Development of denture base resin incorporated with dodecyl quaternary ammonium compound for antifungal efficacy against *Candida albicans*

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Development of denture base resin incorporated with dodecyl quaternary ammonium compound for antifungal efficacy against *Candida albicans*

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Graduate school marked a significant chapter in my life – a journey I had envisioned since childhood, aspiring to become a doctor. Now, on the cusp of earning my doctoral degree, I reflect on the challenges overcome, bolstered by the unwavering support of those around me.

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ABSTRACT

Development of denture base resin incorporated with dodecyl quaternary ammonium compound for antifungal efficacy against *Candida albicans*

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(Directed by Professor Jae-Sung Kwon, M.D., Ph.D.)

Poly(methyl methacrylate) (PMMA) is a widely used materials in denture fabrication due to its low cost, biocompatibility, and aesthetic properties. However, its susceptibility to microbial accumulation and subsequent abrasion-induced denture stomatitis remains a challenge. Quaternary ammonium compounds have been extensively used for surface modifications and are known for their antimicrobial properties. Among them, the quaternary ammonium compound with 12 carbons has demonstrated antifungal properties when used as a surface coating material. Nonetheless, there is a lack of research investigating the antifungal effects of incorporating this compound into denture base resin. Therefore, this study aims to develop a denture base resin incorporating the dodecyl quaternary ammonium compound (pDMAEMAC$_{12}$) and evaluate its antifungal efficacy

against *Candida albicans* and its physical and mechanical properties for clinical applicability.

The denture base resin was modified by incorporating dodecyl quaternary ammonium polymer at various weight percentages (0-control, 0.5, 0.75, and 1.0 wt. %). Polymerization of $pDMAEMAC_{12}$ was confirmed using ^{1}H NMR, and the aggregation of $pDMAEMAC_{12}$ in PMMA was analyzed through scanning electron microscopy (SEM). The surface properties of the denture base resin, incorporated with $pDMAEMAC_{12}$, were evaluated using color measurement, water sorption, and solubility. Mechanical properties were assessed by flexural strength and flexural modulus following ISO 20795-1 standards. The antifungal analysis involved measuring the colony forming unit (CFU) of *Candida albicans* (*C. albicans*). Both non-aged and thermally aged groups were evaluated for CFU tests to assess long-term antifungal efficacy. Given that quaternary ammonium is known for its antimicrobial effect through contact killing, confocal laser scanning microscopy (CLSM) was employed to evaluate adhered fungal viability on the specimens. Finally, biocompatibility was assessed through cytotoxicity on L929 fibroblast cells.

The SEM images indicated no significant differences among the tested groups. Contact angle results also showed no significant differences among the groups ($p > 0.05$). When comparing the control and the groups containing $pDMAEMAC_{12}$, there were no significant differences in color, water sorption, and solubility ($p > 0.05$). Regarding mechanical properties, all groups met ISO standard requirements in flexural strength and flexural modulus, but the C_{12} 1.0% group exhibited significantly decreased strength compared to the control ($p < 0.05$). CFU results on *C. albicans* revealed that the antifungal efficacy significantly increased when more than 0.75 wt.% of $pDMAEMAC_{12}$ was incorporated into the denture base resin ($p < 0.05$). Furthermore, all aged groups containing $pDMAEMAC_{12}$ showed a significant antifungal effect compared to the control ($p < 0.05$), especially when more than 0.75 wt.% of $pDMAEMAC_{12}$ was incorporated. Additionally, CLSM results demonstrated that more dead fungi were observed on the specimen surface as more $pDMAEMAC_{12}$ was incorporated, confirming the contact killing mechanism of quaternary

ammonium. Lastly, despite significant differences in cytotoxicity between the control and the groups containing $pDMAEMAC_{12}$, all values met ISO standard criterion.

Therefore, it can be concluded that the incorporation of up to 0.75 wt.% of $pDMAEMAC_{12}$ into the denture base resin would not adversely affect the physical and mechanical properties of the denture base resin while providing a long-term antifungal effect. Consequently, PMMA incorporated with 0.75 wt.% $pDMAEMAC_{12}$ would exhibit sustained antifungal efficacy, promoting healthy oral conditions for edentulous patients.

Key words: denture, quaternary ammonium, denture stomatitis, long-term antifungal effect, physical properties, mechanical properties

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I. INTRODUCTION

1. Poly(methyl methacrylate) in dentistry

Modern society's longer life expectancy has resulted in an increased population of older people, and despite improved oral hygiene, it has been reported that seniors 85 and older have an average of 14 natural teeth left (Steele et al., 2012). An implant can replace missing teeth, although edentulous people may not always be eligible to use one. Hence, it is crucial to design effective dentures for the restoration of edentulism. Among the various materials employed in dental practices, methacrylate-based acrylic resin, particularly poly(methyl methacrylate) (PMMA), stands out as the primary choice for fabricating dentures. PMMA, initially explored as a fragrance-free acrylic acid polymer, has seen

continuous development over several decades, eventually becoming an essential material in dental clinics since the 1940s for various biomedical applications (Zafar, 2020). Due to its desirable attributes, including aesthetics, comparatively lower toxicity, and strength, PMMA is extensively utilized not only as denture base material but also for applications such as temporary crowns, obturators, and more (Frazer et al., 2005; Gautam et al., 2012; Johnson, 1959; Khindria, Mittal, and Sukhija, 2009). Nevertheless, similar to other materials, PMMA has its limitations. Residual monomers remaining after polymerization can lead to cytotoxicity and diminished color stability (Alla et al., 2015; Kedjarune, Charoenworaluk, and Koontongkaew, 1999). Moreover, it is susceptible to microbial growth within the oral cavity due to the development of anaerobic conditions caused by a decrease in saliva flow to the underlying tissue (Dar-Odeh, Al-Beyari, and Abu-Hammad, 2012; Sivakumar et al., 2014). Oral microbes have a tendency to adhere to the unpolished surface of dentures, which comes into direct contact with the oral mucosa, leading to the formation of denture plaque (Okita et al., 1991). Its vulnerability to microbial growth can lead to oral infections, causing reported issues such as allergic reactions, irritation of the oral mucosa, and hypersensitivity. Consequently, it has been reported that over 50 % of patients over the age of 40 have experienced oral mucosal lesions induced by dentures (Gaur et al., 2015).

2. Denture stomatitis

As dentures interconnect with the oral mucosa, the change in natural oral condition could trigger denture biofilm accumulation which could cause cariogenic or periodontic biofilms (An et al., 2021). As a result, even though PMMA has favorable characteristics for dentures, more than two-thirds of denture wearers suffer from denture stomatitis (Hannah et al., 2017). Denture stomatitis typically arises from a combination of factors, including ill-fitting dentures, mechanical trauma to the oral tissues caused by the dentures, and the potential development of fungal infections in the affected areas (Budtz-Jorgensen and Bertram, 1970; Nyquist, 1952). These factors often contribute to the development and progression of denture stomatitis. In order to address denture stomatitis, various methods are employed, including the use of cleansers, laser treatment, application of a resilient liner or tissue conditioner, and the administration of antifungal therapy (Yarborough et al., 2016). Among the various treatments for denture stomatitis, such as improving oral and denture hygiene and administering antifungal therapy, prevention is the most effective approach (Carl O. Boucher, 1980). Preventive measures involve ensuring the stability of dentures and educating individuals who wear dentures on the importance of maintaining oral hygiene, which includes cleaning their dentures and refraining from wearing them while sleeping (Hannah et al., 2017). Given that denture stomatitis is commonly observed among elderly individuals who may find it challenging to manage their oral health, the introduction of antifungal dentures could prove to be a valuable preventive measure against denture stomatitis (Budtz-Jörgensen, Stenderup, and Grabowski, 1975). Consequently, it is essential for denture base resin to possess inherent antifungal properties to impede the advancement of denture stomatitis.

3. Antimicrobial efforts

To overcome bacterial infection, several therapeutic strategies have been studied. Incorporating antibacterial agents into resin material is one of the antibacterial approaches. Incorporating antimicrobial agents, such as silver and zinc oxide, into dental materials has demonstrated significant antimicrobial effects (Aydin Sevinç and Hanley, 2010; Yin et al., 2020). While the released antimicrobial agents effectively inhibit microbial growth, previous studies have indicated a burst of initial release (Jamuna-Thevi et al., 2011). Moreover, the incorporation of certain materials may impede polymerization or act as stress center, potentially compromising the mechanical properties of the materials (Cyphert et al., 2019; Kim et al., 2019). Consequently, there is uncertainty in ensuring a sustained, long-term antimicrobial effect without compromising mechanical properties.

In addition to applying of antimicrobial agent, surface modification with an antimicrobial coating is another option. As dentures are utilized over the long term, they undergo exposure to abrasion and surface damage caused by activities such as brushing, masticatory exercises, and other external factors (Jin et al., 2022). As a result, previous studies have observed surface deformation over time due to brushing (Mainieri et al., 2011). This surface deformation has the potential to impact the effectiveness of the surface coating, eventually leading to a loss of antimicrobial efficacy. Therefore, denture base resin with a long-lasting antifungal effect is promising to maintain oral health for edentulous patients.

4. Quaternary ammonium

Quaternary ammonium compounds have been used to combat pathogenic microorganisms. They can be immobilized within a resin matrix, which allows for sustained, long-term contact killing of the targeted pathogens (Han et al., 2011). Although the precise mechanism by which antimicrobial activity is achieved remains unclear, it is postulated that the cationic component of the quaternary ammonium interacts with the negatively charged cell membrane. Subsequently, the amine groups penetrate the cell, inducing lysis (Lin et al., 2003; Tiller et al., 2001). This has made them a promising solution for controlling microbial growth in various applications, including medical devices, water treatment, and food packaging. Imazato pioneered the introduction of quaternary ammonium into dental materials, demonstrating its capability to inhibit bacteria (Imazato et al., 1994). Since then, various types of quaternary ammonium compounds have been applied to dental materials.

Among the numerous quaternary ammonium compounds, those with 12 to 16 carbon chains have exhibited the highest biocidal activity (Gerba, 2015). Moreover, the quaternary ammonium compound with a 12-carbon substituent on the amine has been shown to possess antifungal properties (Ravikumar et al., 2006). In a previous study, dodecyl quaternary ammonium compounds have been utilized as a coating material, suggesting their broad-spectrum antimicrobial properties and potential applicability in various settings (Han et al., 2011). Nevertheless, it is important to note that the utilization of dodecyl quaternary ammonium compounds was limited to coating materials and was not incorporated into dental materials in that particular study. As such, the exploration of incorporating dodecyl quaternary ammonium into denture base materials merits further investigation as a potential means of reducing the incidence of oral fungal infections.

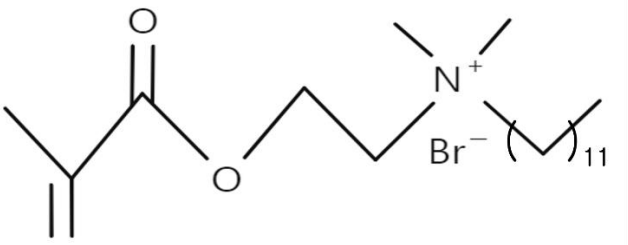

Figure 1. Chemical structure of dodecyl quaternary ammonium used in this study.

5. Research objective and null hypothesis

In this study, dodecyl quaternary ammonium compounds were incorporated into a denture base material to improve the antifungal properties. While cationic quaternary ammonium has been known for its antifungal-contact-killing properties, its use has been limited to surface coatings and other materials. The objective of this study was to develop a denture base resin that incorporates the dodecyl quaternary ammonium compounds and evaluate its antifungal effectiveness against *Candida albicans*. The study also investigated the physical and mechanical properties of the modified denture base material to assess its suitability for clinical use.

The null hypothesis was that there would be no significant differences in the physical and mechanical properties, cytotoxicity, and antifungal effectiveness of denture base resin with and without the dodecyl quaternary ammonium compounds.

II. MATERIALS AND METHODS

1. Materials

2-(Dimethylamino)ethyl methacrylate, 1-bromododecane ($C_{12}H_{25}Br$), acetonitrile, chloroform, diethyl ether, 2,2'-azobisisobutyronitrile (AIBN), methyl mercaptopropionate (MMP), dimethylformamide (DMF) and reagent-grade solvents were purchased from Fisher Scientific (Waltham, MA, USA). The cold-cured methacrylate-based resin (ProBase Cold, Ivoclar-Vivadent, FL) was used in all the experiments in this study.

2. Synthesis of $DMAEMAC_{12}$ compound

2.1. Synthesis of $DMAEMAC_{12}$ monomer

The dodecyl quaternary ammonium ($DMAEMAC_{12}$) monomer was synthesized using a method described in a prior study before producing the copolymer (Han et al., 2011). In a round bottom flask, 10.8 mL of 2-(dimethylamino)ethyl methacrylate was added to 17.5 mL of 1-bromododecane, 50 mL of acetonitrile, and 25 mL of chloroform. The mixture was stirred overnight under N_2 at 40 °C (Figure 2). After mixing, the solvent was evaporated under a vacuum. The residue was then given diethyl ether to precipitate.

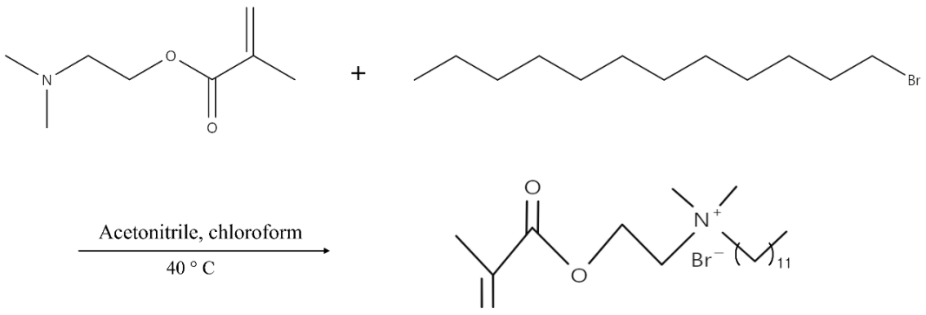

Figure 2. Synthesis of $DMAEMAC_{12}$ monomer.

2.2. Synthesis of $DMAEMAC_{12}$ polymer

To synthesize the $DMAEMAC_{12}$ polymer ($pDMAEMAC_{12}$), $DMAEMAC_{12}$ monomer, AIBN, and MMP were dissolved in DMF in a round bottom flask. Then the flask was covered and the nitrogen gas was purged for 5 min. The mixture was stirred overnight at 60 °C. After stirring, the lid was opened to air to stop the polymerization. The solution was evaporated followed by a high vacuum overnight to remove the resident solvent. The obtained powder, following evaporation, was subsequently ground with a mortar to eliminate excess mass.

3. Characterization of pDMAEMAC$_{12}$

The composition of pDMAEMAC$_{12}$ was determined by analyzing its ^{1}H nuclear magnetic resonance (^{1}H NMR) data. To measure the degree of polymerization, the obtained pDMAEMAC$_{12}$ was dissolved in methanol (Fisher Scientific, Waltham, MA, USA) and subjected to ^{1}H NMR spectroscopy using both Bruker 600 NMR (Bruker Daltonics, Bremen, Germany) and Varian MR400 (400 MHz) (Varian, Inc., Palo Alto, CA, USA). The ^{1}H NMR data was analyzed with MestReNova Software (Mestrelab Research SL, Spain).

4. Preparation of denture base resin incorporated with $pDMAEMAC_{12}$

The $pDMAEMAC_{12}$ was incorporated into the denture base resin at various weight percentages; 0 % (control), 0.5 %, 0.75 %, and 1 %, respectively (Table 1).

The pre-weighted $pDMAEMAC_{12}$ was mixed with PMMA. To achieve a homogeneous distribution of the $pDMAEMAC_{12}$, PMMA and $pDMAEMAC_{12}$ were mixed by ball mill for 48h. The mixed powder was then mixed with MMA in a mass ratio of 3 : 2 according to the manufacturer's instruction with stirring for 15 s by a speed mixer (DAC 150.1 FVZ Speed mixer, Hauschild, Germany). The thoroughly mixed materials were poured into disc- or bar-shaped polyacetal resin molds and polymerized under 0.4 MPa pressure for 15 min.

The polymerized specimens were polished with silicon carbide (SiC) paper up to 1500 grit using an Ecomet 30 grinder/polisher (Buehler, Lake Bluff, IL, USA) before testing their physical, mechanical, and surface properties. For the antifungal and biological analysis, the specimens were polished up to 800 grit.

Table 1. Weight ratio (%) of $DMAEMAC_{12}$ polymer in each group and experimental codes

Groups	**PMMA (wt. %)**	**MMA (wt. %)**	**$pDMAEMAC_{12}$ (wt. %)**	**Group code**
1	60.0	40.0	0	Control
2	59.7	39.8	0.5	C_{12} 0.5 %
3	59.55	39.7	0.75	C_{12} 0.75 %
4	59.4	39.6	1.0	C_{12} 1.0 %

C_{12}: dodecyl quaternary ammonium ($DMAEMAC_{12}$)

$pDMAEMAC_{12}$: $DMAEMAC_{12}$ polymer

5. Characterization of denture base resin incorporated with pDMAEMAC$_{12}$

An aggregation analysis was conducted to verify that the polymers are uniformly disseminated in the denture base resin. The micromorphology and chemical composition of the powder mixed in the ball mill were examined using scanning electron microscopy (SEM; Merin, Carl ZEISS, Oberkochen, Germany) at a magnification of 100 × with an accelerating voltage of 15.0 kV.

6. Surface properties evaluation

6.1. Morphology of polished surface

Scanning electron microscopy (FE-SEM; Merin, Carl ZEISS, Oberkochen, Germany) was used to examine the surface morphology of denture base resin incorporated with pDMAEMAC$_{12}$. The samples were prepared following the technique described in Section 4. The images were captured using a 15 kV acceleration voltage and a 500 × magnification.

6.2. Contact angle

The contact angle was measured to evaluate the experimental specimens' hydrophilic and hydrophobic characteristics. A droplet analysis device (SmartDrop standard, FEMTOFAB, Gyeonggi-do, Korea) and the sessile-drop method were used for the analysis. 5 μL of each distilled water and ethylene glycol (Sigma-Aldrich, St. Louis, MO, USA) were dropped onto the center of the specimen surface. Then, the static contact angle (θ) of both right and left values was measured after 5 seconds, and the calculated average was reported. Five disc-shaped specimens (d = 10.0 mm, h = 2.0 mm) from each group were tested, and the contact angle analysis was performed twice for each specimen.

7. Physical properties analysis

7.1. Water sorption and solubility

The water sorption and solubility analysis were performed according to the ISO 20795-1 (ISO, 2013). Six disc-shaped specimens (d = 15.0 mm, h = 1.0 mm) were prepared for each group using the method described in Section 4. Prior to weighing the specimens, the specimens were first stored in a desiccator at (37 ± 1) °C for (23 ± 1) h and then transferred to another desiccator which was then kept at (23 ± 2) °C for (60 ± 10) min. The prepared samples were then weighed to an accuracy of 0.2 mg, and the cycle described above was repeated until a constant mass (m_1). The volumes of each sample (V) were calculated after measuring the constant mass. Then these conditioned samples were immersed in water at (37 ± 1) °C for 7 d ± 2 h. After the immersion, the samples were cleaned and dried with air for (15 ± 1) s and weighed (60 ± 10) s after the removal from the water to an accuracy of 0.2 mg (m_2). The samples were then reconditioned in the desiccator as described above to measure constant mass (m_3). The values of water sorption, W_{sp}, and the water solubility, W_{sl}, were calculated with the following equations:

$$W_{sp} = \frac{m_2 - m_3}{V} \ , \ W_{sl} = \frac{m_1 - m_3}{V}$$

where,

m_1 is the constant mass of the conditioned mass in micrograms;

m_2 is the mass of the samples in micrograms after immersion in water;

m_3 is the mass of the reconditioned samples in micrograms;

V is the volume of the samples in cubic millimeters.

7.2. Color measurement

Color changes resulting from the incorporation of pDMAEMAC$_{12}$ were compared to the control group using a spectrophotometer (CM-5, Konica Minolta, Osaka Japan). Disc-shaped specimens, prepared according to the Section 4, were used for the color measurements. Before taking the measurement, the spectrophotometer was calibrated according to the manufacturer's instructions. The color shift was assessed with light reflection from a 3 mm diameter region. The following formula calculated the color difference (ΔE) of each specimen from the control (Ly et al., 2020):

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

where,

L^* is lightness (0: black, 100: white);

a^* is green-red chromaticity ($a^* > 0$: shift to red, $a^* < 0$: shift to green);

b^* is yellow-blue chromaticity ($b^* > 0$: shift to yellow, $b^* < 0$: shift to blue)

Five specimens from each group were assessed for this experiment, and three random spots of each specimen were measured. The mean value of the ΔE was then used for the calculation.

8. Mechanical properties analysis

8.1. Ultimate flexural strength and flexural modulus

The flexural strength and modulus testing were conducted according to ISO 20795-1 (ISO, 2013). According to the procedure outlined in Section 4, five bar-shaped experimental samples from each group were prepared and polished with a dimension of 64 × 10 × 3.3 millimeters. Samples were then immersed in water at (37 ± 1) °C for (50 ± 2) h before the flexural test.

A computer-controlled universal testing machine (Model 5942, Instron, Norwood, MA, USA) loaded with a 1 kN load cell gave force to the samples in a three-point flexure. The force on the load was consistently increased from zero with a constant displacement rate of 5 mm/min at 50 mm of span length. The following formulae, in megapascals, were employed to calculate the ultimate flexural strength (σ) and flexural modulus (*E*).

$$\sigma = \frac{3Fl}{2bh^2} \ , E = \frac{F_1 l^3}{4bh^3 d}$$

where,

F is the maximum load forced on the samples in newtons;

F_1 is the load at a point in the straight-line portion of the load/deflection curve in newtons;

l is the distance between the supports (50 mm);

b is the width of the samples in millimeters;

h is the height of the samples in millimeters;

d is the deflection at load F_1 in millimeters.

9. Antifungal analysis

9.1. Fungal strains and culture condition

Candida albicans of fungal strain were used to analyze the antifungal effect of the denture base resin incorporated with pDMAEMAC$_{12}$. The frozen *Candida albicans* fungal colonies were thawed and cultured in Sabouraud dextrose broth (SD broth; MB cell, Seoul, Korea) at 37 ℃ for 24 h. Fungi were cultivated in incubation at 37 ℃ for 24 h by inoculating an isolated colony from SD broth. Approximately 1×10^7 CFU/mL of *Candida albicans* suspension was then prepared by diluting the culture with SD broth.

9.2. Fungal colony forming unit (CFU) evaluation

9.2.1. Antifungal efficacy analysis

The three prepared disc-shaped specimens (diameter 10 mm × thickness 2 mm), following Section 4, were placed on a solid agar plate. 100 μL of fungal suspension (1×10^7 CFU/mL) was applied to each specimen, followed by incubation at 37 ℃. After 24 h, the specimens were gently washed with PBS twice to remove non-adherent fungi before being sonicated in SD broth for 5 min. The harvested fungi were spread on a solid SD agar plate and incubated at 37 ℃ for 24 h. Finally, the total number of colonies was counted after the incubation. These experiments were conducted in triplicate.

9.2.2. Antifungal efficacy of aged denture base resin incorporated with pDMAEMAC$_{12}$

To assess the antifungal effect of aged denture base resin incorporated with pDMAEMAC$_{12}$, disc specimens prepared in accordance with Section 4. These specimens were then subjected to conditioning through thermocycling aging, using thermocycling equipment (Thermal Cyclic Tester, R&B Inc., Daejeon, Korea). The thermocycling process involved cycling between temperatures of 5 °C and 55 °C, with each temperature held for 45 s, resulting in a total of 630 cycles, which corresponds to approximately three weeks of exposure under oral conditions. (Ishihara et al., 1998; Lee et al., 2019).

Following thermal aging, the specimens were subjected to antifungal efficacy testing as outlined in Section 9.2.1.

9.3. Fungal viability

The viability of adhered fungi on the specimen surface was evaluated using a LIVE/DEAD FungaLight Yeast Viability Kit (SYTO 9 and propidium iodide, Molecular Probes, Eugene, OR, USA) following the manufacturer's protocol. Four disc-shaped specimens from each group were prepared following the guidelines outlined in Section 9.2.1. The procedure, from the fungal culture to PBS washing, remained consistent with the steps detailed in Section 9.2.1. SYTO 9 and propidium oxide dye were mixed thoroughly with a volume ration of 1:1. The comprehensive solution was formulated by adding 3 μL of the blended dye per mL of PBS. Subsequently, 1 mL of the solution was applied to each specimen, and the specimens were incubated at room temperature for 20 min in the dark. Observation of the stained specimens was conducted using a confocal laser scanning microscope (LSM 980, Carl Zeiss, Thornwood, NY, USA). Live fungi were stained with SYTO 9, appearing green, while dead fungi were stained with propidium iodide, appearing red.

10. Cytotoxicity analysis

10.1. Cell culture

The investigation into oral cellular activity utilized the mouse fibroblast cell line L929. The L929 cells were cultured in RPMI-1640 cell culture medium (Welgene, Gyeongsangbuk-do, Korea), supplemented with 1 % antibiotic-antimycotic (Gibco, Grand Island, NY, USA) and 10 % fetal bovine serum (FBS; Gibco, Grand Island, NY, USA).

The culture medium was replenished every 2 - 3 days, maintaining a constant incubation temperature of 37 °C and a humidified atmosphere with 5 % CO_2. Adherent L929 cells were harvested using the following procedure: initially, cells were detached using 0.05 % trypsin-EDTA (Gibco, Grand Island, NY, USA); subsequently, centrifugation was performed at 1300 rpm for 3 min, followed by resuspension in the culture medium. Subsequently, a hemocytometer was employed to determine cell counts, providing results in terms of live/dead cell numbers per mL.

10.2. Cytotoxicity evaluation of extracts

The cytotoxicity assessment adhered to the protocols outlined in ISO 10993-5 (ISO, 2009) and ISO 10993-12 (ISO, 2012). To obtain extracts, specimens of denture base resin containing $pDMAEMAC_{12}$ were immersed in RPMI 1640 culture medium for 24 h at 37 °C, maintaining a ratio of 3 cm^2/mL (sample surface area/culture medium volume). For control purposes, RPMI 1640 eluted for 24 h was employed as the blank group, 0.1 % phenol solution was used as the positive control, and a polyethylene film was utilized as the negative control.

In a 96-well plate, L929 cells were seeded at a density of 1×10^4 cells per well and incubated in RPMI 1640 for 24 h at 37 °C with 5 % CO_2. Subsequently, the L929 cells were exposed to the extracted liquid from each sample at a concentration of 100 % for an additional 24 h, post the removal of the medium. The extracted liquid was then discarded, and the assessment of cytotoxicity of the denture base resin incorporating $pDMAEMAC_{12}$ was carried out using the methylthiazole tetrazolium (MTT) assay, comparing the results with the blank group.

An MTT assay was employed to assess the cytotoxicity of the denture base resin incorporating $pDMAEMAC_{12}$. The MTT solution was freshly prepared by dissolving thiazolyl blue tetrazolium bromide (MTT; Sigma-Aldrich, St. Louis, MO, USA) in phenol red-free RPMI1640 medium at a concentration of 1 mg/mL. This solution was filtered through a syringe and syringe filter with a pore size of 0.20 μm (DISMIC-25CS, ADVANTEC, Tokyo, Japan). In each test well, 50 μL of MTT solution was introduced, and the plates were subsequently incubated in a 37 °C incubator with 5 % CO_2 for 2 hours. Following this incubation, the MTT solution was replaced with 100 μL of isopropanol (Sigma-Aldrich, St. Louis, MO, USA) to dissolve the formazan product. A Microplate Spectrophotometer (Epoch, Bio Tek, Winooski, VT, USA) operating at 570 nm was utilized to measure the absorbance. The percentage of optical density (OD) in comparison to the blank (set at 100 %) was employed to calculate cell viability.

11. Statistical analysis

The IBM SPSS 25.0 software (IBM Korea Inc., Seoul, Korea) was employed to conduct all statistical analyses. Both control and experimental groups were subjected to one-way analysis of variance (ANOVA), followed by post hoc Tukey's test to analyze the results. The CFU results within groups for both non-aged and aged conditions were analyzed by paired *t*-test. Statistical significance was determined using a confidence level of 95 % ($p < 0.05$) for all analyses.

III. RESULTS

1. Characterizations of pDMAEMAC$_{12}$

^{1}H NMR spectroscopy was utilized to assess the degree of polymerization of pDMAEMAC$_{12}$. To determine this degree, the protons of the chain transfer agent (MMP, 3.67 ppm) and the $COOCH_2CH_2NCH_2CH_3$ protons of the polymer (d, 0.85 ppm) were analyzed (Figure 3). Based on the analysis of peak d in Figure 2, the chain length of the pDMAEMAC$_{12}$ was calculated to be 22.

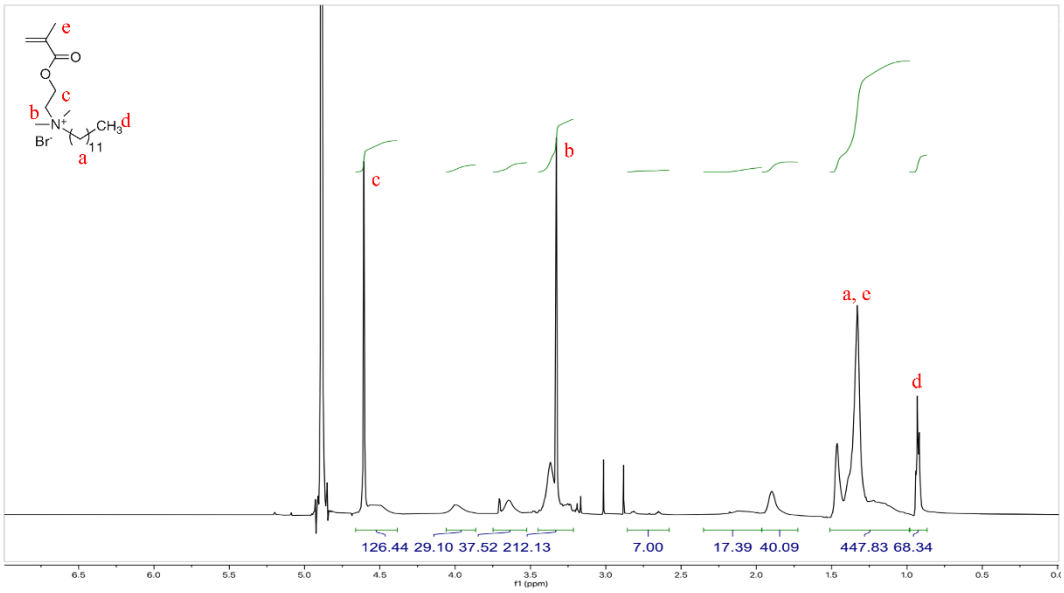

Figure 3. ^{1}H NMR spectrum of DMAEMAC$_{12}$ polymer in $CDCl_3$. Peaks corresponding to the chain transfer agent (MMP, 3.67 ppm) and the $COOCH_2CH_2NCH_2CH_3$ protons of the polymer (d, 0.85 ppm) were used to determine the degree of polymerization.

2. Characterization of denture base resin incorporated with pDMAEMAC$_{12}$

In the SEM results, it was observed that the pDMAEMAC$_{12}$ was present in the denture base resin powder without any observable aggregation. This was evident in all groups containing pDMAEMAC$_{12}$, except for the control group.

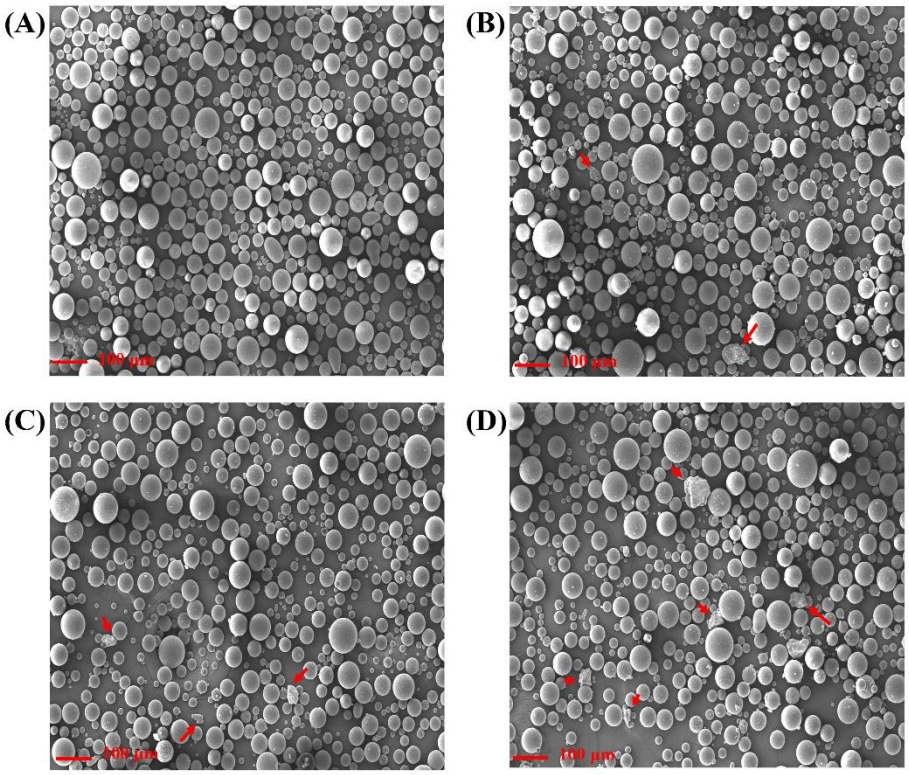

Figure 4. SEM images of denture base resin incorporated with pDMAEMAC$_{12}$ powder; (A) control, (B) C_{12} 0.5 %, (C) C_{12} 0.75 %, and (D) C_{12} 1.0 % at magnification of 100 ×. The arrow highlights the presence of pDMAEMAC$_{12}$.

3. Surface properties evaluation

3.1. Morphology of polished surface

The morphology of the polished surface was examined for all tested groups using FE-SEM, as illustrated in Figure 5. No significant differences were observed among the FE-SEM images.

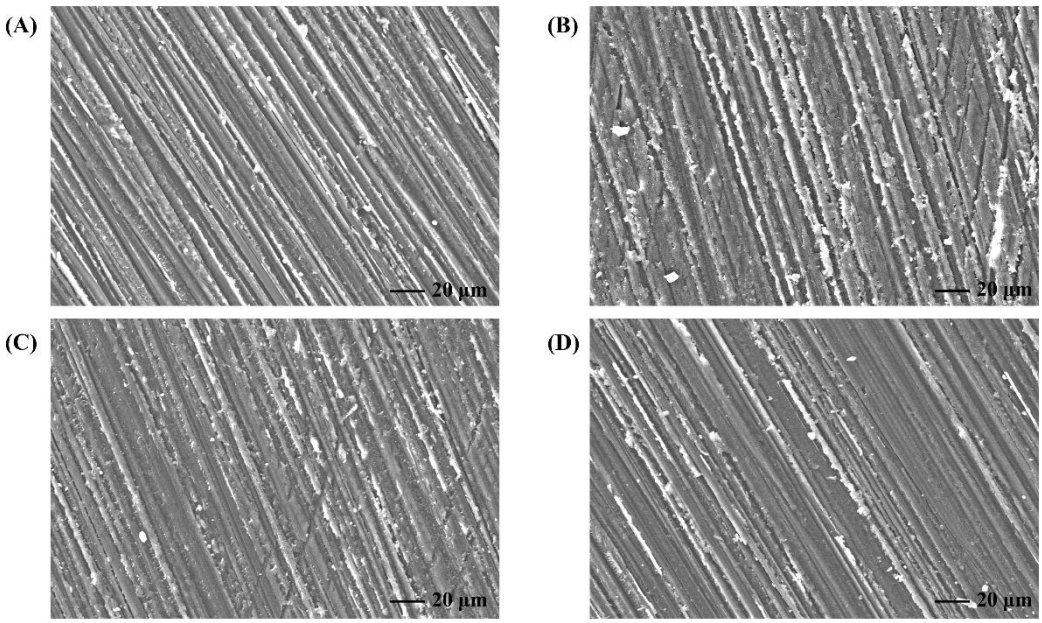

Figure 5. SEM images of the surface of denture base resin incorporated with pDMAEMAC$_{12}$ at a magnification of 500 ×. (A) Control, (B) C$_{12}$ 0.5 %, (C) C$_{12}$ 0.75 %, and (D) C$_{12}$ 1.0 %.

3.2. Contact angle

The contact angle and surface energy results from both the control and test groups are presented in Table 2 and Figure 6. The analysis demonstrated no significant difference in contact angle for all test groups when tested with both distilled water and ethylene glycol ($p > 0.05$).

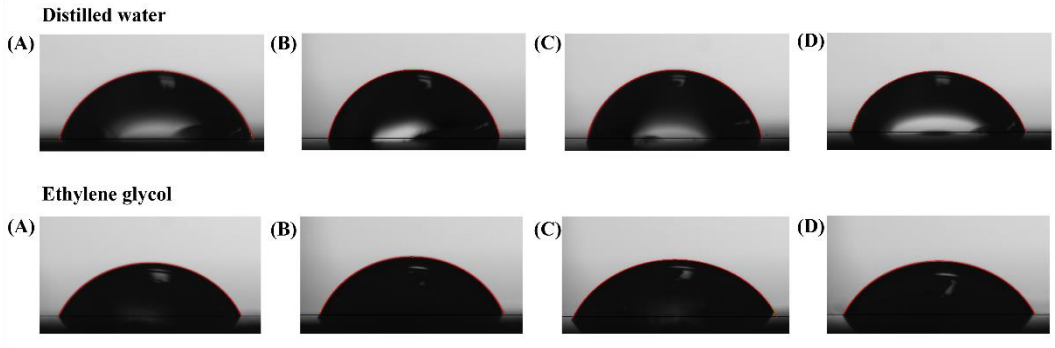

Figure 6. Distilled water and ethylene glycol droplet images of (A) control, (B) C_{12} 0.5 %, (C) C_{12} 0.75 %, and (D) C_{12} 1.0 %.

Table 2. Contact angles of the control and test groups

Groups	Contact angle (°)	
	Distilled water	Ethylene glycol
Control	73.96 (± 1.50) [a]	67.73 (± 3.64) [a]
C_{12} 0.5 %	76.58 (± 5.72) [a]	68.66 (± 4.27) [a]
C_{12} 0.75 %	78.76 (± 1.55) [a]	62.85 (± 4.81) [a]
C_{12} 1.0 %	77.75 (± 4.56) [a]	63.22 (±5.42) [a]

Same lowercase letter in the same column indicated no significant difference ($p > 0.05$).

4. Physical properties analysis

4.1. Water sorption and solubility

The results of water sorption and solubility tests indicate that there were no significant differences among the tested groups ($p > 0.05$, Table 3).

Table 3. Water sorption and solubility of the control and test groups

Groups	Water sorption ($\mu g/mm^3$)	Water solubility ($\mu g/mm^3$)
Control	21.96 (± 0.61) [a]	1.42 (± 0.30) [a]
C_{12} 0.5 %	22.68 (± 0.57) [a]	1.32 (± 0.07) [a]
C_{12} 0.75 %	22.86 (± 0.45) [a]	1.76 (± 0.17) [a]
C_{12} 1.0 %	22.37 (± 0.53) [a]	1.69 (± 0.32) [a]

Same lowercase letter in the same column indicated no significant difference ($p > 0.05$).

4.2. Color measurement

The color differences (ΔE) between the control and each experimental group are presented in Table 4. The obtained data indicates that no significant difference was found among the groups ($p > 0.05$).

Table 4. The mean value and standard deviation of color difference (ΔE) of the control and test groups

Group	Color differences (ΔE)
C_{12} 0.5 %	1.41(± 0.93) [a]
C_{12} 0.75 %	2.27 (± 2.13) [a]
C_{12} 1.0 %	2.14 (± 2.39) [a]

Same lowercase letter indicates no significant difference ($p > 0.05$).

5. Mechanical properties analysis

5.1. Flexural strength and flexural modulus analysis

The average flexural strength and flexural modulus of the control group and groups containing 0.5 %, 0.75 %, and 1.0 % of pDMAEMAC_{12} are shown in Figure 7. The average flexural strength of the control, C_{12} 0.5 %, C_{12} 0.75 %, and C_{12} 1.0 % were 98.41 ± 11.23 MPa, 86.18 ± 5.62 MPa, 84.89 ± 4.41 MPa, and 80.51 ± 7.05 MPa, respectively. There was a significant difference in flexural strength between the control and the C_{12} 1.0 % (p = 0.008). The average flexural modulus of the control, C_{12} 0.5 %, C_{12} 0.75 %, and C_{12} 1.0 % were 3009.03 ± 299.04 MPa, 2781.15 ± 83.0 MPa, 2582.35 ± 123.5 MPa, and 2560.23 ± 71.38 MPa, respectively. The flexural modulus also showed a significant difference between the control and the C_{12} 1.0 % (p = 0.004).

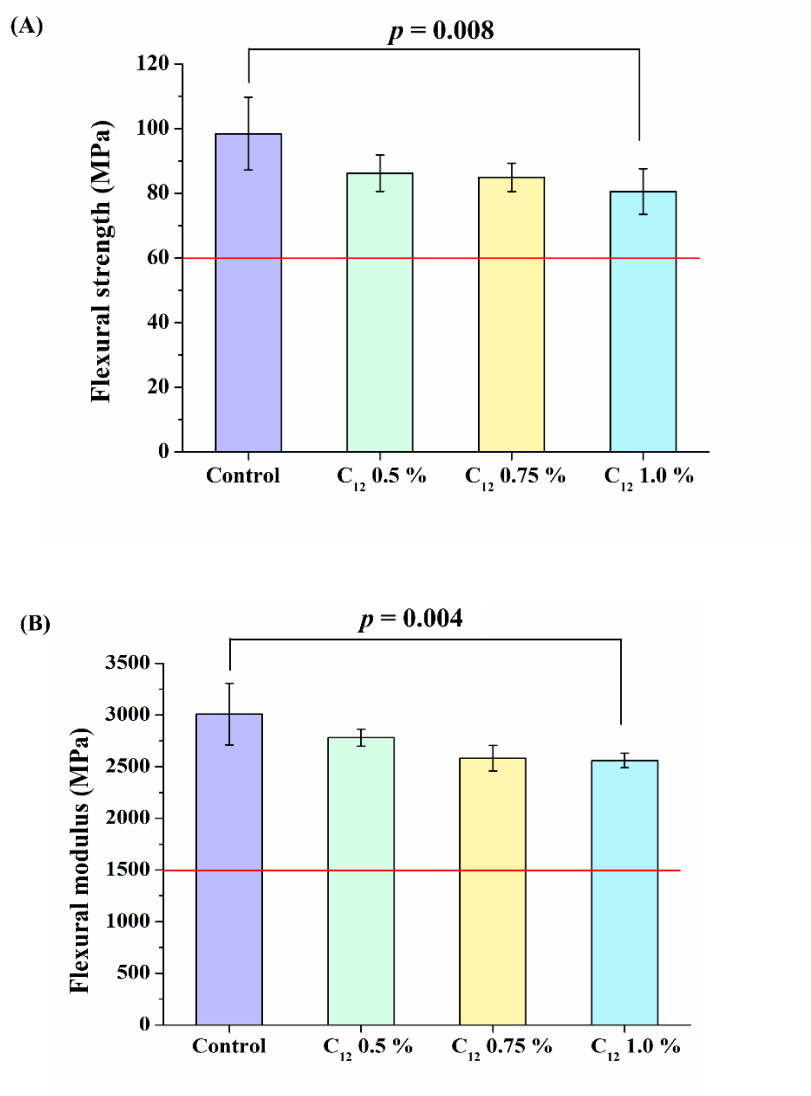

Figure 7. Flexural strength (A) and flexural modulus (B) of the control and the experimental groups. The red line represents the minimum ISO standard (ISO 20795-1, 2013) requirements: 60 MPa for flexural strength and 1500 MPa for flexural modulus.

6. Antifungal analysis

6.1. Evaluation of fungal counting colony forming unit (CFU) of non-aged and aged denture base resin incorporating pDMAEMAC$_{12}$

The antifungal outcomes for both non-aged and thermally aged groups against *Candida albicans* were assessed using the CFU method. The results confirmed a significant antifungal efficacy when incorporating more than 0.75 wt.% of pDMAEMAC$_{12}$ into the denture base material for the non-aged groups (p = 0.002). In addition, the CFU results from the thermally aged denture base resin specimens indicated that the pDMAEMAC$_{12}$-incorporated denture base resin had a significantly lower count of adhered *Candida albicans* than the control ($p < 0.05$, Figure 8).

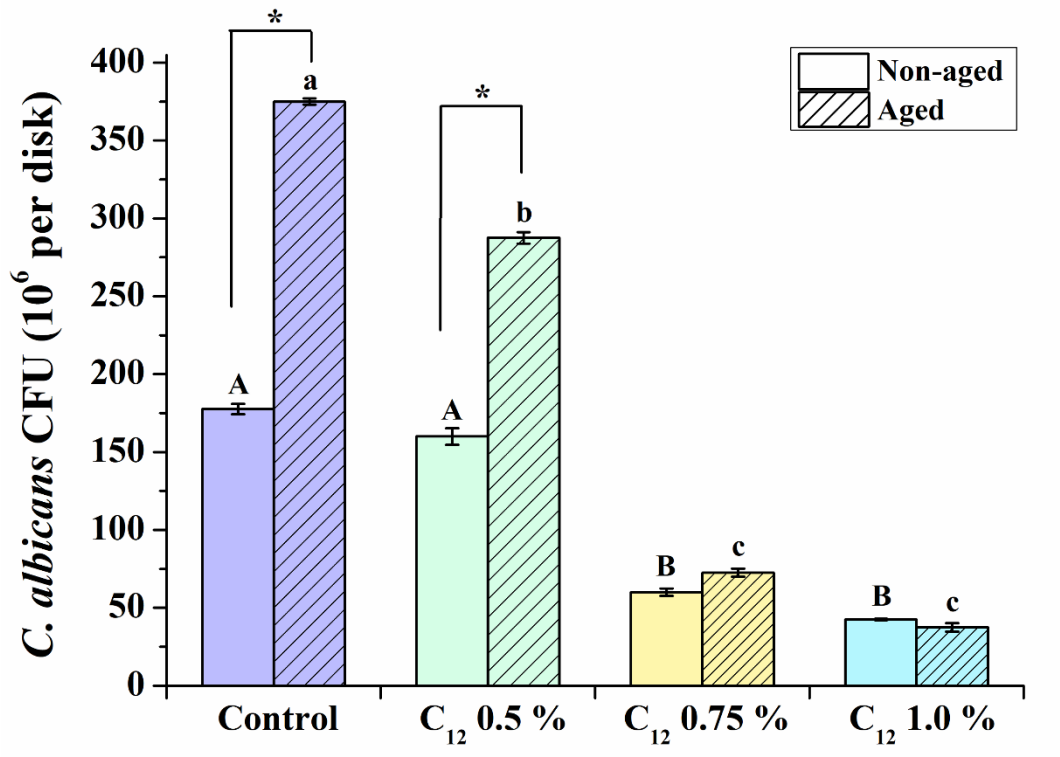

Figure 8. The colony forming unit (CFU) results indicate the count of adhered *Candida albicans* in both non-aged and aged groups. Upper case alphabetical letters above the bar graph signify significant differences among non-aged groups, while lower case letters indicate significant differences among the aged groups ($p < 0.05$). Asterisks (*) denote significance for comparison between the non-aged and aged groups within each group, as analyzed by paired *t*-test ($p < 0.05$).

6.2. Fungal viability

The fungal viability of *Candida albicans* on the tested specimens was assessed using the FungaLight live/dead assay kit. Dead fungi were indicated by red fluorescence, whereas live fungi were represented by green fluorescence. The results demonstrated an increase in the observance of dead fungi as more pDMAEMAC12 was incorporated (Figure 9).

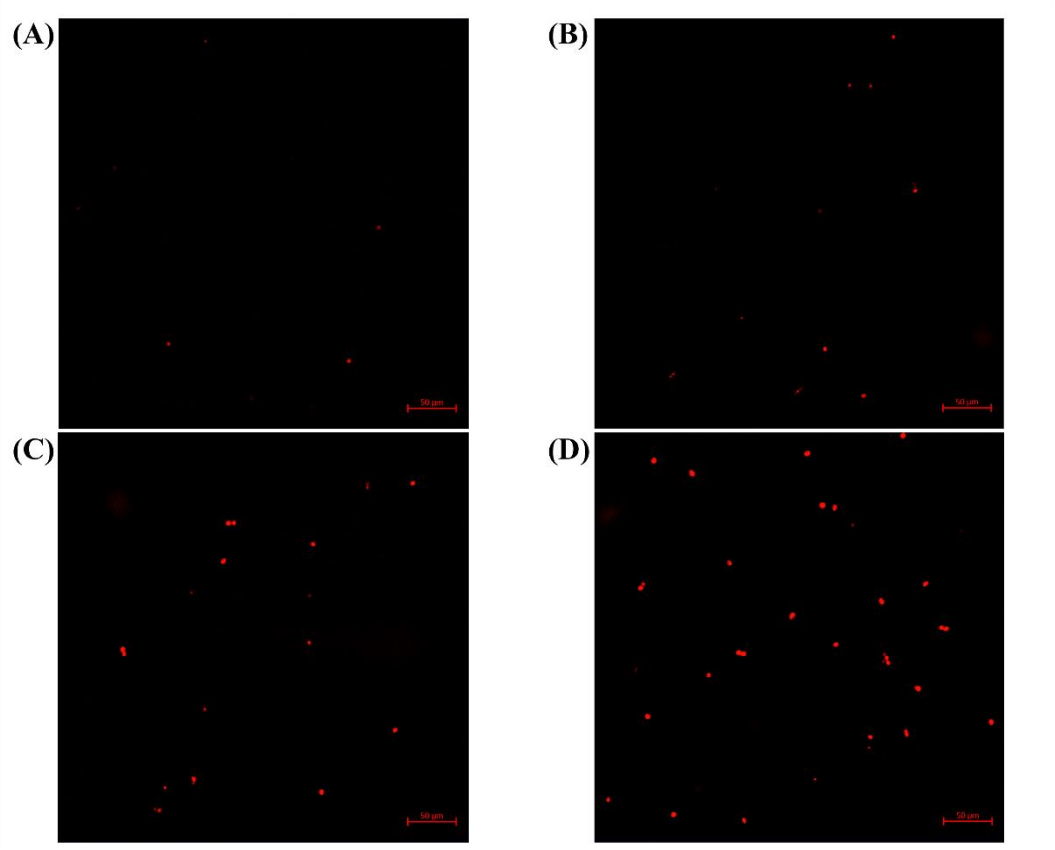

Figure 9. Fluorescent images depict dead fungi (red) adhering to denture base resin; (A) control, (B) C_{12} 0.5 %, (C) C_{12} 0.75 %, and (D) C_{12} 1.0 %.

7. Cytotoxicity analysis

7.1. Cell viability

Figure 10 displays the cell viability data for the tested groups. The cell viability values were as follows: (62.74 ± 0.12) % for the positive control, (95.86 ± 0.08) % for the negative control, (90.07 ± 0.10) % for the control group, (78.35 ± 0.10) % for C_{12} 0.5 %, (77.35 ± 0.14) for C_{12} 0.75 %, and (73.65 ± 0.11) % for C_{12} 1.0 %, in comparison to the blank. There was a significant difference between the control and all the groups containing pDMAEMAC_{12} ($p < 0.05$).

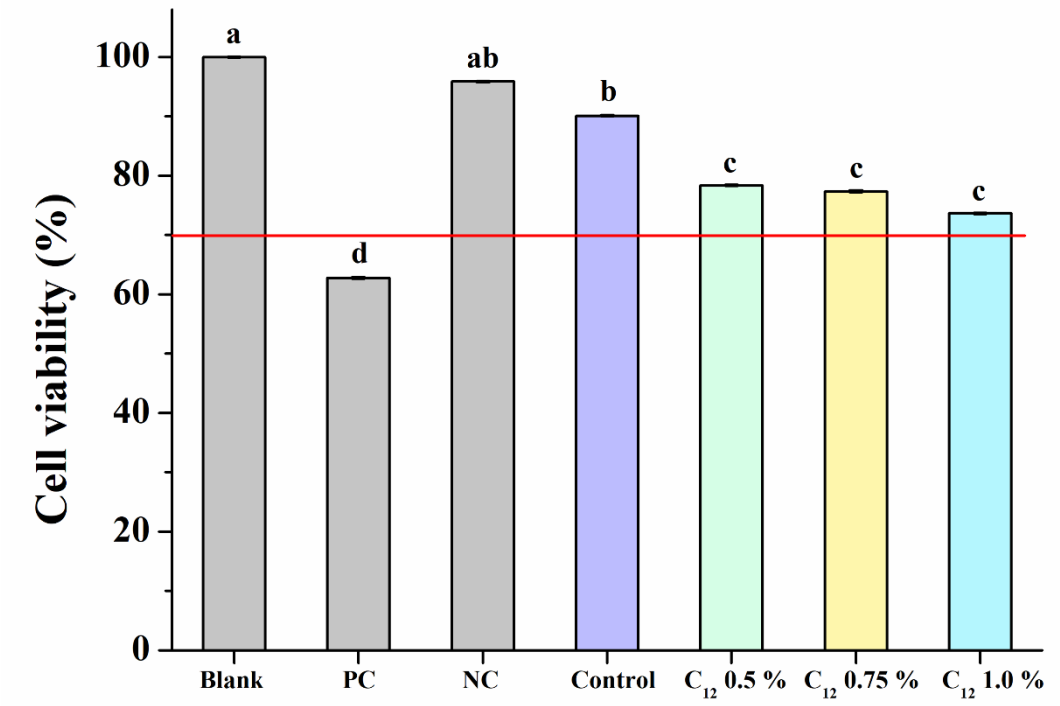

Figure 10. Cell viability of pDMAEMAC$_{12}$-incorporated denture base resin compared to the blank (%). The red line represents the minimum ISO standard (ISO 10993-5, 2009) criterion: a cell viability less than 70 % is considered cytotoxic. When lowercase letters above the bar graph are the same, it signifies that there are no significant differences between the groups ($p > 0.05$). Conversely, differing lowercase letters indicate significant differences between the groups ($p < 0.05$).

IV. DISCUSSION

A complete denture is a frequently used treatment option for individuals who are edentulous, and the primary material used for creating dentures is acrylic resin, which contains methyl methacrylate. The popularity of this material can be attributed to its affordability, ease of fabrication, and cosmetic appeal. Despite their advantages, dentures are associated with a significant drawback: the increased risk of developing fungal infections, including candidiasis (Gleiznys, Zdanavičienė, and Žilinskas, 2015). Superimposed fungal infection and trauma are common causes of denture stomatitis, with approximately 70 % of individuals who wear dentures experiencing this condition (Budtz-Jorgensen and Bertram, 1970; Gendreau and Loewy, 2011). Among the various treatments available for denture stomatitis, which include strategies like improving oral and denture hygiene and administering antifungal therapy, the most effective approach is prevention (Carl O. Boucher, 1980; Cawson, 1963; Nyquist, 1952). Preventive measures are key to avoiding the occurrence of denture stomatitis in the first place, which can be more effective and less burdensome than treating it after it has developed.

Cationic quaternary ammonium is widely recognized for its antimicrobial properties, as it can interact with the negatively charged cell membranes of bacteria and other microorganisms, leading to their destruction (Gilbert and Moore, 2005). One cationic quaternary ammonium compound, in which the nitrogen atom was substituted with 12-carbon chains, exhibited a surface-active antifungal effect when compared to other similar compounds (Ravikumar et al., 2006). Despite its efficacy, the application of this compound has thus far been restricted to surface coating and other materials. In this study, therefore, the incorporation of dodecyl quaternary ammonium into denture base resin was investigated as a means of enhancing its antifungal properties, while also evaluating other relevant properties to determine the feasibility of using the experimental materials in future clinical applications.

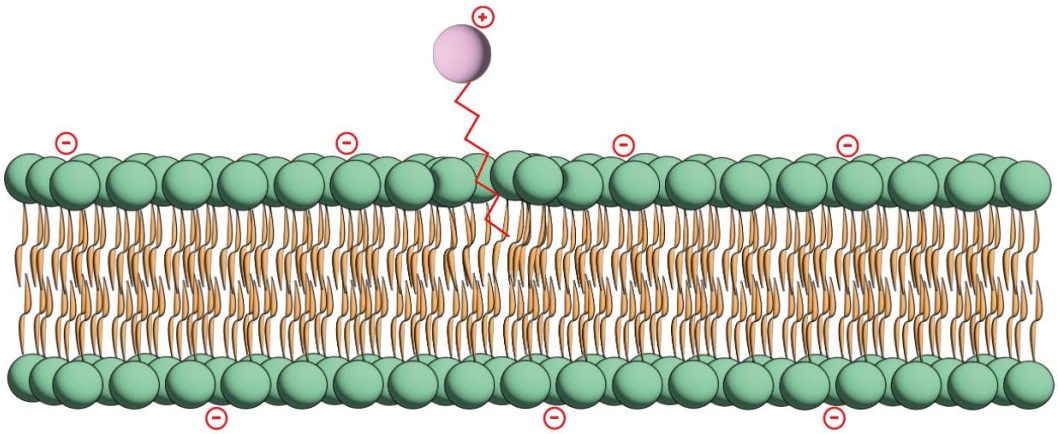

Figure 11. The antimicrobial mechanism of quaternary ammonium involves the disruption of cell membranes. The positively charged amine group interacts with the negatively charged cell membrane, and the alkyl group penetrates the cell membrane, leading to cell lysis.

Research has investigated the modification of surfaces from hydrophobic to hydrophilic to prevent the adhesion of Candida albicans, a primary causative agent of denture stomatitis, which is characterized by hydrophobic properties (Lazarin et al., 2014; Zamperini et al., 2010). To confirm the alteration of surface physical properties, therefore, the contact angles of the experimental groups were measured. It came out that there were no significant differences between the control and the groups containing $pDMAEMAC_{12}$ when measured with both distilled water and ethylene glycol ($p > 0.05$). Despite the hydrophobic nature of $pDMAEMAC_{12}$, the limited incorporation of the polymer into the denture base resin at 1 wt.% suggests that it would not lead to significant changes in the contact angle.

Given the extended duration that dentures are exposed to the oral environment, ensuring their dimensional stability is crucial, and this can be evaluated through water sorption and solubility test. When water is absorbed, it exerts a force on the macromolecules, which can

lead to resin expansion and compromise the integrity of the denture (Wong et al., 1999). There were no significant differences in water sorption among the tested groups. However, the higher average water sorption values in groups containing $pDMAEMAC_{12}$ could be attributed to the presence of both positive and negative charges in quaternary ammonium, potentially contributing to water adsorption (Liang et al., 2013). Furthermore, water has potential to be accommodated at the interface between the filler and the polymer matrix, offering an easily accessible diffusion path (Kalachandra, 1989; Sideridou et al., 2004). In this context, despite the inherently hydrophobic nature of dodecyl quaternary ammonium, it functions as a filler, facilitating water absorption. The water absorbed by the polymer causes the polymer network to swell, allowing unreacted monomers to leach out from the material, thereby contributing to water solubility (Huang et al., 2016). In this study, it was noted that the inclusion of $pDMAEMAC_{12}$, particularly when incorporated at a concentration exceeding 0.75 wt. %, resulted in slightly higher water solubility compared to the control group. According to ISO 20795-1 (2013), both water sorption and water solubility should not exceed 32 $\mu g/mm^3$ and 8.0 $\mu g/mm^3$, respectively. Furthermore, despite the slight changes observed between the control and the groups containing $pDMAEMAC_{12}$, the limited amount of the incorporated polymer did not significantly alter the physical properties of the denture base resin. Therefore, it was found that both water sorption and water solubility values of all tested groups fell below the ISO standard requirements, indicating that these materials are suitable for use as denture base resin.

As dentures serve to replace both teeth and gingiva, their aesthetic properties are of paramount importance when considering denture materials. Poor color stability in denture materials, therefore, could potentially diminish the expected outcome of restorative procedures. Various studies have reported that color changes in denture materials can occur due to alterations in the matrix, staining, or chemical degradation (Hong et al., 2009). The color of five specimens from each group was measured and compared to the control group. The results indicated no significant difference among groups, with ΔE values measuring less than 3. It is recognized that a ΔE value less than 3.3 is considered a clinically

acceptable (Ruyter, Nilner, and Möller, 1987). Therefore, based on the results of the color analysis, it can be inferred that the incorporation of cationic quaternary ammonium into denture base resin material would not adversely impact the visual acceptability of dentures.

Flexural fatigue is a common cause of denture failure in clinical settings, and therefore, it is imperative that the denture base material possesses sufficient flexural strength to withstand such forces (Chitchumnong, Brooks, and Stafford, 1989). In comparing the test groups, it was found that both flexural strength and flexural modulus were significantly different between the control and C_{12} 1.0 % groups ($p < 0.05$). Additionally, as more pDMAEMAC_{12} was added to the denture base material, there was a trend toward a decrease in both flexural strength and modulus. The observed decrease in both flexural strength and modulus with increasing concentrations of pDMAEMAC_{12} in the denture base material could be due to the additives acting as impurities (Sodagar et al., 2013). The interference of impurities with the polymerization process has the potential to yield a less homogeneous material and compromise mechanical properties. However, the ability of pDMAEMAC_{12} to co-polymerize with the resin matrix suggests that it did not disrupt the polymer network (Imazato et al., 1994). This is supported by the results indicating no significant difference when up to 0.75 wt.% of pDMAEMAC_{12} was incorporated. Moreover, all groups with the additive demonstrated clinically acceptable values. According to ISO 20795-1 (ISO, 2013), Type 2 materials, autopolymerizable materials, should have a flexural strength of no less than 60 MPa and a flexural modulus of no less than 1500 MPa. As all groups containing pDMAEMAC_{12} exceeded these minimum requirements, it can be concluded that the denture base material with pDMAEMAC_{12} is suitable for clinical applications. The incorporation of additional components often compromises the properties of denture base resin. However, based on surface, physical, and mechanical analyses, the inclusion of pDMAEMAC_{12}, particularly up to 0.75 wt.%, would not significantly impact the surface, physical, and mechanical properties of denture base resin.

Antimicrobial properties are a critical consideration in denture materials as a significant number of denture wearers experience denture stomatitis. To address issues related to *Candida albicans*, numerous studies have investigated incorporating antimicrobial agents, coatings, or surface modifications into denture materials to develop materials with antimicrobial effects (Chladek et al., 2011; Kamikawa et al., 2014). The antifungal results showed that the inclusion of $pDMAEMAC_{12}$ into denture base resin led to a significant reduction in adhered fungi on the specimens, as evidenced by the CFU count. Within the non-aged groups, a significant difference in CFU count was observed when incorporating more than 0.75 wt.% of $pDMAEMAC_{12}$ compared to the control. The antifungal properties of the denture base resin containing $pDMAEMAC_{12}$ remained effective following exposure to thermal aging through a thermocycling procedure. This thermocycling method replicates the physical conditions within the oral cavity, which might otherwise lead to degradation of biological and mechanical attributes due to harm inflicted on the polymer chains (Finer and Santerre, 2004; Gale and Darvell, 1999). In this study, 630 thermocycling cycles were conducted to simulate aging of the specimens, corresponding to approximately three weeks in oral conditions, given that dentures are typically worn over the long term (Kwon et al., 2021). While a three-week duration may seem short for evaluating long-term antifungal efficacy, it is important to note that a minimum of 500 cycles is considered sufficient to mimic a long-term effect (De Munck et al., 2005). Hence, the findings from the aged groups in the antifungal analysis can be considered reliable for predicting the long-term antifungal effect. Based on the CFU results, it is evident that groups incorporating $pDMAEMAC_{12}$ demonstrated a sustained antifungal effect even after the aging process. The incorporation of pDMAEMAC12, particularly exceeding 0.75 wt.%, demonstrated the ability to maintain low CFU results even after thermocycling. This persistence can likely be attributed to $pDMAEMAC_{12}$'s immobilized properties within the polymer structure (Han et al., 2011). The methacryloyl structure of quaternary ammonium compounds facilitates their copolymerization with other methacrylate

monomers (Imazato et al., 1994). Consequently, the dodecyl quaternary ammonium was chemically bound to the resin matrix, imparting a long-term antifungal ability.

The antifungal activity resulting from direct contact can be verified using confocal laser scanning microscopy (CLSM). The CLSM captured images of the biofilm grown on the specimens, illustrating the membrane integrity of the fungi. Consequently, CLSM facilitates the investigation of the biocidal properties of quaternary ammonium, believed to disrupt the cytoplasmic membrane (Filoche et al., 2007; Van der Mei et al., 2006). The CLSM results revealed the presence of adherent dead *C. albicans* on the specimens. Quaternary ammonium is recognized for its antimicrobial properties through contact killing. The positively charged amine group attracts the negatively charged cell membrane, ultimately causing cytoplasmic leakage, facilitated by the long and lipophilic alkyl chain. (Kawabata and Nishiguchi, 1988; Namba et al., 2009). Furthermore, the influence of counter-ions was studied, revealing that bromine is more effective in antimicrobial ability than chloride anions (Chen et al., 2000). However, the specific effect of the counter-ion of quaternary ammonium on antimicrobial effect is not known to date. For this reason, the adhered *C. albicans* were found to be dead due to the incorporation of pDMAEMAC$_{12}$. Moreover, an increased incorporation of pDMAEMAC12 resulted in the observation of more dead fungi, likely due to a higher likelihood of contact between fungi and the quaternary ammonium compounds. The CLSM results are consistent with a previous study suggesting that dodecyl quaternary ammonium acted as surface-active biocides, effectively preventing fungal proliferation. (Ravikumar et al., 2006). Taking into account both CFU and CLSM results, the antifungal effect of the denture base resin incorporated with pDMAEMAC$_{12}$ is attributed to the immobilized quaternary ammonium, resulting in fungal lysis upon contact. The C_{12} 0.75% group, in particular, exhibited significant antifungal efficacy against *C. albicans* in both non-aged and aged conditions. The co-polymerization of pDMAEMAC$_{12}$ with the resin matrix remained active and demonstrated its ability to express antifungal effects even after aging, inhibiting the regrowth of fungi (Daood et al., 2020).

For a denture base resin to function appropriately and elicit an appropriate host response when used as intended, it is crucial to assess its biocompatibility through testing (Schmalz and Arenholt-Bindslev, 2009). In this study, biocompatibility was evaluated through a cytotoxicity test, which involved analyzing the biological response of mammalian cells (Jorge et al., 2003). The results indicated that significant differences between the control and the groups containing $pDMAEMAC_{12}$ ($p < 0.05$). Despite the significant decrease in cell viability, it is important to note that according to ISO 10993-5 (ISO, 2009), a reduction in cell viability of less than 30 % is not considered cytotoxic. Furthermore, given that $pDMAEMAC_{12}$ had limited mobility within the resin matrix, the leaching of dodecyl quaternary ammonium was constrained, ultimately leading to clinically acceptable cytotoxicity (Li, Weir, and Xu, 2013). This suggests that the incorporation of $pDMAEMAC_{12}$ into denture base resin would not compromise the biocompatibility.

This study explored the incorporation of dodecyl quaternary ammonium into denture base resin to confer a long-term antifungal effect without compromising the physical and mechanical properties. However, the evaluation was limited to three weeks of simulated aging, and a longer duration would be necessary to ensure the sustained long-term antifungal efficacy.

V. CONCLUSION

In the current research, denture base resin was formulated by incorporating dodecyl quaternary ammonium, and the study examined its antifungal properties against *Candida albicans*, as well as its physical and mechanical properties.

The results were as follows;

(1) The study found no significant differences in surface and physical properties, suggesting that the inclusion of pDMAEMAC12 did not impact these characteristics.
(2) There was a significant decrease in flexural strength when 1.0 wt.% of $pDMAEMAC_{12}$ was incorporated into the denture base resin. Therefore, incorporation of pDMAEMAC12 up to 0.75 wt.% into the denture base resin would not significantly compromise the mechanical properties.
(3) The denture base resin containing $pDMAEMAC_{12}$ exhibited a significant reduction in *Candida albicans* CFU for C_{12} 0.75 % and C_{12} 1.0 % in the non-aged groups. Moreover, all groups containing $pDMAEMAC_{12}$ demonstrated a significant antifungal effect against *Candida albicans* when aged to simulate three weeks in oral conditions. Thus, the incorporation of $pDMAEMAC_{12}$ more than 0.75 wt. % into denture base resin would confer sustained antifungal efficacy.
(4) A significant difference in cell viability was observed between the control and the groups containing $pDMAEMAC_{12}$. Despite the decrease, all groups met the ISO standard requirements, indicating that the incorporations of $pDMAEMAC_{12}$ into denture base resin would not induce cytotoxicity.

The integration of $pDMAEMAC_{12}$ into denture base resin exhibited sustained antifungal properties with minimal to no adverse effects on the physical and mechanical properties, with the optimal concentration of $pDMAEMAC_{12}$ being 0.75 %. This This innovative approach holds promise for the prevention and management of denture stomatitis, offering potential benefits for preserving oral health among denture wearers.

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ABSTRACT (IN KOREAN)

*Candida albicans*에 대한 항진균 효과를 지닌 4차 암모늄을 함유한 의치 레진 개발

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한 아 름

의치에 주로 사용되는 폴리메틸메타크릴레이트(이하 PMMA)는 생체 적합하고 가격이 저렴하며 심미적이지만 장기간 사용 시 미생물이 자라기 쉬운 환경이 조성되어 구강 내 감염을 유발한다. 맞지 않는 의치나 *Candida albicans*에 의해 유발되는 의치성 구내염은 의치 사용자 중 2/3 정도에서 발병된다고 보고된다. 이에 치과 재료의 항균을 증진시키는 방법으로 이온 방출 및 재료 코팅이 거론되어 왔지만 항균 물질을 적용하면 물리적 성질이 저하되며 장기적 사용이 어렵고, 표면 손상 시 그 효과가 저하된다는 문제가 있다. 4차 암모늄은 레진 기질에 고정되어 미생물에 대한 세포 사멸을 일으켜 장기적인 항균 효과에 대한 기대를 받고 있다. 이에 본 연구의 목적은 의치의 물리·기계적 성질을 저하시키지 않는 최적 양의 4차 암모늄을 PMMA에 적용하고,

이에 대한 장기적인 항진균 효과에 대해 확인하는 것이다.

본 연구에서는 dodecyl quaternary ammonium을 합성 후 PMMA에 질량비에 따라 각각 0 %(대조군), 0.5 %, 0.75 %, 1.0 %씩 첨가하여 제작하였다. 먼저, ^{1}H NMR을 통해 4차 암모늄 합성을 확인하였고, SEM을 통해 4차 암모늄이 PMMA에 전체적으로 고르게 잘 분산되어 있는 것을 확인하였다. 표면 특성은 SEM을 이용한 연마 후 시편 표면 관찰과 접촉각 측정을 통해 평가하였다. 물리적 특성은 시편 표면의 색차 및 물 흡수·용해도를 이용해 측정하였다. 기계적 성질은 ISO 20795-1에 따라 굴곡 강도 및 계수를 통해 평가하였다. 또한 항진균 평가는 *Candida albicans*에 대한 CFU를 통해 평가하였다. 이 때 장기적인 항진균 효과에 대해 평가하기 위해 중합 후 항진균력을 평가한 군과 thermocyce aging을 통해 구강 내에서 3주 간의 환경을 모사한 군으로 나눠 항진균력을 평가하였다. 또한 contact killing으로 알려진 4차 암모늄의 항진균성을 측정하기 위해 CLSM을 통해 시편 표면에 부착된 진균의 활성도를 관찰하였다. 마지막으로, 본 재료의 생체 적합성을 평가하기 위해 L929 세포에 대한 세포 독성을 진행하였다.

연마된 시편을 SEM을 통해 관찰했을 때 모든 그룹에서 뚜렷한 차이가 나지 않는 것을 확인하였고, 접촉각 측정 결과 모든 그룹에서 유의한 차이가 나타나지지 않았다($p > 0.05$). 4차 암모늄을 함유한 그룹들은 대조군과 비교했을 때 유의한 색차값을 갖지 않았으며($p > 0.05$), 물 흡수·용해도 또한 그룹 간 유의차가 나타나지 않았다($p > 0.05$). 굴곡 강도 측정 결과 모든 그룹이 ISO 기준에 부합하는 강도를 갖지만, 4차 암모늄을 1.0 % 함유한 그룹은 대조군과 유의한 차이를 가졌다($p < 0.05$). *Candida albicans*에 대한 항진균 평가 결과 4차 암모늄이 0.75 % 이상 함유됐을 때 대조군과 유의한 차이를 갖는 항진균 효과를 보였으며($p < 0.05$), 구강 내 3주에 해당하는 기간만큼

aging 시켰을 때는 항진균 효과에 있어서 모든 그룹에서 대조군과 유의한 차이를 보였다($p < 0.05$). 특히 0.75 % 이상의 그룹에서 그 차이가 더 뚜렷하게 나타났다. 또한, CLSM 결과 4차 암모늄의 함유량이 높아질수록 시편의 표면에서 사멸된 항진균의 수는 높아졌고, 이를 통해 4차 암모늄의 contact killing 기전을 확인할 수 있었다. 마지막으로, 세포 독성에 있어서 실험군은 대조군과 유의한 차이가 있었지만 ISO 표준에 부합하는 값을 가졌다($p < 0.05$).

본 연구를 통해 4차 암모늄을 0.75 wt.%까지 적용했을 때 PMMA의 물리·기계적 특성에 영향을 미치지 않으며, 0.75 wt% 이상 적용했을 때 장기적인 항진균 효과를 갖는 것을 확인하였다. 이를 통해 0.75 wt%의 4차 암모늄을 함유한 PMMA를 사용하면 지속되는 항진균 효과로 인해 무치악 환자의 구강 위생이 보다 건강하게 유지될 것으로 사료된다.

핵심되는 말: 의치, 4차 암모늄, 의치성 구내염, 장기 항진균 효과, 물리 · 기계적 성질