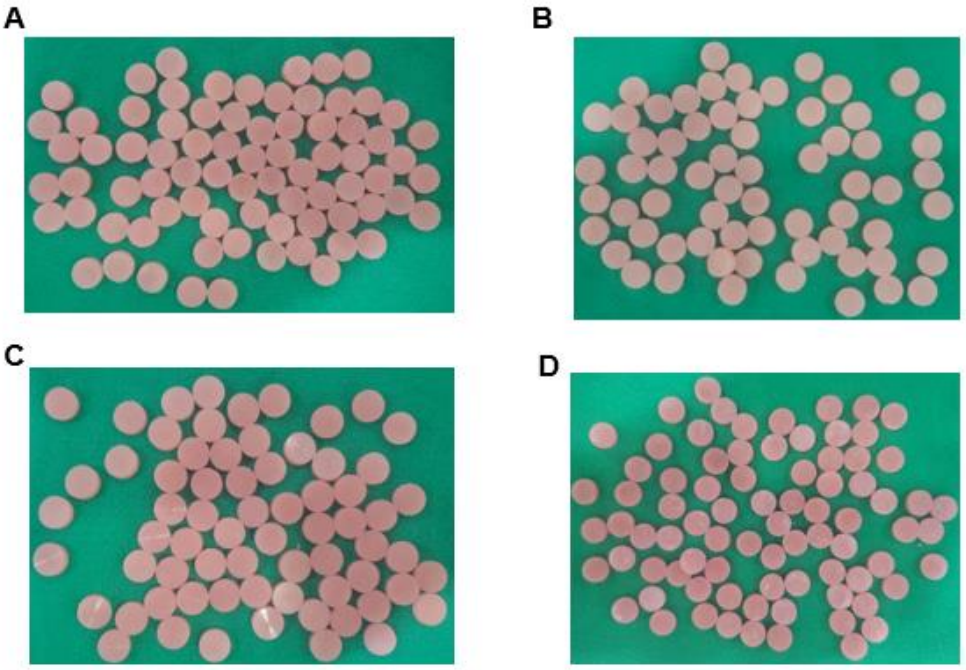


**Figure 1.** Denture base arcylic resin specimens prepared.
(A) Cold Curing, (B) Heat Curing, (C) Milling, (D) 3D-printing

## 2. Biofilm formation and removal treatment on acrylic resin specimens

### 2. 1. Biofilm formation on acrylic resin specimens

To create a biofilm, resin specimens were coated with saliva (Sanchez et al. 2011). Unstimulated saliva was collected in sterile plastic tubes at least 1.5 hrs after eating, drinking or tooth brushing. This study was approved by the Ethic Committee of Seoul National University Dental Hospital (CRI22008), and

informed written consent from all participants was obtained prior to the research. Collected saliva was centrifuged (12,000 rpm, 10 mins, 4°C) and only supernatant was mixed with phosphate-buffered saline (PBS, pH = 7.4) in 1:1 (v/v) ratio and was filtered through 0.2 μm pore size Minisart syringe filter (Sartorius Stedim Biotech GmbH, Göttingen, Germany). The sterile resin specimens were placed in 24-well tissue culture plate, and incubated with filtered saliva/PBS mix for 2 hrs at 37 °C. After the saliva was removed, 1 mL of *C. albicans* (ATCC 18804) cultured in YM broth (0.3% yeast extract, 0.3% malt extract, 0.5% peptone and 1.0% dextrose, concentration 1 x $10^6$ cells/mL) was added and incubated aerobically for 24 hrs at 37 °C.

## 2. 2. Biofilm removal treatments on acrylic resin specimens

A total of 240 denture base acrylic resin specimens were prepared by using four fabricating techniques (60 disks for each fabricating technique). 60 resin specimens prepared with the same fabrication technique were randomly assigned to six groups (n=10). After the biofilm formation, the resin specimens were gently washed with PBS and treated according to the protocol corresponding to each group. The concentration and application time of the agents used in each group were determined based on previous studies or on the manufacturer's instructions: Phosphate-buffered saline group, phosphate-buffered saline for 10 mins (Martínez-Serna et al. 2021); Polident group, Polident 5-Minute

(GlaxoSmithKline, Philadelphia, PA, USA) for 5 mins (manufacturer's instructions), Chlorhexidine group, 0.12% (w/v) chlorhexidine group for 10 mins (Martínez-Serna et al. 2021); Hydrogen peroxide group, 3% (v/v) hydrogen peroxide for 10 mins (Martínez-Serna et al. 2021); DM group, co-treatment of 3 mg/mL of DM and 3% hydrogen peroxide for 5 mins (Seo et al. 2018); negative control (no contamination to verify asepsis of the experiment) for nothing.

The remaining biofilms after each treatment were quantified using crystal violet assay (Ellepola et al. 2017). The resin specimens were washed with PBS and incubated with 1 mL of 1% (w/v) crystal violet solution (Junsei Chemical, Tokyo, Japan) for 10 mins to stain remaining biofilm on the resin specimens. After that, the specimens were rinsed with PBS for three times to remove residual dye. The remaining crystal violet dye in the biofilms was extracted by using 95% ethanol. The optical density (OD) of dissolved crystal violet dye was quantified by using microplate reader (Epoch 2, Bio-Tek Instruments, Winooski, VT, USA) at 570 nm.

## 3. Analysis of removal treatment

### 3. 1. Scanning Electron Microscope (SEM) analysis

After each treatment, the resin specimens were fixed for 4 hrs with 1 mL of 4% paraformaldehyde (Biosesang, Seongnam, Korea) and washed with 1 mL

PBS for 15 mins three times. After that, the specimens were fixed with 1 mL of 1% osmium tetroxide in PBS for 60 mins and rinsed with 1 mL PBS for 15 mins three times. The specimens were dehydrated in a successively increasing concentration of ethanol for 15 mins each at 70%, 80%, 90%, 95% and 100%. Subsequently, the resin specimens were treated in 1 mL of 100% hexamethydilazane (HMDS, Sigma-Aldrich, St. Louis, MO, USA) for 20 mins. After the specimens were completely dry, platinum coating was performed. Each resin specimens were examined by using SEM at a voltage of 10 kV.

## 3. 2. Biofilm Analysis by Confocal Laser Scanning Microscopy (CLSM)

The resin specimens after each treatment were stained in broth containing 5 μg/mL Hoechst 33342 (Invitrogen-Life Technologies, Carlsbad, CA, USA) and 5 μg/mL propidium iodide (PI, Invitrogen-Life Technologies, Carlsbad, CA, USA) for 30 mins at 4 °C as previously described (Yang et al. 2019). Each resin specimen was washed with PBS three times and placed upside-down on a glass bottomed confocal dishes (SPL Life Science, Kyong-Gi, Korea) with BacLight mounting oil (Thermo Fisher Scientific, Waltham, MA, USA). Images were obtained by confocal laser scanning microscopy (CLSM, LSM700, Carl Zeiss, Oberkochen, Germany) equipped with 405 and 555 nm excitation lasers.

### 3. 3. Statistical analysis

All data are presented as the mean value ± SEM (standard error of the mean). The significance of the differences among groups was determined by the Kruskal-Wallis test followed by Dunn's multiple comparisons test ($\alpha = 0.05$), since the normality and homoscedasticity assumptions of the data had been violated. GraphPad Prism 9 (GraphPad, La Jolla, CA, USA) was used for the statistical analyses.

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# III. RESULTS

## 1. Physicochemical characterization of the DMs

### 1. 1. Physicochemical characterization of the DMs

Fossilized *Aulacoseira* diatom particles in the form of hollow cylinders (approximately 10 μm in diameter and 18 μm in length) with many holes (approximately 500 nm in diameter) on the surfaces were used in this study (Fig. 2A). Elemental analysis through SEM revealed that $MnO_2$ nanozymes were uniformly doped on the silica surfaces of the diatom particles (Fig. 2B, 2C).

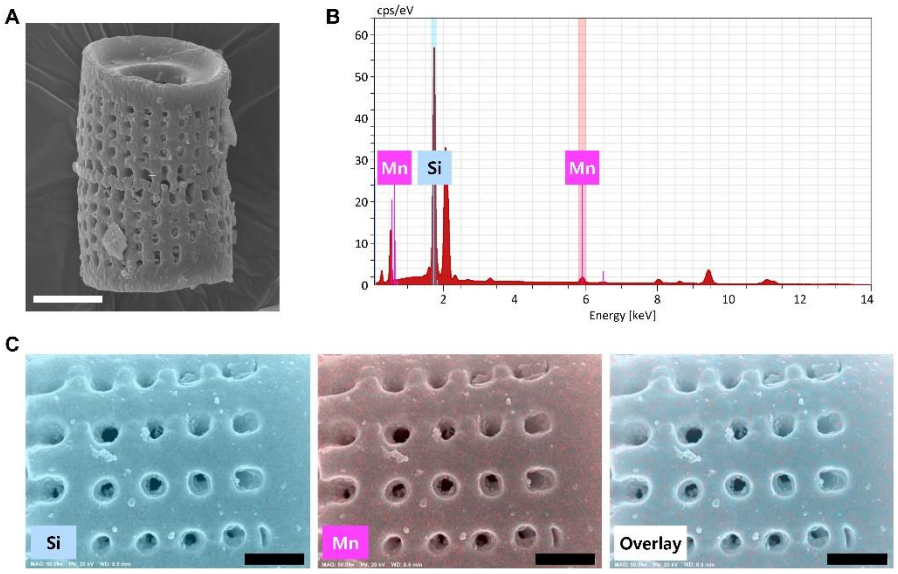


**Figure 2.** Fabrication of the $MnO_2$ nanozyme-doped diatom microbubbler. (A) Scanning electron microscopy images of diatom microbubbler (magnification: 15,000 times, white scale bar = 5 μm), (B) A representative elemental spectrum of diatom microbubbler obtained from energy dispersive spectrometer, (C) Element mapping images showing homogenous distribution of $MnO_2$ nanozyme sheets on diatom microbubbler (black scale bar = 800 nm).

## 2. Biofilm removal effect of the DMs

### 2. 1. Crystal violet assay

The results of the crystal violet assay represent total *C. albicans* biofilms remaining on the acrylic resin specimens after each treatment (Fig. 3). In the case of cold-cured, and milled acrylic resin, only the DM groups showed significantly less biofilms than the PBS groups ($p < 0.05$), and Polident, Chlorhexidine and

hydrogen peroxide groups showed no significant difference from the PBS groups (Fig. 3A, 3C). In the case of biofilms of heat-cured and 3D-printed acrylic resin specimens, the hydrogen peroxide groups and DM groups had significantly lower values than the PBS groups ($p < 0.05$), but there was no significant difference between the other groups and the PBS groups (Fig. 3B, 3D). In addition, when 3D-printed acrylic resin specimens were used, higher OD values were observed than when other polymerization methods were used (Fig. 4).

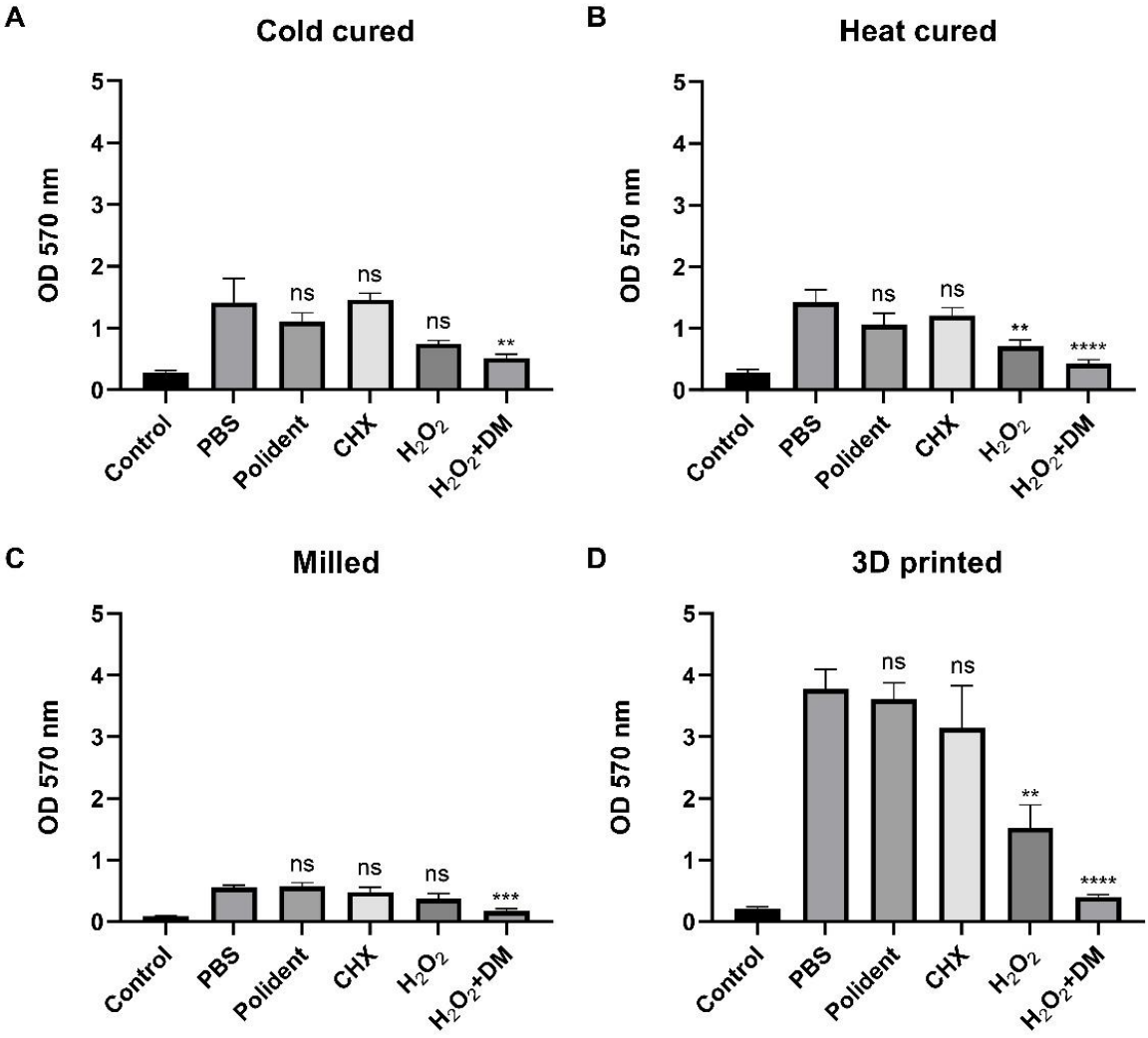


**Figure 3.** *Candida albicans* biofilm removal effect evaluated using crystal violet analysis. Denture base acrylic resin specimens were prepared by using four fabricating techniques. (A) Cold curing, (B) Heat curing, (C) Milling, (D) 3D-printing. Data are expressed as mean value ± SEM. Kruskal-Wallis test is performed with Dunn's multiple comparisons test. Symbol represents statistically significant differences between the PBS group and experimental groups (ns: not significant, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$).

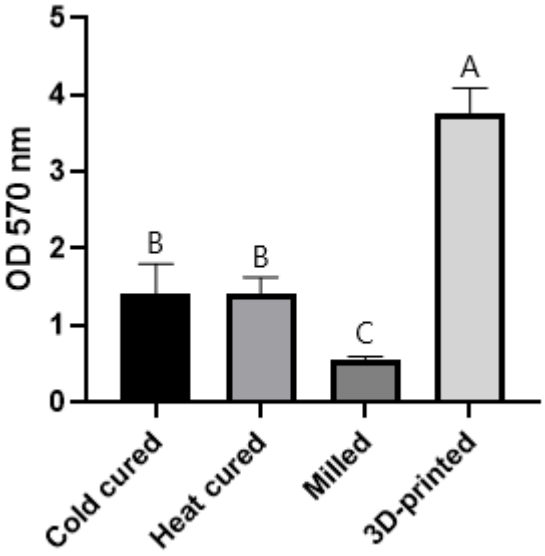


**Figure 4.** *Candida albicans* biofilm removal effect evaluated using crystal violet analysis in PBS group. Capital letters on the graph indicate the statistically significant differences in order between different kind of resin specimens (Cold cured vs. Heat cured : not significant, Milled vs. 3D-printed : $p < 0.0001$, Other comparisons : $p < 0.05$).

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## 2. 2. SEM analysis

To visually confirm the removal efficiency of *C. albicans* biofilm, the 4 types of acrylic resin specimens were subjected to SEM imaging after each treatment (Fig. 5). The cluster of *C. albicans* were similarly observed in the PBS, Polident, and chlorhexidine groups. A relatively small number of *C. albicans* was observed in the hydrogen peroxide groups. In the DM groups, very few *C. albicans* and fragmented or unrecognizable biofilm remnants were observed.

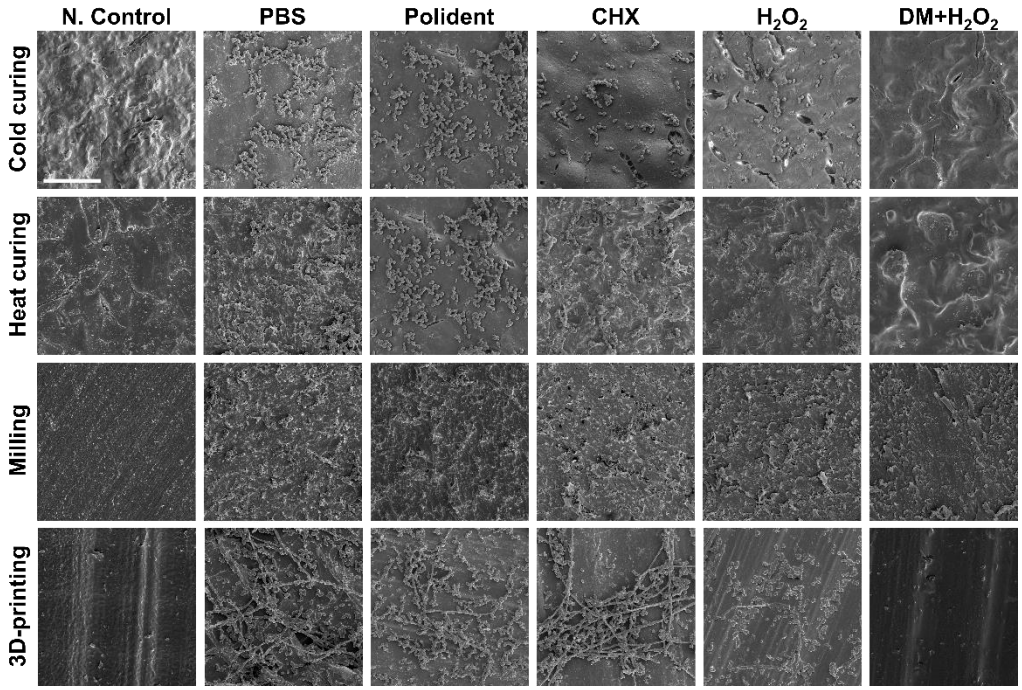


**Figure 5.** Scanning electron microscopy images of denture base acrylic resin specimens after each biofilm removal treatment. Each type of resin was arranged in each row, and each type of treatment was arranged in each column (magnification: 2,000 times, scale bar = 50 μm).

## 2. 3. Biofilm Analysis by CLSM

According to the CLSM images of all types of acrylic resins (Fig. 6), *C. albicans* stained with PI were clearly more observable in the chlorhexidine groups. In the hydrogen peroxide group, a relatively small number of cells was observed compared to the PBS group, and there were even fewer remaining cells in the DM group.

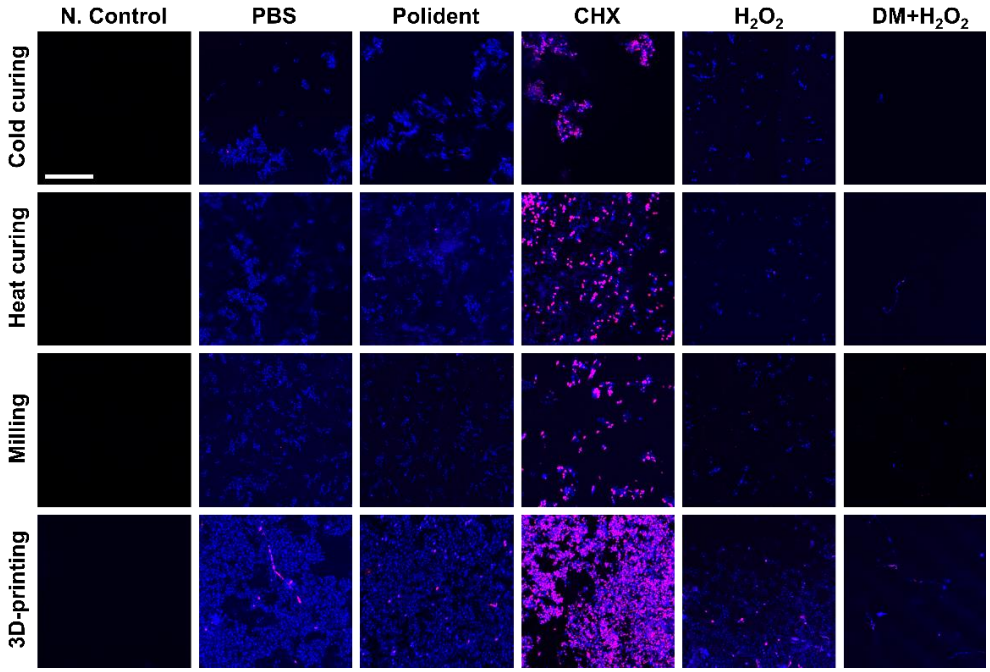


**Figure 6.** Confocal Laser Scanning Microscopy images of remaining biofilms on the denture base acrylic resin specimens after each biofilm removal treatment. Dual-staining method using Hoechst 33342/PI was used to evaluate the viability of remaining *Candida albicans* after treatment. Both live and dead cells are stained with Hoechst 33342 and dead cells are stained with PI. Each type of resin was arranged in each row, and each type of treatment was arranged in each column (scale bar = 50 μm).

# IV. DISCUSSION

The results of the present study demonstrated that each denture cleansers tested showed a different biofilm reduction on the acrylic resin specimens;

therefore, the null hypothesis was rejected. According to crystal violet assay, the Polident groups and the chlorhexidine group did not show a significant difference with the PBS group (Fig. 3). Polident and chlorhexidine could not effectively remove the biofilms of the denture base resin specimens. hydrogen peroxide effectively removed the biofilms of heat cured and 3D-printed resin specimens, but showed no significant difference from the PBS group in other types of resins (Fig. 3). DM groups are the only groups to have significantly more *C. albicans* removed from four kinds of acrylic resin specimens when compared to the PBS groups (Fig. 3). More *C. albicans* can inhabit the 3D-printed denture surface than other polymerized denture base resins (Fig. 4). In these days when 3D-printed dentures are being used more frequently, using DM and hydrogen peroxide to clean these denture surfaces can be a good idea (Fig. 3D, Fig. 4).

The SEM images were observed to visually confirm the biofilm removal efficiency, which supported the results of the crystal violet assay (Fig. 5). The remaining biofilms of the Polident and chlorhexidine groups were similar to those of the PBS groups, but relatively fewer biofilms were observed in the hydrogen peroxide groups. In the DM groups, only fragmented or unrecognizable biofilm remnants were observed. To evaluate the viability as well as the amount of *C. albicans* after each treatment, the specimens were stained and observed with CLSM (Fig. 6). To evaluate the viability of *C. albicans*

remaining after treatment, dual-staining method using Hoechst 33342/PI was used (Carolina et al. 2011; Yang et al. 2019). Hoechst 33342 can cross cell membranes and stain the DNA of living and dead cells. In contrast, PI selectively labels dead cells as it only enters cells with damaged plasma membranes (Carolina et al. 2011). Chlorhexidine groups did not significantly remove biofilms, but effectively killed *C. albicans* (Fig. 6).

Polident is a denture cleansing tablet that has been reported to be effective in previous studies (Li et al. 2010). However, in this study, Polident could not effectively remove the biofilms or kill *C. albicans*, and this results are similar to those reported by some previous studies (Drake et al. 1992; Vasconcelos et al. 2020). Chlorhexidine has been reported to show high effectiveness against *C. albicans* (Da Silva et al. 2008), but this agent did not effectively remove the biofilms according to the result of crystal violet assay. This study conformed the same results with CLSM images. Therefore, chlorhexidine can kill *C. albicans*, however, the dead *C. albicans* and exopolymeric substances were not removed. Hydrogen peroxide could remove biofilms on heat cured and 3D-printed resins, but did not show significant effect on the other two types of resins. Martínez-Serna et al. reported that the effect of reducing *C. albicans* was insufficient when hydrogen peroxide was used alone (Martínez-Serna et al. 2021).

For the treatment of denture stomatitis, photodynamic therapy (PDT)/photodynamic inactivation (PI), which is effective for drug-resistant

Candida and safe, is emerging as an alternative (Hamblin. M. R, 2016). Compared to the conventional nystatin washing, PDT/PI significantly reduced the number of *C. albicans* in the oral cavity. However, both nystatin washing and PDT washing failed to significantly reduce *C. albicans* in the inner surface of the denture (Alves, F. et al, 2020). This is due to the fact that the amount of Oxygen required to kill Fungi is higher than that required to sterilize bacteria (Alves, F. et al, 2020). Therefore, it is worth paying attention to the denture cleaning potential of Manganese Oxide Nanozyme-doped diatom by generating continuous oxygen.

Oxygen bubbles generated from Manganese Oxide Nanozyme-doped diatoms form microbubbles by nucleating inside the hollow space of diatoms (Seo et al. 2018). The generated Oxygen gas bubbles nucleate and form microbubbles inside the hollow space of diatoms (Seo et al. 2018). As the bubbles build up the pressure, the DM particles continuously eject microbubbles and move randomly, propelled by driving forces. DM particles penetrate into the biofilms, and continuously generate Oxygen gas and hydrogen peroxide readily diffuses within the biofilms(Seo et al. 2018). Since DM particles penetrated into the biofilm and continuous production of Oxygen gas and diffusion of hydrogen peroxide were possible within the biofilms, DM removed the bilofilms more effectively than hydrogen peroxide.

In this study, resin specimens were prepared by four fabricating techniques to evaluate the effect of removing biofilms on various types of denture base acrylic resin used in the general dental practice. Unpolished resin specimens were used in this study because the main reservoir of *C. albicans* is the surface of the dentures covering the mucosa and *C. albicans* can more easily penetrate the unpolished surface (Ramage et al. 2004; Van Reenen 1973). DM effectively removed biofilms regardless of the resin fabricating technique.

In this study, the DM concentration for the experiment was set to 3 mg/mL, and the treatment period was 5 mins according to the previous study (Seo et al. 2018). This study is the first to report feasibility of DM application as a novel candidate for denture cleaner. Further studies are needed on the appropriate concentration and application time of DM for practical use as a denture cleanser in clinical practice. It is also necessary to study the effect of removing multispecies biofilms and the biofilms on the dentures in use by patients. And it is necessary to confirm the biofilm removal effect of DM even on metal base dentures.

# V. CONCLUSIONS

This study analyzed the removal effect of *C. albicans* from the inner surface of dentures using Manganese Oxide Nanozyme-doped diatom compared to conventional denture cleaners. Specimens were fabricated using four types of methods using denture base acrylic resin, and the removal effect of *C. albicans* was investigated using crystal violet assay, SEM analysis, and confocal laser scanning microscopy. Manganese Oxide Nanozyme-doped diatom and hydrogen peroxide combined showed to be the most effective method in removing biofilm and reducing the number of *C. albicans* on denture base acrylic resin. The denture cleaning method using Manganese Oxide Nanozyme and hydrogen peroxide solution more effectively removed the *C. albicans* biofilm on the denture base resin specimen compared to the existing chemical methods, and it could be an alternative that can solve the limitations of the existing denture cleaning method.

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**ABSTRACT IN KOREAN**

– 국문초록 –

# 의치상 레진에서 Manganese Oxide Nanozyme-doped diatom microbubbler 의 캔디다 균 제거 효과에 대한 연구

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**전 윤 호**

의치를 사용하는 환자에게서 많이 관찰되는 캔디다균에 의한 의치성 구내염을 예방하기 위해서는 의치에서 캔디다균막을 제거하는 것이 매우 중요하다. 그러나 일반적인 의치세정제는 칸디다균에 의해 형성된 생물막을 제거하는 데에 충분한 효과를 나타내지 못한다. 최근 연구에 따르면, 산화망간 나노자임이 도핑된 규조류 미세 기포 발생자(Manganese Oxide Nanozyme-doped diatom microbubbler, DM)는 과산화수소 용액에서 촉매제 모방 활성에 의해 미세 산소 가스 기포를 생성하고, 지속적으로 생성되는 미세 기포의 추진력으로 생물막을 침범하고 파괴할 수 있다. 이에 본 연구에서는 의치 세정의 방법으로써 의치 레진상 표면에서 DM 의 생물막 제거 효과를 기존 의치세정제와 비교하고 새로운 의치세정제로서의 가능성을 평가하고자 한다. 이산화망간 나노시트가 도핑된 속이 빈 원통 형태의 미세한 크기의 규조류 입자를 제작하고, 주사전자현미경을

이용하여 형태와 성분을 분석하였다. 의치상 레진 시편을 직경 10mm, 두께 2mm 의 원판 형태로 자가중합형, 열중합형, 밀링 및 3D-프린팅 레진을 이용하여 제작하고, 시편 표면은 임상에서의 의치상 내면과 유사하게 연마하지 않았다. 준비된 시편에 섭씨 37 도, 24 시간 동안 캔디다균을 배양하여 생물막을 형성한 후, 인산완충식염수군(PBS group), 폴리덴트군(Polident group), 0.12% 클로로헥시딘군(0.12% Chlorohexidine gluconate group), 3% 과산화수소수군(3% Hydrogen peroxide group), DM 군(DM & 3% Hydrogen peroxide group)으로 각각 선행 연구와 제조사의 지침에 의해 처리하였다. 각 군의 생물막 제거 효과는 Crystal violet assay 로 정량 분석 하였으며, 주사전자현미경 영상으로 확인하였다. 캔디다균을 공초점 레이저 주사 현미경으로 관찰하여 생존력을 평가하였다. 95% 신뢰수준에서 Kruskal-Wallis 분석과 Dunn 의 다중 비교 분석으로 통계처리 하였다.

폴리덴트군과 0.12% 클로로헥시딘군에서 생물막이 효과적으로 제거되지는 않았지만 클로로헥시딘은 캔디다균을 효과적으로 살균하였다. 3% 과산화수소수군은 열중합 및 3D 프린팅 레진 시편의 생물막을 제거할 수 있었다. DM 군(DM 과 3% 과산화수소수 처리)은 모든 유형의 레진 시편에서 캔디다균 생물막을 통계적으로 유의하게 제거하였다. DM 과 과산화수소수를 이용한 의치 세척 방법은 기존 방법에 비해 의치상 레진 시편의 캔디다균 생물막을 더 효과적으로 제거하였고, 이는 기존의 의치세정제의 한계를 극복할 수 있는 대안이 될 수 있을 것이다.

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**주요어** : 의치 세정제, 생물막, 캔디다증, 클로로헥시딘, 과산화수소, 산화망간 나노자임이 도핑된 규조류 미세 기포 발생자

**학 번** : 2017-29488