Antifungal effect of poly(methyl methacrylate) containing zinc doped mesoporous silica nanoparticles

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Antifungal effect of poly(methyl methacrylate) containing zinc doped mesoporous silica nanoparticles

Directed by Professor Jae-Sung Kwon

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Antifungal effect of poly(methyl methacrylate) containing Zinc doped mesoporous silica nanoparticles

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

Denture stomatitis is caused by the accumulation of biofilm on the oral tissues and denture surface, characterized by inflammation and erythema of the oral mucosa beneath dentures, affecting many denture wearers. Zinc oxide nanoparticles (ZnO NPs) are known for their antifungal effects against *Candida albicans*, a common cause of denture stomatitis. However, incorporating ZnO NPs can negatively impact the mechanical properties of denture base resins. To address this issue, mesoporous silica nanoparticles (MSNs) are introduced due to their ability to enhance mechanical properties by forming a micromechanical interlocking interface between the fillers and the resin matrix. In this study, we incorporated Zn-doped mesoporous silica nanoparticles (Zn-MSNs) into poly(methyl methacrylate) (PMMA) and assessed their impact on surface properties, mechanical strength, and antifungal effects against *C. albicans*.

MSNs and Zn-MSNs were synthesized by a sol–gel method and characterized by transmission electron microscopy (TEM) and energy-dispersive X-ray spectroscopy (EDS). N_2 adsorption/desorption measurements were conducted to obtain the specific surface area and pore size. PMMA specimens were prepared by incorporating ZnO NPs, MSNs, and Zn-MSNs at 1.0 and 5.0 weight percentages (wt%). The PMMA surface gloss was evaluated using a gloss meter with a 2 mm × 2 mm area and a 60° geometry. Translucency was measured using a spectrophotometer operating in reflectance mode and calibrated using a white tile and a black trap following standard procedures. The flexural strength and flexural modulus were measured by a universal mechanical testing machine according to the International Standard ISO 20795–1. The fungal viability test was conducted following ISO 10993-12. Each specimen was introduced into a *C. albicans* suspension and incubated at 37 °C in a shaking incubator. The anti-adhesion assessment was performed by a colony-forming unit (CFU).

TEM images of MSNs and Zn-MSNs were successfully prepared and exhibited spherical morphology with a well-ordered mesoporous structure. ZnO NPs were found to be irregular in shape and had varying sizes and aspect ratios, with some of the particles agglomerating. EDS mapping results of the Zn-MSNs showed the composition and distribution of the Si, O, and Zn elements. Zn was successfully doped with an amount of approximately 7.34 wt.% and homogeneously distributed. According to the IUPAC classification, the N_2 adsorption–desorption curves showed typical characteristics of the type IV isotherm of mesoporous materials. BET analysis confirmed the porosity of the Zn-MSNs with a pore size of 3.41 nm, pore volume of 0.77 cm^3/g, and surface area of 848 m^2/g. The surface gloss observed no significant difference between the control group, the 1% MSN, and the 1% Zn-MSN groups ($p > 0.05$) . In contrast, the 1% ZnO group showed a lower gloss value than the 5% MSN and 5% Zn-MSN groups. Moreover, the 5% ZnO group had the lowest

surface gloss value among all the groups ($p < 0.05$). Regarding translucency results, no significant difference was observed among all groups with the incorporation of MSNs and Zn-MSNs ($p > 0.05$) . However, the 1% and 5% ZnO groups showed significant differences from the control group ($p < 0.05$). The flexural strength showed no significant difference in the incorporation of 1 wt% of MSNs and Zn-MSNs groups compared to the control group ($p > 0.05$). However, when the concentration of MSNs, Zn-MSNs, and ZnO NPs was increased to 5 wt%, a significant decrease in flexural strength was observed ($p < 0.05$). Among all groups, the lowest flexural strength value was observed in the 5% ZnO group. The fungal viability results indicated no significant differences ($p > 0.05$) when comparing 1% MSN, 1% Zn-MSNs, 1% ZnO NPs, and 5% MSNs groups to the control group. However, 5% Zn-MSN and 5% ZnO groups exhibited significantly lower fungal viability against *C. albicans* than the control group ($p < 0.05$). In terms of fungal anti-adhesion, the control group demonstrated the highest value. The denture base resin containing 5 wt% ZnO NPs, 5 wt% MSNs, and 5 wt% Zn-MSNs showed a significant difference from the control group ($p < 0.05$). Notably, 5 wt% Zn-MSNs showed the lowest value among all values.

In this study, denture base resins incorporating ZnO NPs, MSNs, and Zn-MSNs were prepared, and the antifungal effect against *C. albicans* and clinical applicability were investigated. Incorporating Zn-MSNs into denture base resin exhibited acceptable surface properties, mechanical strength, and effective antifungal activity. Therefore, the denture base resin containing 5 wt% Zn-MSNs is expected to be a valuable material for denture.

Key words; ZnO nanoparticles, mesoporous silica nanoparticles, mesoporous nanoparticles, Zn-doped mesoporous nanoparticles, Denture, PMMA, Antifungal effect

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I. INTRODUCTION

1. Edentulous patients and dentures

1.1. Edentulism

Globally, the elderly population is on the rise, owing to improved quality of life and declining mortality rates (Lunenfeld & Stratton, 2013). Despite a global decrease in the rate of edentulism, the number of individuals with tooth loss remains substantial, especially as more people reach old age. Edentulism is a complete loss of teeth caused by biological disease processes such as dental caries, periodontal disease, trauma, and oral cancer and is considered an irreversible oral condition. The prevalence of this condition is the highest

among elderly populations with unfavorable demographic and socioeconomic contexts (Borg-Bartolo et al., 2022). In particular, economic status, age, and level of education are among the contributing factors most often associated with edentulism (Al-Rafee, 2020; Roberto et al., 2019). Tooth loss significantly reduces the quality of life in physiological (speech and mastication), social (connected to esthetics/appearance), nutritional, and psychological aspects (Gupta et al., 2019; McGrath & Bedi, 2001). A commonly used solution for restoring oral function in edentulous patients is rehabilitation through dental implants or complete dentures. However, many patients choose dentures for general health, expense, and anatomical issues.

1.2. Denture base resin

Acrylic polymers such as poly(methyl methacrylate) (PMMA) are the most widely used material for denture base resin fabrication of dental prostheses (Alqutaibi et al., 2023). The liquid mainly comprises the monomer methyl methacrylate, and the powder consists primarily of poly(methyl methacrylate) (Zafar, 2020). Because this poly(methyl methacrylate) is translucent, it creates a color similar to the gingiva through pigment additives. It adds colored nylon or acrylic synthetic fibers to reproduce the thread veins of the oral mucosa (Kostić et al., 2022). PMMA is suitable for use as a denture base resin because it is aesthetically acceptable, easy to fabricate, relatively inexpensive, lightweight, and biocompatible, which means that it does not cause any harm to the soft tissues in the oral cavity, and is stable in the oral environment (Pagano et al., 2021; Srinivasan et al., 2021). However, PMMA lacks antifungal properties and can easily act as a reservoir and incubator for microorganisms, accelerating oral infections. The rough surface of PMMA increases the available surface area for microbial attachment and provides a scaffold for adhesion. Additionally, rough surfaces can shield microbes against shear forces, thereby resisting the detachment of bound microbes. Consequently, microbial adhesion and biofilm formation intensify with increasing surface roughness.

1.3. Denture stomatitis

Denture stomatitis, a chronic inflammatory condition, is caused by the accumulation of biofilm on the oral tissues and denture surface and results in inflammation of the oral mucosa in contact with dentures, accompanied by erythema (Gendreau & Loewy, 2011). It is the most common multifactorial oral condition among denture wearers, affecting approximately 50-70%, and also affects people who use obturators and intraoral removable orthodontic appliances. The etiology of denture stomatitis is influenced by various factors, such as dry mouth, poor oral hygiene, wearing ill-fitting dentures, prolonged denture use, and wearing dentures during sleep (Abuhajar et al., 2023). One of the primary factors contributing to denture stomatitis is the presence of *Candida albicans*, a type of fungus that is commonly found in the oral cavity (Mylonas et al., 2022). *C. albicans* is a significant opportunistic fungal pathogen that typically exists as a harmless commensal organism. However, it can become pathogenic and cause infections, especially in individuals with weakened immune systems or when mucosal barriers are compromised. *C. albicans* contributes to palatal inflammation by adhering to oral mucosal surfaces, including those beneath dentures. This adherence leads to the accumulation of Candida colonies and the formation of biofilms. Furthermore, wearing dentures can alter the oral environment, providing favorable conditions for biofilm formation and the growth of *C. albicans*. These biofilms can act as reservoirs for various microbial species, increasing the risk of denture stomatitis. (Funari & Shen, 2022; Monteiro et al., 2021).

2. Zinc Oxide nanoparticles

Various studies have been conducted to improve the antifungal ability of PMMA, which is the most widely used material for denture base resin. (Mirizadeh et al., 2018). One of the approaches involves incorporating antimicrobial agents and metal oxide nanoparticles into the PMMA matrix (Jo et al., 2017; Totu et al., 2017). Among the various metal nanoparticles investigated, zinc oxide nanoparticles (ZnO NPs) have garnered considerable attention due to their excellent properties (Toledano et al., 2021). ZnO NPs are white powder, odorless, non-toxic, and biocompatible, making them suitable for use in the human body (Cierech et al., 2018; Szerszeń et al., 2022). Several studies have investigated using ZnO NPs in PMMA denture base resins to improve their antifungal properties. Furthermore, ZnO NPs exhibit antifungal and antimicrobial properties against various pathogens, including *C. albicans* (Kairyte et al., 2013; Sharma et al., 2010). These studies have shown that incorporating ZnO NPs can significantly reduce the growth of *C. albicans* on denture surfaces (Cierech et al., 2016).

3. Mesoporous silica nanoparticles

Mesoporous silica nanoparticles (MSNs) have been proposed as potential nano-additives to improve the properties of PMMA-based denture base resins. MSNs are biocompatible, stable, durable, and cost-effective, making them attractive for biomedical applications (Attik et al., 2017; Yan et al., 2018; Yu et al., 2016). In a previous study, it was reported that the addition of MSNs improved the mechanical properties of dental composites. MSNs have a unique mesoporous structure that allows the resin matrix to penetrate the pores of the particle, forming a micromechanical interlocking interface between the filler and the resin matrix, thereby improving the bond between the filler and the matrix. These mechanically interlocking interfaces can improve the durability and mechanical properties of resin materials (Wang et al., 2017). Moreover, the incorporation of MSNs into PMMA was demonstrated to have an antibacterial effect on the attachment of *C. albicans* (Lee et al., 2016). In addition, Zn-doped MSNs have an excellent antimicrobial effect on bacteria, fungi, etc., by creating a nano additive capable of ion release (Bai et al., 2020).

4. Research objective and null hypothesis

ZnO NPs are a material known for their antimicrobial properties and have been used for an extended period due to their numerous advantages. However, when directly added to denture materials, it can introduce aesthetic issues related to such as color and translucency, challenging its utilization. Moreover, it may negatively impact the mechanical properties of the dentures.

To address these challenges, zinc was doped into mesoporous silica nanoparticles, a material with a porous structure known to improve mechanical properties. This modified material, Zn-MSNs, was employed in the experiment. The primary objective of this study is to fabricate dentures by incorporating Zn-MSNs and subsequently evaluate their potential for clinical applicability.

Therefore, the null hypothesis of this study is that there would be no significant difference in mechanical strength, surface properties, and antifungal effect against *C. albicans* between the denture base resin with and without Zn-MSNs.

II. MATERIALS AND METHODS

1. Materials

1.1. Commercially available materials

The commercially available, self-curing acrylic resin material (ProBase Cold, Ivoclar Vivadent, Schaan, Liechtenstein) was used in all the experiments.

ZnO NPs were purchased from Sigma-Aldrich (St. Louis, MO, USA). According to the information provided by the manufacturer, the ZnO NPs were white with an average particle size of less than 100 nm.

1.2. Synthesis of MSNs and Zn-MSNs

1.2.1. Synthesis of MSNs

MSNs and Zn-MSNs were synthesized using the sol-gel method through a two-step Stöber process. The synthesis employed the cationic surfactant cetyl trimethyl ammonium bromide (CTAB) as the template and tetraethyl orthosilicate (TEOS) as the silica precursor. First, CTAB and ammonium fluoride were added to distilled water and dissolved using magnetic stirring. While stirring, the reaction mixture was heated and maintained at 80 degrees Celsius (°C) using a refrigeration and heating circulator (RW-0525G, Lab Companion, Daejeon, Korea). Subsequently, TEOS was added dropwise to the reaction mixture and stirred for 4 hours. The molar composition ratio used to produce MSNs was CTAB: ammonium fluoride: TEOS= 1: 15.72: 5.89. Then, centrifugation at 3000 rpm for 5 minutes was performed to precipitate the MSNs. The resulting MSNs were thoroughly washed, involving three cycles of ethanol and deionized water. The obtained MSNs were dried overnight in an oven set at 50°C. To eliminate the CTAB template, the dried samples underwent calcination in a furnace (BF51866C-1, Thermo Fisher Scientific, Waltham, MA, USA) at 650°C for 4 hours.

1.2.2. Synthesis of Zn-MSNs

Zn-MSNs were prepared in a similar method to MSNs. CTAB and ammonium fluoride were put into distilled water and stirred magnetically at 80 °C until complete dissolution. In a separate beaker, zinc nitrate was dissolved in ethanol at a concentration of 0.5 g/mL and subsequently mixed with TEOS, forming a clear solution. The mixture was then dropped into the previously prepared mixture and stirred for 4 hours. The molar composition ratio applied to synthesize the Zn-MSNs was CTAB: ammonium fluoride: $Zn(NO_3)_2.6H_2O$: TEOS = 1: 16.25: 0.926: 8.13. Centrifugation separated the precipitated Zn-MSNs for 5 minute at 3000 rpm. The Zn-MSNs were washed thrice with ethanol and deionized water, respectively. The Zn-MSNs were dried overnight in an oven at 50 °C and calcinated at 650 °C for 4 hours in a furnace to remove CTAB.

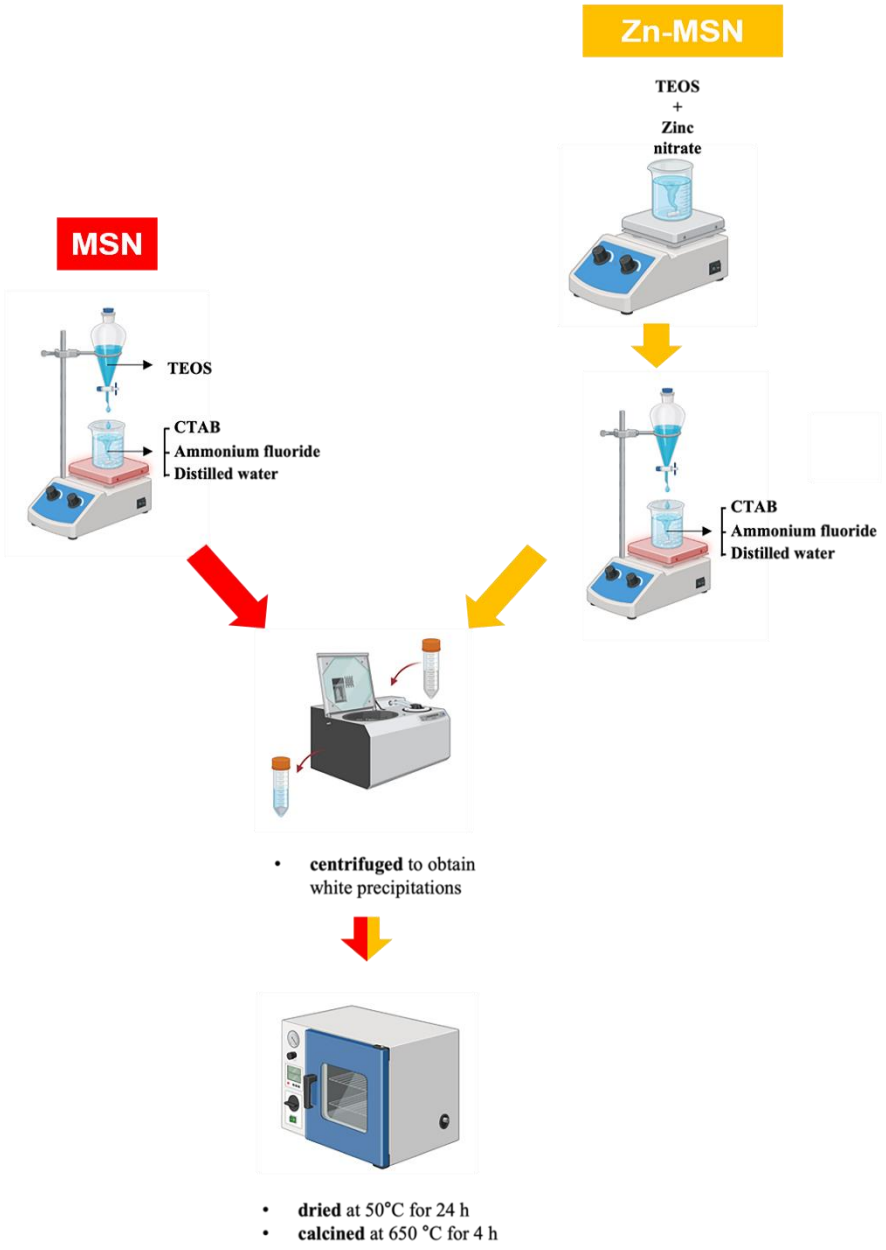

Figure 1. Schematic illustration of the preparation process of MSNs and Zn-MSNs.

2. Characterizations of ZnO NPs, MSNs, and Zn-MSNs

2.1. Morphology and elemental composition

Transmission electron microscopy (TEM; JEM-200F, JEOL, Tokyo, Japan) was employed to examine the morphology of ZnO NPs, MSNs, and Zn-MSNs. The elemental composition of ZnO NPs, MSNs, and Zn-MSNs was determined by energy-dispersive X-ray spectroscopy (EDS). The powder samples were evenly dispersed in ethanol by sonication to produce a suspension at a concentration of 0.1% (w/v). A drop of the suspension was carefully dropped onto a copper grid and dried at 25 °C in a desiccator cabinet.

2.2. Crystal structure

The X-ray diffraction (XRD; Ultima IV, Rigaku, Tokyo, Japan) analysis was conducted to identify the crystal structure of MSNs and Zn-MSNs by examining the diffraction pattern and the position of the Bragg peaks. MSNs and Zn-MSNs was screened within the 2θ angle range of 10° to 80°, using Cu Kα1 radiation at 40 kV and 30 mA, with a scanning speed of 2˚/min.

2.3. Characterization of mesoporous structure

The pore volume and size were analyzed from N_2 adsorption–desorption measurements at -196.15 °C using a Surface Area & Pore Size Analyzer (Autosorb IQ, Quantachrome Instruments, Boynton Beach, FL, USA) based on the non-local density functional theory (NLDFT) method. The Brunauer–Emmett–Teller (BET) method was utilized to calculate the surface area.

3. Preparation of denture base resin specimens

The ZnO NPs, MSNs, and Zn-MSNs were incorporated into denture base resin at 1.0 and 5.0 weight percentage (wt%), respectively. Denture base resin specimens without adding any powder were considered as a control group. The detailed proportions of powder in the denture base resin were listed in Table 1. For homogeneous dispersion, High-power probe sonication (Qsonica LLC, Newtown, CT, USA) was used to disperse the experimental powder in the MMA liquid at 40% amplitude for 10 minute with a 10 second on, 5 second off pulse regimen. After that, the suspension was mixed with a PMMA powder using a speed mixer (DAC 150.1 FVZ, Hauschild SpeedMixer, Hamm, Germany) at 3500 rpm for 30 second.

Table 1. Compositions of the control and experimental groups in this study. Weight ratio (%) of materials in each group and experimental codes

Group	PMMA	MMA	MSNs	ZnO NPs	Zn-MSNs
PMMA	60	40			
1% MSN	59.4	39.6	1		
5% MSN	57	38	5		
1% ZnO	59.4	39.6		1	
5% ZnO	57	38		5	
1% Zn-MSN	59.4	39.6			1
5% Zn-MSN	57	38			5

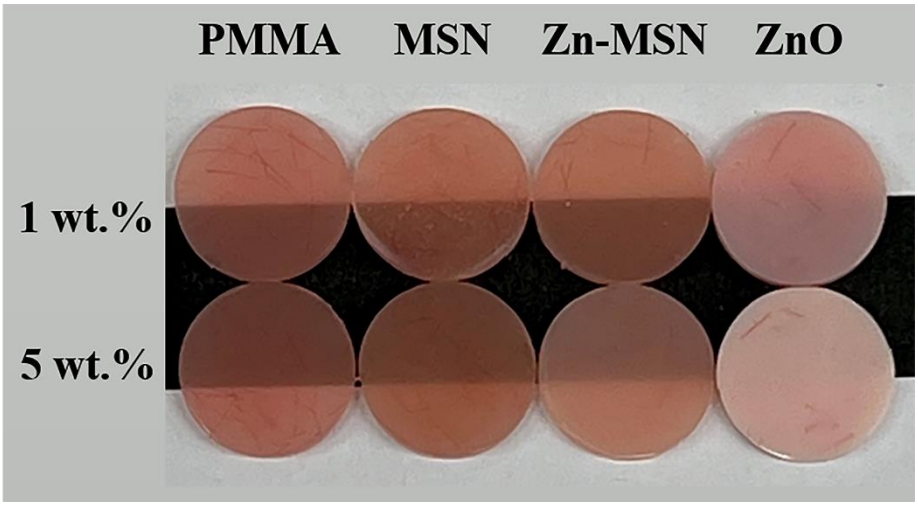

Figure 2. Photograph of PMMA and PMMA-incorporated MSNs, Zn-MSNs, and ZnO NPs respectively. The two specimens of PMMA are denture base resin without any incorporation. The denture base resin specimens (10 × 2 mm) in the first row were added powder of 1 wt%, excluding PMMA. Speciments in the second row were added powder of 5 wt%, excluding PMMA.

4. Characterizations of denture base resin specimens

4.1. Surface gloss

The measurement of surface gloss was conducted using a Novo curve gloss meter (Novo – Curve, Rhopoint Instruments, East Sussex, UK) with a 2 mm × 2 mm area and a 60° geometry for light incidence. The gloss values were expressed in gloss units (GU). Before measurement, the specimens of 10 mm in diameter and 2 mm in thickness were polished sequentially using silicon carbide paper with grit sizes of 800, 1200, 1500, and 2000, followed by rinsing with deionized water. Three random sites on each specimen were measured, and five specimens were analyzed for each group.

4.2. Translucency

A spectrophotometer (CM-5, Konica-Minolta, Tokyo, Japan) was utilized for measuring translucency. The instrument operated in reflectance mode and underwent calibration using a white tile and a black trap in accordance with standard procedures. The disc-shaped specimens were assessed at three randomly chosen locations on each individual specimen, and a total of five specimens were examined for each experimental group. Measurements of the three parameters (L*, a*, and b*) within the Commission Internationale de l'Eclairage (CIELab) system were recorded for each specimen against both white and black backgrounds. In the CIELab system, L* values range from 0 (representing black) to 100 (representing white). Negative a* values indicate green, while positive a* values indicate red. Negative b* values indicate blue, and positive b* values indicate yellow. The collected data were then tabulated, and the translucency value was calculated using the following formula:

$$\text{Translucency} = \sqrt{(L_b - L_w)^2 + (a_b - a_w)^2 + (b_b - b_w)^2}$$

where the subscripts "b" and "w" in the formula refer to color coordinates measured over the black and white backgrounds, respectively.

4.3. Zn ion release behavior

Zinc ion release behavior was tested according to ISO 10993-12. The 5% ZnO and 5% Zn-MSN specimens were extracted in deionized water with an extraction ratio of 3 cm^2/mL, calculated as the specimen surface area to deionized water volume. The 5% ZnO and 5% Zn-MSN specimens were immersed in distilled water at 37 °C for specific durations (12 h, 24 h, 2 d, 4 d, 8 d, 16 d, and 32 d) and stored at the same temperature in a shaking incubator (IST 4075R, Jeio Tech Co., Ltd., Seoul, Korea). After the set duration, the solution was collected, and the release behavior of zinc was confirmed using inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7900, Agilent Technologies, Tokyo, Japan).

5. Mechanical properties

The three-point flexural strength test was considered as a standard method to evaluate the mechanical properties of denture base resin materials. In accordance with the International Standard ISO 20795–1 (2013), the flexural strength and flexural modulus were assessed and calculated. The specimens were prepared to an average size of 64 mm × 10 mm × 3.3 mm and polished using a grit silicon carbide paper sequence. Next, they were immersed in water at a temperature of 37 °C for a duration of 50 hours. A universal testing machine (UTM; Model 5942, Instron, Norwood, MA, USA) with a span length of 50 mm was used to measure the specimens. The crosshead was pressed continuously at a rate of 5.0 mm/min. The load was applied until the denture base resin specimen fractured. The flexural strength (σ) and modulus (E) were calculated using the following equations:

$$\sigma = \frac{3Fl}{2bh^2}$$

$$E = \frac{F_1 l^3}{4bh^3 d}$$

Where F is the maximum load exerted on the specimens (N), l is the distance between the supports which is 50 mm, b is the width, h is the height of the specimens (mm), F_1 is the load at a point on the linear portion of the load/displacement curve (N), and d is the deflection at load F_1 (mm).

6. Analysis of the antifungal effect

6.1. Fungal viability assay

The fungal strain was obtained from the Korean Collection for Type Cultures (KCTC; Jeollabuk-do, Korea) to evaluate the antifungal effect. *C. albicans* was cultured in Sabouraud dextrose broth (SD broth, MB Cell, Los Angeles, CA, USA) at 37 ℃ for 24 hours under aerobic conditions.

The fungal viability test was assessed according to ISO 10993-12 (specimen surface area/SD broth volume: 3 cm^2/ml). After preparing disk-shaped specimens with a diameter of 10 mm and a thickness of 2 mm, the specimens were sequentially polished to 800 grit on silicon carbide paper. Then, each specimens was added to a fungal suspension (10^7 CFU/ml) and incubated at 37 °C in a shaking incubator. After 24 hours, 100 μL of the fungal suspension was spread onto a solid SD agar plate and incubated at 37 ℃ for 24 hours. The total number of colonies was then counted.

6.2. Fungal anti-adhesion

A denture base resin specimen was manufactured using a mold with a diameter of 10 mm and a thickness of 2 mm. The specimens underwent a polishing process and were subsequently sterilized. Each specimen was then placed on an SD solid agar plate. Next, 100 μL of fungal suspension (1×10^6/ 100 μL) was carefully placed on the surface of each specimen and incubated at 37 ℃. After 24 hours incubation, the specimens were gently washed twice with PBS to remove non-adherent fungi. Subsequently, the adherent fungi from the specimen surfaces were harvested in 1 mL of SD broth through sonication (SH-2100; Saehan Ultrasonic, Seoul, Korea) for 5 minute. Then, 100 μL of fungal suspension was spread onto a solid SD agar plate and incubated at 37 ℃ for 24 hours. The CFU counts obtained for each dilution were utilized to calculate the original concentration of the suspension and subsequently compared.

6.3. Live and dead assay

The denture base resin specimens (diameter 10 mm × thickness 2 mm, $n = 5$) were used for Fungal viability test. The viability of adherent *C. albicans* was determined through a staining process applied to the surface of each specimen, employing the LIVE/DEAD Fungalight yeast viability kit (Invitrogen, Thermo Fisher Scientific, Waltham, USA). Live fungi were stained with SYTO9, resulting in green fluorescence, while dead fungi were stained with propidium iodide, resulting in red fluorescence. The staining process followed the protocols provided by the manufacturer of the kit. Equal volumes of SYTO9 dye and propidium iodide from the kit were mixed thoroughly. Then, 3 μL of this mixture was added to each mL of the PBS, and 1 mL of the completed solution was placed on each specimen in a 24 well plate for 30 minute in the dark environment at 37 ℃. After incubation with the dyes, the stained specimens were observed using confocal laser scanning microscopy (LSM 980, Carl Zeiss, Thornwood, NY, USA).

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7. Statistical analysis

The statistical analyses were performed using the IBM SPSS statistics 26.0 software program (IBM Corp., Armonk, NY, USA) by one-way analysis of variance (ANOVA). The differences between the groups were analyzed by a Tukey's post hoc test. All statistical significance levels were set at a confidence 95 % ($p < 0.05$).

III. RESULTS

1. Characterizations of ZnO NPs, MSNs, and Zn-MSNs

1.1. Morphology and elemental composition

The morphology and elemental composition of the experimental powders, including ZnO NPs, MSNs, and Zn-MSNs are presented in Figure 3. In the TEM image, commercially available ZnO NPs displayed irregular shapes with varying sizes and aspect ratios, with some particles forming agglomerates (Figure 3(A)). In contrast, both MSNs and Zn-MSNs exhibited a well-ordered mesoporous structure with spherical morphology (Figure 3(B), (C)). The EDS analysis of Zn-MSNs revealed a successful doping of approximately 7.34 wt% of Zn, which was evenly distributed within the Zn-MSNs structure. The remaining 92.66 wt% consisted of silicon (Si) and oxygen (O).

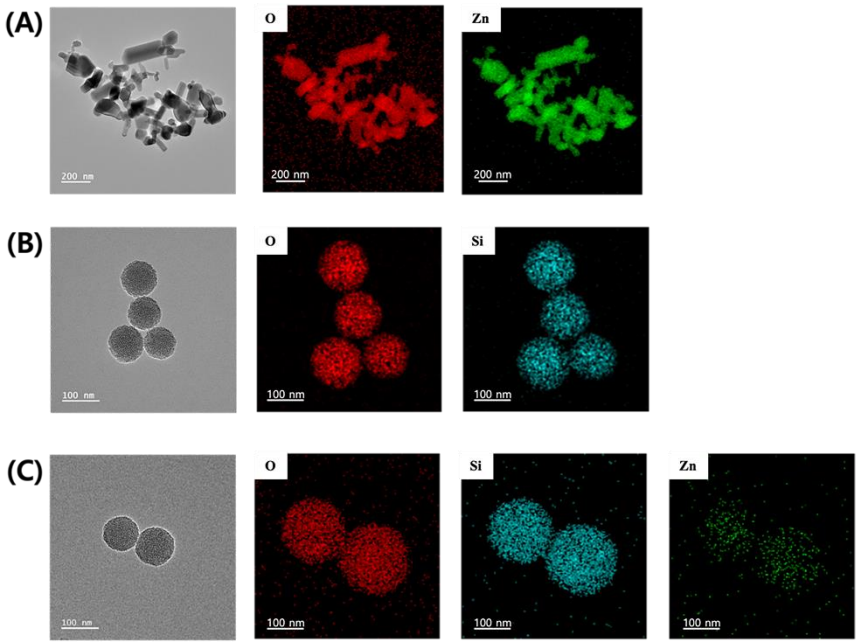

Figure 3. TEM images and EDS elemental mapping images (A) MSNs, (B) Zn-MSNs, and (C) ZnO NPs.

1.2. Crystal structure

The X-ray diffraction (XRD) analysis of MSNs and Zn-MSNs revealed a broad peak within the 20° to 30° range, as depicted in Figure 4. The observed peak had a smooth shape, indicating an amorphous structure.

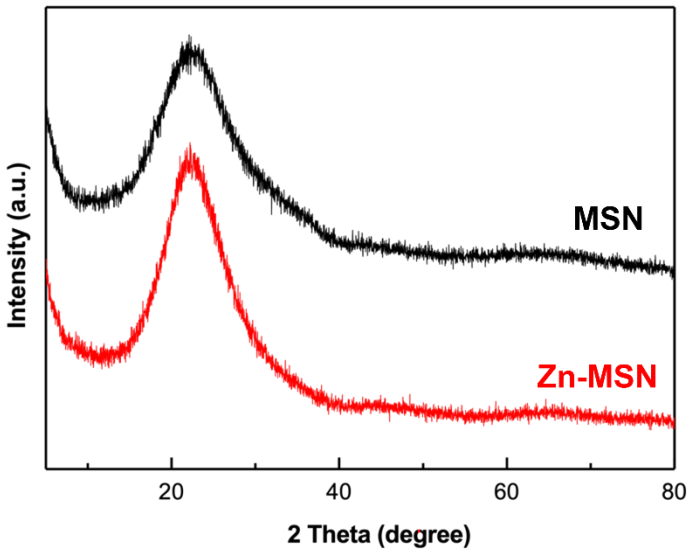

Figure 4. X-ray diffraction patterns of the MSNs and Zn-MSNs.

1.3. Characterization of mesoporous structure

To gain a deeper understanding of the internal structure of Zn-MSNs, we conducted N_2 adsorption/desorption measurements to determine specific surface area and pore size characteristics. The results revealed that Zn-MSNs exhibited a type IV isotherm accompanied by a typical H3-hysteresis loop following the IUPAC classification (Figure 5). BET analysis further confirmed that the Zn-MSNs possessed mesoporous properties, with a pore size measuring 3.41 nm, a pore volume of 0.77 cm^3/g, and a surface area of 848 m^2/g.

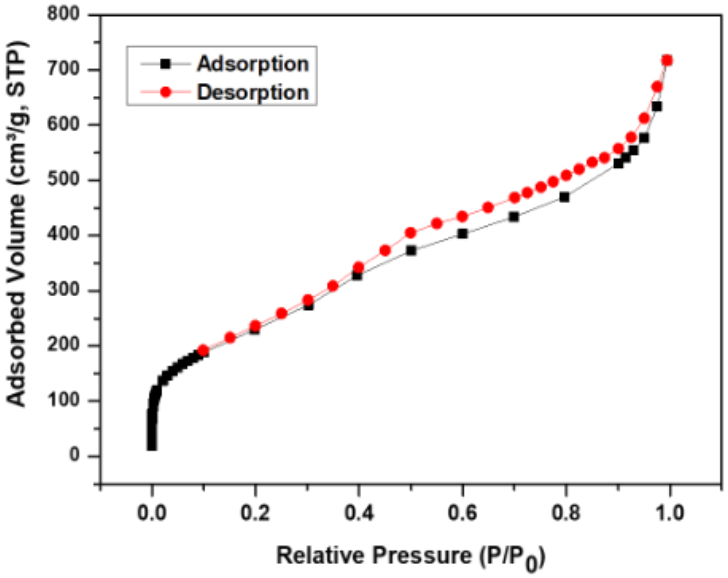

Figure 5. Nitrogen adsorption/desorption isotherms of the Zn-MSNs.

2. Characterizations of denture base resin specimens

2.1. Surface gloss

The mean values and standard deviations of surface gloss for the control and test groups are presented in Figure 6. No significant difference was observed between the control group, the 1% MSN group, and the 1% Zn-MSN group, with values of 97.56 (± 0.97), 97.32 (± 1.00), and 95.45 (± 2.15), respectively ($p > 0.05$). Notably, 1% ZnO showed a lower gloss value than the 5% MSN and 5% Zn-MSN groups ($p < 0.05$). Among the groups, the 5% ZnO group had the lowest surface gloss at 81.62 (± 1.27) ($p < 0.05$).

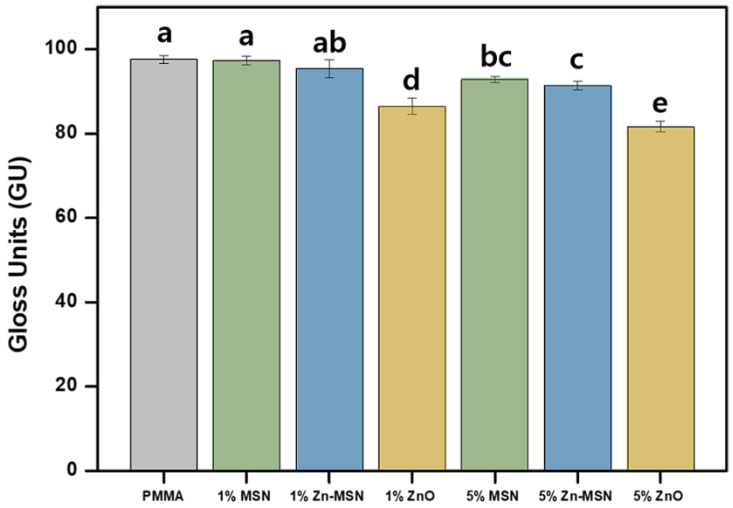

Figure 6. Comparison of surface gloss values between the different groups of denture base resin incorporating MSNs, Zn-MSNs, and ZnO NPs. The same lowercase alphabetical letters above the bar graph indicate there are no significant differences between the groups ($p > 0.05$).

2.2. Translucency

The mean and standard deviation values of translucency are presented in Table 2. There was no significant difference in translucency between the control group (15.29 ± 1.6), 1% MSN group (15.07 ± 0.72), 1% Zn-MSN group (13.51 ± 1.44), 5% MSN group (13.07 ± 0.22), and 5% Zn-MSN group (13.51 ± 1.44) ($p > 0.05$). However, a significant difference was observed between the control group, the 1% ZnO group (7.25 ± 0.78), and 5% ZnO group (2.98 ± 0.48) ($p < 0.05$).

Table 2. Comparison of translucency values between the different groups of denture base resin. The same lowercase alphabetical letters indicate there are no significant differences between the groups ($p > 0.05$)

Group	Mean (SD)
PMMA	15.29 (1.6)[a]
1% MSN	15.07 (0.72)[a]
1% Zn-MSN	13.51 (1.44)[a]
1% ZnO	7.25 (0.78)[b]
5% MSN	13.28 (1.88)[a]
5% Zn-MSN	13.07 (0.22)[a]
5% ZnO	2.98 (0.48)[c]

2.3. Zn ion release behavior

Figure 7 shows the cumulative release of Zn ions from denture base resin specimens of the 5% Zn-MSN and 5% ZnO groups when immersed in deionized water. During the initial 4 days, both groups exhibited a relatively rapid burst release. It was observed that the cumulative release of Zn ions continued to increase with longer incubation time. After 32 days of incubation, the total release amounts were 11.95 μg/mL for the 5% Zn-MSN group and 21.84 μg/mL for the 5% ZnO group, as shown in Table 3.

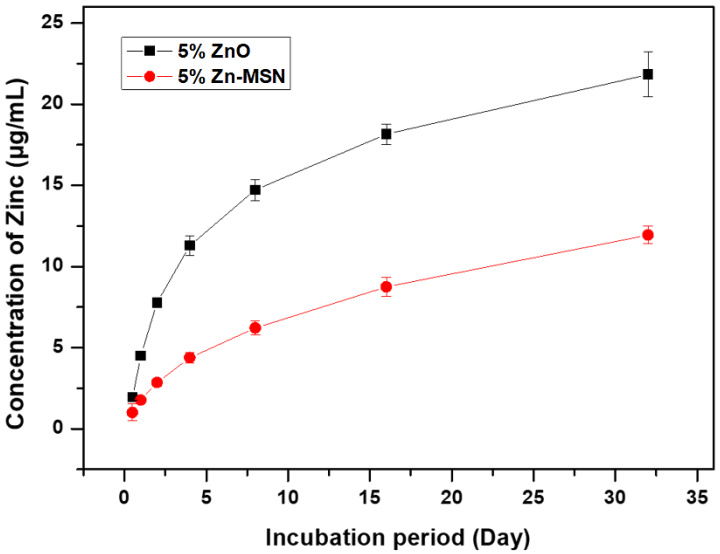

Figure 7. The Concentration of Zn ions released from 5% ZnO and 5% Zn-MSN groups during 12h, 24h, 2d, 4d, 8d, 16d, and 32d.

Table 3. Zn ion concentration released from 5% Zn-MSN and 5% ZnO groups over a period of time

Group		12h	1d	2d	4d	8d	16d	32d
5% Zn-MSN	Mean (μg/mL)	1.01	0.76	1.07	1.53	1.83	2.53	3.21
	SD	0.53	0.14	0.22	0.34	0.42	0.59	0.54
5% ZnO	Mean (μg/mL)	1.94	2.55	3.29	3.51	3.44	3.43	3.68
	SD	0.19	0.28	0.30	0.59	0.64	0.62	1.38

3. Mechanical properties

The results indicated that there was no significant difference ($p > 0.05$) in flexural strength between the control group, 1% MSN group, and 1% Zn-MSN group, with values of 89.28 (± 3.57) MPa, 88.19 (± 2.87) MPa, and 88.46 (± 3.12) MPa, respectively (Figure 8A, $p > 0.05$). However, incorporating 1% ZnO group significantly reduced the flexural strength to 84.16 MPa (± 4.54) compared to the control group ($p < 0.05$). Moreover, a significant decrease was observed compared to the control group ($p < 0.05$) when 5% MSN and 5% Zn-MSN groups were incorporated, with values of 77.56 (± 5.22) MPa and 76.08 (± 5.05) MPa, respectively. The lowest flexural strength value was recorded in the 5% ZnO group, measuring 70.37 (± 2.06) MPa. The mean values and standard deviations of the Flexural modulus for control and test groups are given in Figure 8B. There were no significant differences among the different groups ($p > 0.05$).

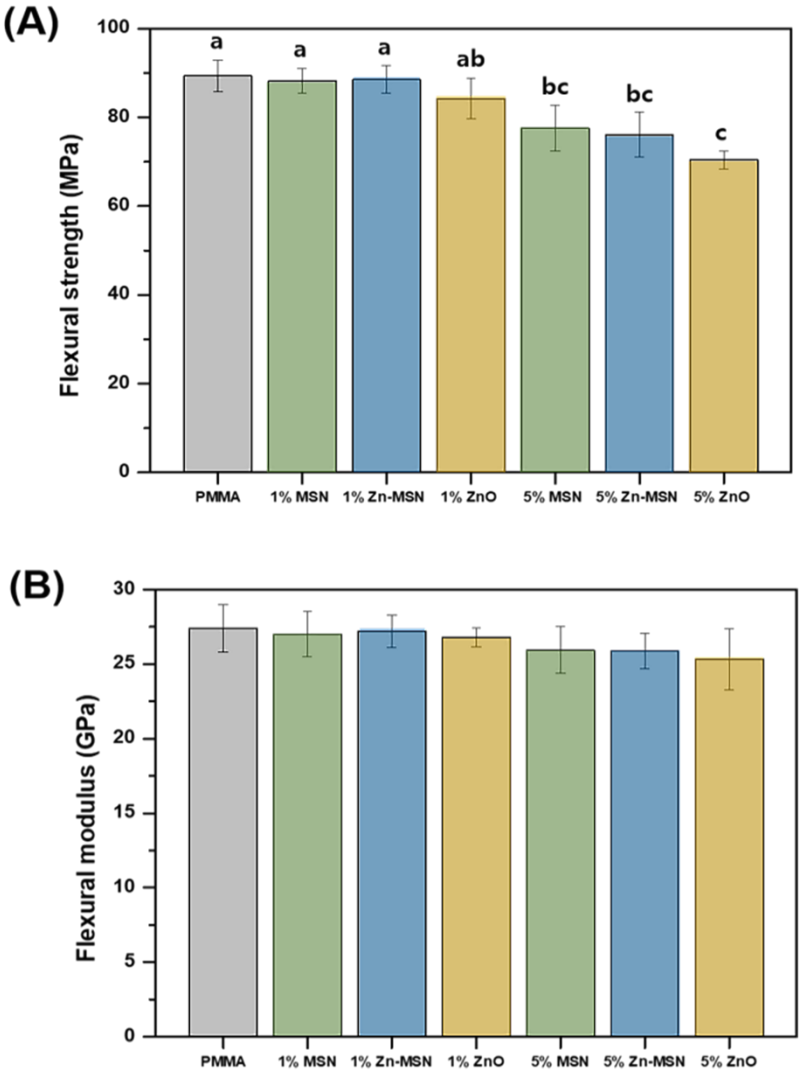

Figure 8. Comparison of flexural strength (A) and flexural modulus (B) among the different groups of denture base resin incorporating MSNs, Zn-MSNs, and ZnO NPs, respectively. The same lowercase alphabetical letters above the bar graph indicate there are no significant differences between the groups ($p > 0.05$). The flexural modulus showed no significant differences among the different groups ($p > 0.05$).

4. Analysis of antifungal effect

4.1. Fungal viability

The results of the fungal viability are shown in Figure 10. In the case of denture base resin incorporating 1 wt% of MSNs, Zn-MSNs, ZnO NPs, and 5 wt% MSNs, no significant differences were observed compared to the control group ($p > 0.05$). However, specimens incorporating 5 wt% of Zn-MSNs and 5 wt% ZnO NPs exhibited significantly lower fungal viability against *C. albicans* compared to the control group ($p < 0.05$). In particular, The 5% ZnO group had the lowest Fungal viability value among all the groups ($p < 0.05$).

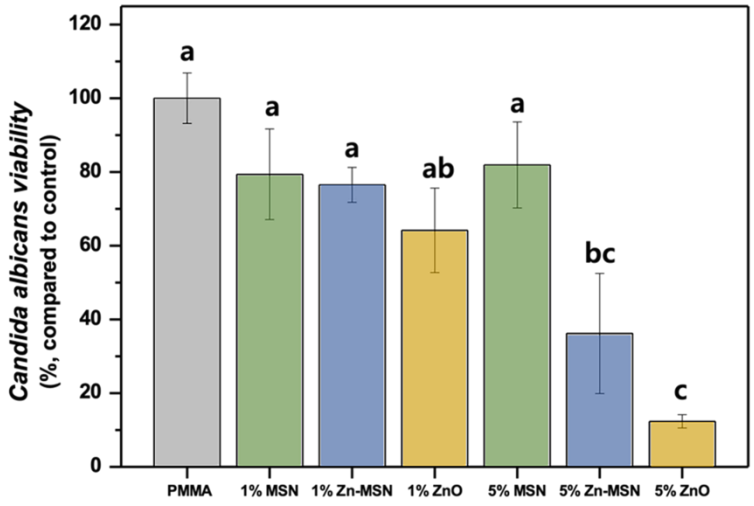

Figure 9. The fungal viability against *C. albicans* of denture base resin incorporated MSNs, Zn-MSNs, and ZnO NPs. The same lowercase alphabetical letters above the bar graph indicate there are no significant differences between the groups ($p > 0.05$).

4.2. Fungal anti-adhesion effect

The anti-adhesion results of *C. albicans* on both control and test specimens were assessed using the CFU method. In the case of denture base resin incorporating 1 wt% of MSNs, Zn-MSNs, and ZnO NPs, no significant differences were observed when compared to the control group ($p > 0.05$). However, specimens incorporating 5 wt% of MSNs, Zn-MSNs, and ZnO NPs exhibited significant anti-adhesive effects against *C. albicans* after 24 hours of attachment compared to the control group ($p < 0.05$), as shown in Figure 10.

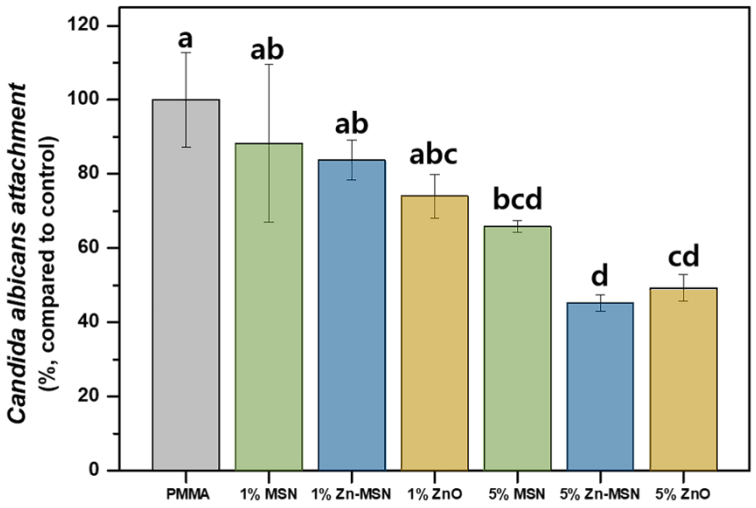

Figure 10. Anti-adhesive effect against *C. albicans* of denture base resin incorporated MSNs, Zn-MSNs, and ZnO NPs. The same lowercase alphabetical letters above the bar graph indicate there are no significant differences between the groups ($p > 0.05$).

4.3. Live and dead assay

The results of fungal viability were notably discernible among the different groups with fluorescence microscopy, as shown in Figure 11. Based on the live/dead staining results, the specimen surface of the control group was covered with many live fungi, which were stained green (Figure 11(A)). The specimen had fewer fungi attached to its surface for the MSN group. However, most fungi in these specimens exhibited green fluorescent signals, indicating the presence of living fungi (Figure 11(B), (E)). For the Zn-MSN and ZnO groups, while fungi were observed on the specimens, the number of attached fungi was lower than that in the control group (Figure 11(C), (D)). In certain areas, dead fungi were identified, displaying red or yellow fluorescence. Moreover, it was noted that an increase in the Zn content, from 1 wt% to 5 wt%, increased dead fungi (Figure 11(F), (G)).

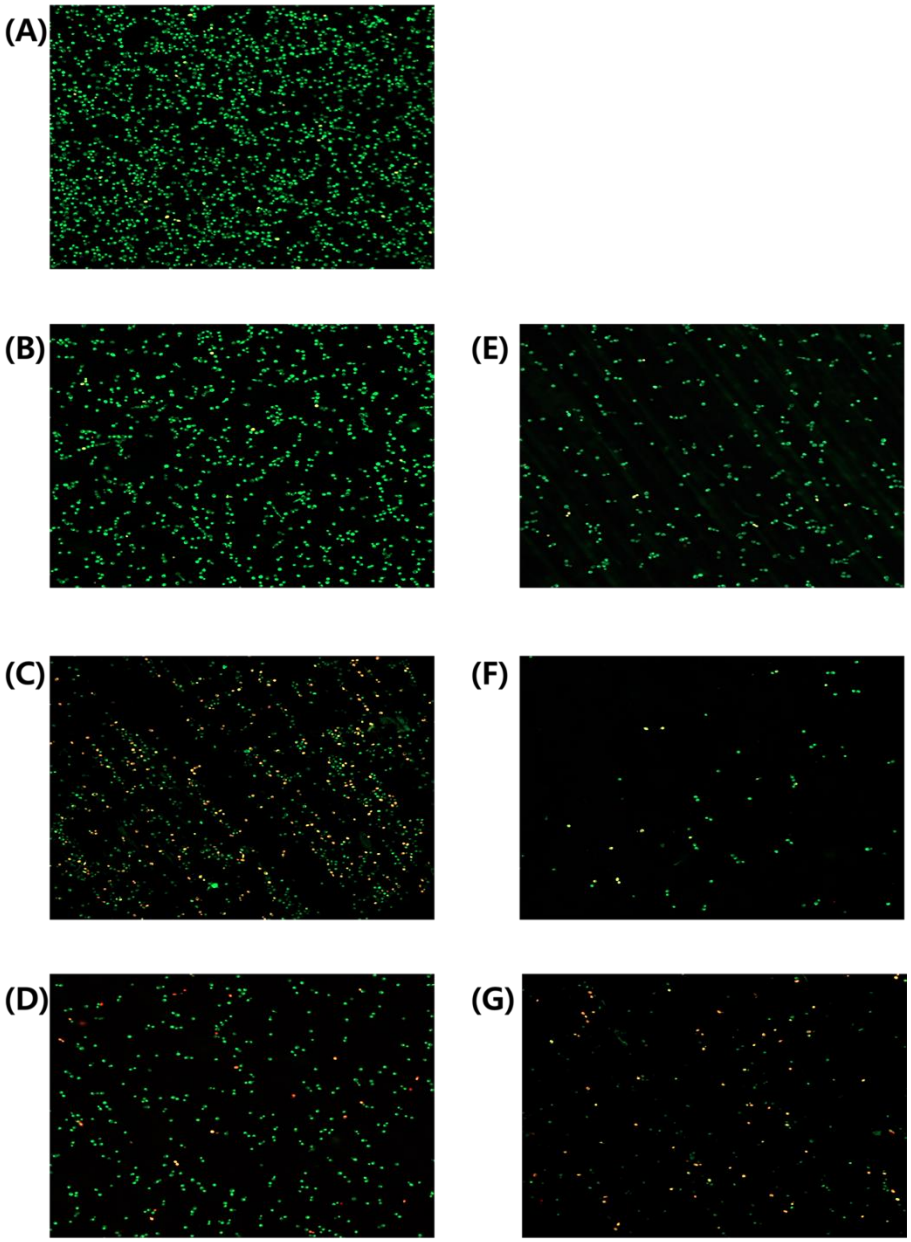

Figure 11. Fluorescent images showing the live (green) and dead (red) stained fungi adherent to denture base resin following control group (A); 1% MSN (B); 1% Zn-MSN (C); 1% ZnO (D); 5% MSN (E); 5% Zn-MSN (F); and 5% ZnO (G).

IV. DISCUSSION

Dentures serve as prosthetic devices designed to replace missing teeth, aiming to restore both functionality and aesthetic appearance for patients. Poly(methyl methacrylate) (PMMA) stands out as one of the most commonly utilized materials for denture base resin due to its appealing aesthetics, cost-effectiveness, biocompatibility, and stability within the oral environment. Despite these advantages, denture stomatitis, a chronic inflammatory condition affecting individuals who wear dentures, poses a significant concern in the oral cavity (Jeon et al., 2022).

ZnO NPs are well-known for their antifungal properties, and MSNs can improve mechanical properties due to their mesoporous structure and exhibit anti-adhesion effects. Considering the advantageous properties of MSNs in reinforcing denture base resin and the broad antifungal activity of zinc, Zn-MSNs were successfully synthesized. The primary objective of this study was to enhance the antifungal properties of denture base resins while minimizing any adverse effects on other essential properties.

The morphology and elemental composition of the synthesized particles were investigated using TEM and EDS analyses. The results showed that Zn-MSNs had a spherical morphology and homogeneous dispersibility, with approximately 7.34 wt% of Zn successfully doped and evenly distributed in the particles. Furthermore, the Zn-MSNs exhibited a well-ordered mesoporous structure with a pore size of 3.41 nm, pore volume of 0.77 cm^3/g, and surface area of 848 m^2/g. The XRD pattern of Zn-MSN showed a broad diffraction peak in the range of 20° to 30°, suggesting that the presence of zinc did not significantly affect the crystallization of silica. These findings align with the previous studies investigating the doping of mesoporous silica (Bai et al.,

2020; Wei et al., 2009).

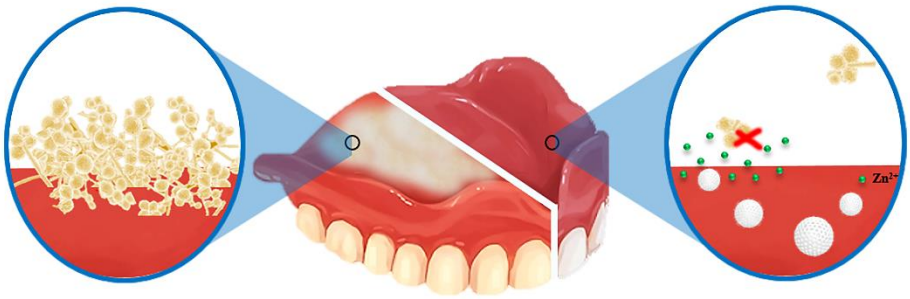

Figure 12. The schematic representation of *C. albicans* attachment to a denture (left) and the antifungal effect of denture base resin incorporated Zn-MSNs (right).

Consideration of optical properties becomes crucial when incorporating antifungal substances into PMMA. Even if substances exhibit excellent antifungal effects, their applicability may be restricted if they adversely affect translucency or introduce undesirable color changes when added to PMMA (Zafar, 2020). Hence, this study investigated the optical properties of PMMA incorporated with MSNs, Zn-MSNs, and ZnO NPs. Gloss is an optical property to be considered when evaluating the visual characteristics of a surface. The level of gloss is related to the surface texture or finish. Smooth surfaces reflect light uniformly and maintain the relative position of the reflected light, while rough surfaces reflect light in a random pattern, resulting in a dull or matte appearance (Keyf & Etikan, 2004). As a result of this study, no significant differences were observed between the control group, the 1% MSN group, and the 1% Zn-MSN group regarding gloss values. However, the gloss values for the 1% ZnO group were significantly lower than those of the control group. Furthermore, the 5% ZnO group exhibited the lowest surface gloss, with a significant difference among all groups.

Regarding the translucency experiment, there was no significant difference between the control group, 1% MSN group, 1% Zn-MSN group, 5% MSN group, and 5% Zn-MSN group. However, significant differences were observed between the control group, 1% ZnO, and 5% ZnO groups. The morphology of the nanoparticles may have prevented the passage of light or caused more light reflection than transmission, making the nanocomposite more opaque (Gad et al., 2021). According to the TEM results, MSNs and Zn-MSNs are spherical particles, while ZnO NPs consists of particles of non-uniform shape and size. The differences in refractive indices between the nanoparticles and the PMMA matrix can impact light refraction and reflection at the interface, affecting translucency. If the difference in refractive index between the two materials is large, the specimen tends to be more opaque. In this case, the refractive index of SiO2 (1.475) was closer to that of PMMA(1.49) than ZnO (2.029) (Lee & Chen, 2001; Norton et al., 2004; Peña-Rodríguez et al., 2012). This suggests that directly adding ZnO to denture base resin can lead to negative optical properties, causing aesthetic concerns.

Denture base resin must have appropriate mechanical properties to withstand mechanical stress caused by masticatory forces in the oral cavity (Dejak et al., 2003; Bacali et al., 2019). The mechanical strength results showed that the incorporation of 1 wt% of MSNs, Zn-MSNs, and ZnO NPs groups did not significantly affect the flexural strength of the denture base resin. However, a significant decrease in flexural strength was observed when the powder concentration was increased to 5 wt%. The 5% ZnO group displayed the lowest flexural strength value among all groups. Nevertheless, the flexural strength of all groups met the minimum requirements (60 MPa) specified in ISO 20795-1.

The ability of denture materials to prevent denture stomatitis is critical

because pathogenic microorganisms on dentures can cause various diseases (Pereira-Cenci et al., 2008). In this study, *C. albicans,* a fungus commonly found on dentures, was selected to assess the antifungal effect of denture base resin. The results of fungal viability showed that denture base resin incorporating 1 wt% of MSNs, Zn-MSNs, ZnO NPs, and 5 wt% MSNs exhibited no significant differences compared to the control group ($p > 0.05$). However, specimens incorporating 5 wt% of Zn-MSNs and ZnO NPs demonstrated significantly lower fungal viability against *C. albicans* compared to the control group ($p < 0.05$).

The anti-adhesion results against *C. albicans* after 24 hours indicated a reduction in the attached *C. albicans* with an increased amount of experimental powder. Denture base resins incorporating 1 wt% of experimental powder showed no significant difference compared to the control group. However, the anti-adhesion effect was observed in the 5% MSN, 5% Zn-MSN, and 5% ZnO groups, demonstrating a significant difference from the control group. A previous study showed that adding MSNs increases surface energy and changes the surface to hydrophilicity (Lee et al., 2016; Shim et al., 2020). Their hydrophilic/hydrophobic properties influence the adhesion of microbial species to biomaterials with high surface energy. Microbial species with hydrophobic characteristics prefer hydrophobic surfaces and are less likely to adhere to hydrophilic surfaces. *C. albicans*, a hydrophobic strain, demonstrates a greater affinity for hydrophobic biomaterials compared to hydrophilic strains. Consequently, hydrophobic microbial species like *C. albicans* face difficulty anchoring to denture base resin that incorporates hydrophilic MSNs.

In the case of ZnO and Zn-MSN, it was confirmed that the number of molds was noticeably reduced on the surface of the specimen in the 5% group compared to the 1% group, and the remaining fungi were also confirmed to be

dead(stained red). The antifungal mechanism of action of ZnO has not yet been established in detail. The reactive oxygen species (ROS) and a poisoning effect due to Zn^{2+} release represent the two main contributions to the antifungal activity of ZnO NPs. Specifically, the fungicidal mechanisms of ZnO NSs account for disrupting the cellular structure, such as cell walls or membrane and organelles, prohibiting biological macromolecular activity, such as protein enzyme, preventing DNA replication, and disrupting the antioxidative system. (Izzi et al., 2023; Sun et al., 2018). These findings indicate that Zn-MSNs is promising as an effective antifungal material suitable for incorporation into denture base resins. However, additional research is required to understand the exact antifungal mechanism of Zn-MSNs fully.

V. CONCLUSION

In this study, we prepared denture base resins incorporating MSNs, Zn-MSNs, and ZnO NPs and conducted investigations into their antifungal effects against *C. albicans* as well as surface properties and mechanical strength. The results were as follows:

(1) Regarding translucency results, there was no significant difference observed among all groups with the incorporation of MSNs and Zn-MSNs ($p > 0.05$). However, the 1% ZnO and 5% ZnO groups showed significant differences when compared to the control group ($p < 0.05$).

(2) The mechanical properties and surface gloss test results showed that the control group exhibited the highest value, a significant difference from the 5% MSN and 5% Zn-MSN groups ($p < 0.05$). The 5% ZnO group exhibited the lowest value among all the groups ($p < 0.05$).

(3) The antifungal effect was evaluated in terms of the number of colony-forming units present in *C. albicans*. As a result of viability, 5% Zn-MSN and 5% ZnO groups exhibited significantly lower fungal viability compared to the control group ($p < 0.05$). For fungal anti-adhesion, the control group showed the highest value, and the denture base resin containing 5% ZnO, 5% MSN, and 5% Zn-MSN showed a significant difference from the control group ($p < 0.05$). Among all values, 5% Zn-MSN showed the lowest value.

The incorporation of 5 wt% Zn-MSNs into denture base resin demonstrated superior surface properties and mechanical strength compared to denture base resin containing 5 wt% ZnO NPs. It also exhibited effective antifungal

activity against *C. albicans*. Thus, a denture base resin incorporating 5 wt% Zn-MSNs is expected to be a valuable material for dentures.

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ABSTRACT (IN KOREAN)

아연이 도핑된 메조포러스 실리카 나노입자를 함유한 폴리(메틸 메타크릴레이트)의 항진균 효과

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서 영 빈

의치 구내염은 의치 착용자에게 흔하게 발병하는 질환으로, 구강 점막의 염증과 홍반을 특징으로 한다. 산화아연나노입자 (ZnO NPs)는 의치 구내염의 주요 원인 중 하나인 칸디다 알비칸스(*Candida albicans*)에 대한 항진균 효과를 가지고 있지만, 의치상 레진에 직접 첨가할 경우 기계적 특성에 부정적인 영향을 미칠 수 있다. 한편, 메조포러스 실리카 나노입자(MSNs)는 필러와 레진 기질 사이에 미세 기계적 연동 인터페이스를 형성하여 기계적 특성을 향상시키는 능력으로 인해 다양한 분야에서 연구가 활발히 진행되고 있다. 따라서 본 연구에서는 아연이 도핑된 메조포러스 실리카 나노입자(Zn-MSNs)를 의치상 레진인 폴리(메틸 메타크릴레이트)(PMMA)에 첨가하여 표면 특성, 기계적 강도, 및 항진균 효과에 미치는 영향을 평가하였다.

MSNs 과 Zn-MSNs 은 졸-겔 방법으로 합성되었으며, 합성된 입자는 메조다공성 구조를 갖는 구형 형태를 나타냈다. PMMA 에 준비된 MSN, ZnO, Zn-MSN 나노입자를 각각 1 wt%, 5 wt% 첨가하여 실험군을 제작하였고, 아무것도 첨가하지 않은 PMMA 를 대조군으로 하였다. 반투명도 결과에서 MSNs 과 Zn-MSNs 을 첨가한 그룹에서는 대조군과 유의한 차이를 보이지 않았으나 1% ZnO 과 5% ZnO 은 모두 유의한 차이를 나타냈다 ($p < 0.05$).

기계적 특성 실험 결과, 대조군이 가장 높은 값을 보였으며 1% ZnO, 5% MSN, 5% Zn-MSN, 5% ZnO 군과 유의한 차이를 나타냈다 ($p < 0.05$). 그 중 5% ZnO 은 모든 그룹 중에서 가장 낮은 값을 나타냈다. *C. albicans* 에 대한 항진균 실험 결과, 5% Zn-MSN 및 5% ZnO 그룹은 대조군에 비해 곰팡이 생존율이 유의미하게 낮은 것으로 나타났다 ($p < 0.05$). 곰팡이 부착에 대한 항진균 실험에서는 대조군이 가장 높은 값을냈으며, ZnO 5%, MSN 5%, Zn-MSN 을 5% 함유한 의치상 레진이 대조군과 유의한 차이를 나타냈다 ($p < 0.05$).

5 wt% Zn-MSNs 을 의치상 레진에 첨가하면 5 wt% ZnO 나노입자를 함유한 의치상 레진에 비해 우수한 표면 특성과 기계적 강도를 나타냈다. 또한 *C. albicans* 에 대한 항진균 실험 결과, 효과적인 항진균 활성을 나타냈다. 결과적으로 Zn-MSN 을 5 wt% 함유한 의치상 레진은 의치용 재료로 활용할 수 있는 잠재력이 있음을 시사한다.

핵심되는 말: ZnO 나노입자, 메조포러스 나노입자, 실리카 나노입자, 메조포러스 나노입자, 아연 도핑 메조포러스 실리카 나노입자, 의치, PMMA, 항진균 효과