



Original article

Antifungal and physical characteristics of modified denture base acrylic incorporated with silver nanoparticles

Ki-Young Nam¹, Cheong-Hee Lee² and Chul-Jae Lee³

¹Department of Dentistry, College of Medicine, Keimyung University, Dongsan-dong, Jung-gu, Daegu, Korea; ²Department of Prosthodontics, School of Dentistry, Kyungpook National University, Samduk-dong, Jung-gu, Daegu, Korea; ³Division of Chemical Industry, Yeungnam College of Science & Technology, Hyeonchung-ro, Nam-gu, Daegu, Korea

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Objective: This study evaluated the antifungal and physical characteristics of denture base acrylic combined with silver nanoparticles.

Materials and methods: Polymerized denture acrylic disc specimens containing 0 (control), 1.0, 5.0, 10.0, 20.0 and 30.0 wt% of silver nanoparticles were placed on separate culture plate dish and 100 μ L samples of yeast suspension of *Candida albicans* strain were inoculated on each specimens and incubated at 37°C, for 24 h. The antifungal effects were evaluated as a number of viable cells in retrieved fungal suspension. To characterize physical aspects, specimens were tested for elution of silver cation (Ag^+) at 24 h and 30th day, thermal analysis (TG/DTA), scanning electron microscope and energy dispersed X-ray analysis (SEM/EDX) and color stability.

Results: Significant reduced CFU was exhibited at 20.0 and 30.0 wt% of silver nanoparticles incorporated ($p < 0.01$) and Ag^+ elution from specimens (maximum 0.356 ± 0.11 mg/L) contributed little to the antifungal activity considering MIC of Ag^+ in this study (3.0 mg/L). The successful synthesis of modified denture acrylic containing silver nanoparticles was accessed by TG/DTA and EDX analysis.

Conclusion: The modified denture base acrylic combined with silver nanoparticles displayed antifungal properties and acted like latent antifungal material itself with low-releasing Ag^+ , however, the improvement of poor color stability was still required.

Keywords: silver nanoparticles, denture base acrylic, antifungal effect.

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Introduction

Chronic denture stomatitis is an erythematous pathogenic condition of the denture-bearing mucosa and a relatively common disease among denture wearers with various incidences in epidemiological studies and as 11–67%¹ or 25–42.4%². One of the major aetiological factors in the pathogenesis of this condition is the presence of numerous yeasts, usually *Candida albicans* (*C. albicans*) on the fitting surface of the denture^{3–5} and fungal adherence to inert polymer such as denture acrylic resin is regarded to be an essential prerequisite for colonisation^{6,7}.

Generally, denture cleansing and oral hygiene care are essential for the prevention of this lesion^{8,9}; however, for some geriatric or hospitalised patients^{10,11}, denture cleansing might be compromised owing to cognitive impairment, reduced motor dexterity and memory loss. Systemic or local antibiotic agents have been prescribed for eliminating the fungal population; however, with microbial resistance and healthcare costs, research on antimicrobial denture base materials is needed for its prevention and care^{12–14}.

Silver (Ag) has been well known for its antimicrobial characteristic and has a long history¹⁵ of application in medicine. It has a well-tolerated

tissue response and low-toxicity profile and is a highly active compound against a broad spectrum of bacteria and fungi which colonise the acrylic surface¹⁶. Such characteristics have led to the resurgence of the use of silver-based antiseptics that may have a lower propensity to induce microbial resistance than antibiotics. Ag in polyurethane or silicone, such as the technology of central venous catheter impregnated by Ag, has been developed and used in various clinical fields^{17,18}, and nano-sized (nm) inorganic particle form of silver, with its rapid and broad-spectrum efficacy and its sustained silver cation (Ag⁺) release^{17,18}, appears to be a more effective means of prophylaxis than microsized silver powder (µm) which shows lower antimicrobial activity owing to the limited surface¹⁹.

Therefore, the purposes of this *in vitro* study were to determine whether the modified denture acrylic combined with different concentrations of Ag⁰ exhibits antifungal activity against *C. albicans* and to assess whether the incorporation of Ag⁰ influences the physical properties of the denture acrylic base.

Material and methods

Preparation of Ag⁰ and specimen (Ag⁰-denture acrylic)

An aqueous silver solution was prepared with 10.0 mM of analytical grade AgNO₃ in distilled water, and 2.0% PVP (Polyvinyl Pyrrolidone) was used as a stabiliser. All solutions were deaerated by bubbling with argon gas for 1 h and then they were irradiated in the field of 20 KGy ⁶⁰Co gamma-ray sources. To fabricate modified denture base specimens incorporated with Ag⁰, colloidal Ag⁰ was preliminary combined with the acrylic denture powder (Lucitone 199; Dentsply, York, PA, USA) at a concentration ranging from 0 (control), 1.0, 5.0, 10.0, 20.0–30.0 wt%, respectively. After complete drying for 120 h at room temperature, combined powders were mixed with resin liquid (Lucitone 199; Dentsply) at designated P/L ratio. Ninety specimens were prepared, and they were divided into six groups (*n* = 15) according to the concentration of Ag⁰ incorporated. Specimens were fabricated to uniform disk shape (20 mm × 3.0 mm) with a smooth surface by using a custom-made brass mould covered by two glass frames. All specimens were cured by wet-heat polymerisation method following the manufacturer's instructions.

Before microbial assay, specimens were immersed in sterilised distilled water for 3 days to leach excess residual monomer and treated for 1 h with ultrasonic cleansing (Branson 2200; Branson, Danbury, CT, USA). To ensure the initial sterility of

specimens, sterilisation with ethylene oxide gas for 24 h was also performed.

Bacterial strains

Candida albicans strain, ATCC 66026, was obtained as a stock culture, and the strains were grown in Schaedler broth and onto Sabouraud agar plates under reciprocal shaking at 37°C. After incubating fungal cells at 37°C overnight, optical density (OD) of the suspension at 600 nm was adjusted to 1.0 using a spectrophotometer (Milton Roy spectrophotometer 20D⁺; Milton Roy, Ivyland, PA, USA). The suspension was diluted with phosphate-buffered saline (pH 7.4) to 1:100 and suspended to final concentration of 1.0 × 10⁷ cells/ml.

Determination of minimal inhibitory concentration (MIC)

The MIC of silver cation (Ag⁺) against *C. albicans* was determined visually and spectrophotometrically at OD 600 after 24 h of incubation at 37°C. Ag⁺ was added to 100 ml of fungal suspension prepared to 1.0 × 10⁷ cells/ml in Brain Heart Infusion broth at 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l. A 1-ml aliquot of each suspension was taken, and the absorbance at OD 600 was measured using a spectrophotometer. The tests were performed in triplicate for each concentration.

Microbial assay

Each Ag-denture acrylic specimen was placed on the flat bottom of the separate 12-well cell culture plate (Costa[®]; Corning, New York, NY, USA) (well diameter: 22.1 mm), and then fungal suspensions (100 µl) were inoculated into the each well and incubated at 37°C for 90 min to settle the yeasts down to the specimen. After incubation, 1 ml of Sabouraud broth was dispensed into each well and incubated for 24 h at 37°C in humid conditions. Suspension (100 µl) was withdrawn through serial dilution, and viable cells (CFU, colony-forming unit) in the suspension were determined using the spread plate method at a level of detection within 500 CFU per plate. Assays were independently performed with three repetitive tests, and data were analysed by one-way ANOVA and Student's *t*-test at a significance level of 0.01.

Elution of Ag⁺ from the specimens

For this work, an atomic absorption spectrophotometer (Analyst 100; Perkin-Elmer, Boston, MA,

USA) and shaking incubator (SI-600R; Jeio Tech, Seoul, Korea) were used. Each disc specimen (20 × 3.0 mm, $n = 5$) was put into 100 ml of sterile distilled water and stored at 37°C under agitation. The value of Ag^+ elution from specimens was determined at two different periods, 24 h and 30th day, replacing distilled water daily. The quantity of Ag^+ was expressed as the amounts of Ag^+ in the solution per unit of surface area of the disc (cm^2), and the investigations were conducted in three independent experiments.

Thermogravimetric and differential thermal analysis (TG/DTA)

Thermal analysis (TG/DTA 320, SSC5200H; Seiko, Chiba, Japan) was performed under constant dried nitrogen gas flow at 100 ml/min. Specimens were scanned at constant heating rate (2°C/min) up from 20 to 800°C, respectively.

Evaluation of colour stability

To compare colour changing of Ag-denture acrylic with control, a spectrophotometer colour-reader (CR-10; Minolta, Tokyo, Japan) was used. Disc specimens (20 × 3.0 mm, $n = 5$) were measured in three randomly selected areas in reflectance mode against the white background of an opacity card at 24 h and 30th day from the onset of the curing process. The mean of three readings was recorded, and the value of each specimen was calculated using the CIE- $L^*a^*b^*$ -colour system²⁰ with a colorimeter. The tested samples were exposed to light within a wavelength range of 380–780 nm via a measuring head positioned at a right angle. For each specimen, three repeated measurements were taken to determine the colorimetric values, i.e. L^* (brightness), a^* (red-green proportion) and b^* (yellow-blue proportion). The differences to the zero value were calculated from the means of the colorimetric values ΔL^* , Δa^* and Δb^* . From that difference, the total colour difference ΔE for each sample was calculated using the following equation²¹:

$$\Delta E = (\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2$$

$$\Delta L^* = L_1 - L_0, \Delta a^* = a_1 - a_0, \Delta b^* = b_1 - b_0$$

L_1, a_1, b_1 : Ag^0 impregnated, L_0, a_0, b_0 : no Ag^0 (control).

Mean values and standard deviations were calculated from the total colour difference ΔE , and

differences were analysed by one-way ANOVA and Student's t -test at a significance level of 0.01.

Scanning electron microscope and energy dispersed X-ray (SEM/EDX) analysis

SEM/EDX (Hitachi S-4100 FE-SEM/EDS, Tokyo, Japan) analysis of Ag-denture acrylic was carried out in a field emission electron microscope attached to SEM at an accelerated voltage of 20 keV.

Results

The antifungal activities of Ag-denture acrylic against *C. albicans* were determined by the number of fungal cell recovered, and the results are shown in Fig. 1. When compared to control, a significant antifungal effect was shown at 20.0 and 30.0 wt% Ag incorporated; however, there were no statistical differences between the two concentrations ($p = 0.01$).

The MIC of silver cation against *C. albicans* in the present study was revealed to 3.0 mg/l, while the concentrations of eluted Ag^+ from the modified specimens were 0.176–0.356 mg/l at 24 h and 0.119–0.268 mg/l at 30th day (Table 1). Thermal property of Ag-denture acrylic specimens was investigated by TG/DTA (Fig. 2), compared to control, Ag-incorporated groups showed similar patterns of two-stage weight loss behaviour observed from 400 to 450°C (Fig. 2a) and similar heat absorption responses at the range of 360–390°C exhibiting vaporisation peaks as the temperature increased (Fig. 2b). SEM/EDX analysis indicated the typical signals of silver appearing, confirming the presence of Ag in the tested specimens. Figure 3a is a backscatter electron image of

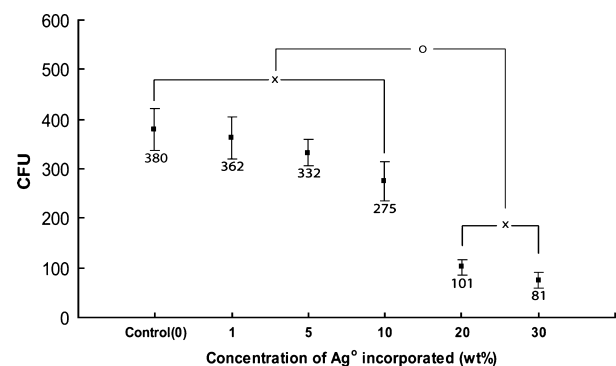


Figure 1 Mean viable cells (colony-forming unit) in retrieved fungal suspension according to each concentration of Ag^0 incorporated after 24 h incubation. O represents values significantly different and X represents values not significantly different ($p = 0.01$).

Table 1 The concentrations of eluted Ag⁺ from specimens at two designated intervals.

Groups (wt%)	Eluted Ag ⁺ (mg/l)	
	24 h	30th day
1.0	0.176 ± 0.04 ^a	0.119 ± 0.06
5.0	0.181 ± 0.08	0.124 ± 0.05
10.0	0.219 ± 0.08	0.192 ± 0.09
20.0	0.261 ± 0.12	0.243 ± 0.08
30.0	0.356 ± 0.11	0.268 ± 0.13

^aMean ± SD.

the morphology and surface composition of Ag-denture acrylic, and EDX spectrum showed the characteristic peaks for Ag at 3 keV (Fig. 3b). Table 2 shows that the values of colour differences (ΔE) from control range from 15.6 ± 0.69 to 28.6 ± 1.38 , and specimens combined with more than 10.0 wt% Ag⁰ showed statistically higher colour differences than 1.0 and 5.0 wt% at both intervals. No statistically different colour change was revealed between 24 h and 30th day ($p = 0.01$).

Discussion

As for the amount of fungal suspension to inoculate on the acrylic specimen, the oral microbe would appear to be in a stationary phase rather than in a growing phase, because nutrition is limited under the antibodies and the antimicrobial enzymes existing in the oral cavity²². The assays that were carried out with resin discs immersed in a large volume of microbial suspension could not reproduce *in vivo* dentures closely fitting the gingival mucosa^{23,24}, and microorganisms in suspension (planktonic phase) are sensitive to lower antiseptic concentrations than microorganisms colonising surfaces and protected by a biofilm such as *Candida*²⁵. In present microbial assay, using Ag⁰ directly polymerised in the denture material to be colonised with a limited volume of inoculum (100 μ l) confirmed the fungal inhibitory effects of Ag-denture acrylic at the concentrations of 20.0 and 30.0 wt%.

Ag⁺ elution from specimens were detected maximum 0.356 ± 0.11 mg/l in concentration of 30.0 wt% Ag⁰ incorporated at 24 h; although the value of each Ag⁺ elution was decreased slightly at the time of 30th day, prolonged and persistent releasing of Ag⁺ could be expected. However, given the MIC value (3.0 mg/l) of Ag⁺ against *C. albicans* in this experiment, we assumed that eluted Ag⁺

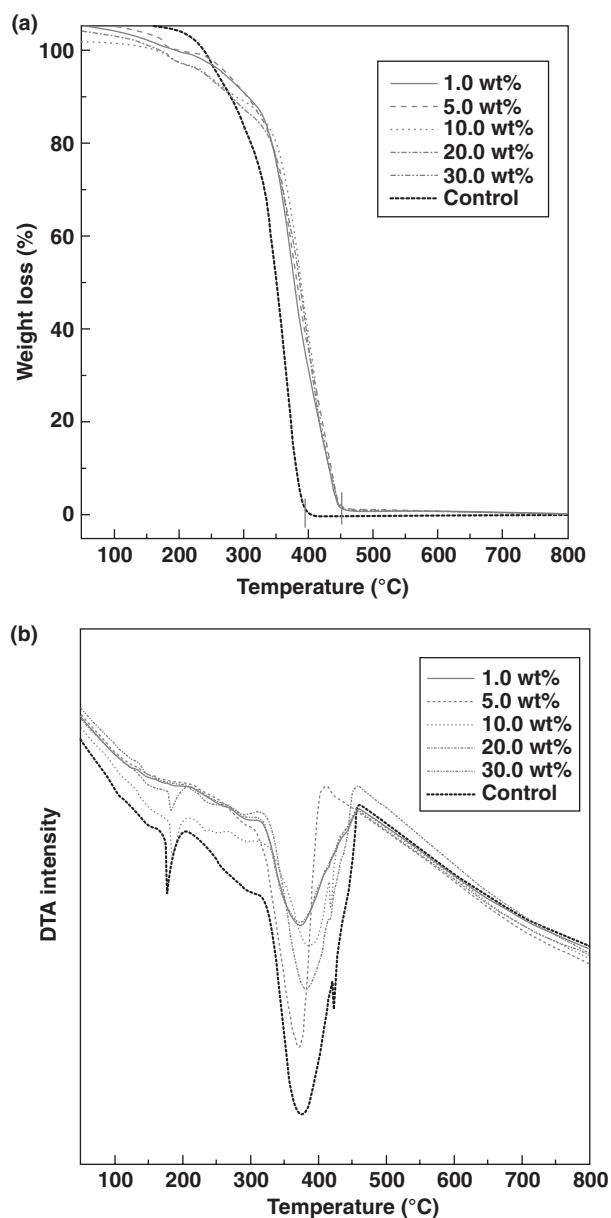


Figure 2 Results of thermogravimetric curves and differential thermal analysis thermograms of experimental groups. (a) Ag- incorporated groups showed two-stage weight loss behaviour from 400 to 450°C. (b) Similar pattern of vaporisation peaks at the temperature range of 360–390°C as compared to control.

from the specimens contributed little to the antifungal effect of modified denture acrylic, rather the effect could be explained as direct contact between Ag-denture acrylic surface and fungal cells at 20.0 wt% above. Similarly, a previous study demonstrated that the inhibitory effect of silver-supported (Novaron and Amenitop) restorative resin composite against *Streptococcus mutans* was

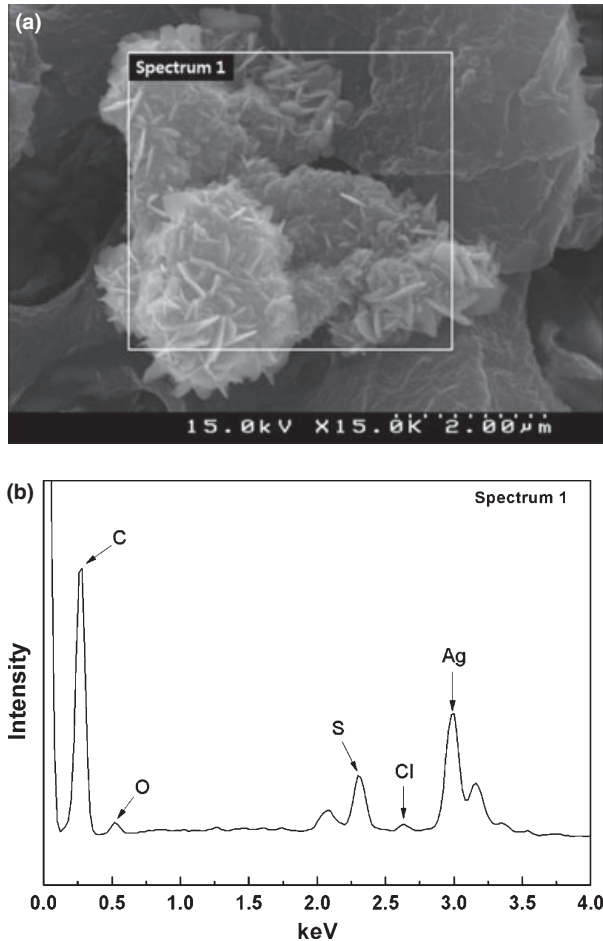


Figure 3 (a) SEM image of denture base acrylic with 20.0 wt% of silver nanoparticles ($\times 15\,000$). (b) In energy dispersed X-ray analysis of silver-loaded denture base acrylic, the spectrum shows the characteristic peaks for Ag at 3 KeV. Unassigned peaks originate from polymer or external contaminants.

Table 2 The colour differences of Ag-denture acrylic specimens with control at the time of 24 h and 30th day from the onset of curing process.

Groups (wt%)	ΔE	
	24 h	30th day
1.0	$15.6 \pm 0.69^{*a}$	15.9 ± 0.42^a
5.0	20.4 ± 0.60^b	21.0 ± 0.82^b
10.0	24.9 ± 0.62^c	25.2 ± 0.62^c
20.0	26.8 ± 0.82^c	27.4 ± 0.56^c
30.0	28.6 ± 1.38^c	28.2 ± 1.54^c

*Mean \pm SD.

Different superscript letters denote statistical differences within groups ($p = 0.01$).

gained with no or extremely little release of Ag^{+26} ; other experiments also revealed that when anti-septic agents such as quaternary ammonium com-

pound²⁷ and methacryloyloxydodecyl pyridinium bromide²⁸ were combined to solid resin material, the inhibitory effects on bacterial growth were because of not releasing these inorganic ions but the molecule of those compounds immobilised on the surface of the filler particle. Unlike fungicidal or inhibitory effects of tissue conditioner combined with silver-zeolite assayed by some studies^{23,29}, Ag^0 bound to the solid denture acrylic seemed difficult to diffuse out, and this limited mobility of the Ag covalently bound to the polymer backbone reduced their fungicidal activities²⁸ in the present study though it was clear that Ag appeared in EDX spectrum. The mechanism of the antimicrobial effect of the silver-supported compound has not been fully explained; it was suggested that as a result of the catalytic action of silver, oxygen is converted into active oxygen (including hydroxyl radicals) by the action of light energy and/or H_2O in the air or water only at polar surfaces and that this active oxygen causes structural damage in bacteria, the so-called 'oligodynamic action of silver.'³⁰

Thermal stability is a general term used to describe the potential changes of a material, and it has a significant implication as far as polymer fabrication processes are concerned³¹. Thermal analysis revealed that Ag-incorporated groups showed a higher decomposition temperature than the control, and incorporated inorganic silver might play the role of reinforcement; however, when compared to the control, Ag-denture acrylic showed stable thermal stability and a similar copolymerisation phase, suggesting that it is acceptable for clinical use as a modified denture acrylic base.

The colour difference (ΔE) of tested samples ranged from 15.6 ± 0.69 to 28.6 ± 1.38 compared with control, whereas a colour change is considered very small if ΔE is <1 ³²; it is clinically acceptable if the colour change is between 1 and 2. It is considered clinically perceptible if the ΔE is >3.3 ³³ and indicates a poor match and clinically unacceptable if the ΔE is >3.7 ^{34,35}. It is well known that discoloration is a common problem for silver-containing materials because of the oxidative reaction; therefore, further studies are required to investigate the improvement of colour stability for the clinical application possibility so as not to compromise the aesthetics of the prosthesis.

Resin voids and roughness of the fitting surface of denture base acrylic basically promote initial microbial adhesion. The mature denture plaque on the fitting surface is associated with a protective biofilm, and biofilm-related chronic infections, such as candidiasis, are inherently difficult to treat and fully eradicate with routine therapy²³. As

elderly denture wearer's ability to perform oral self-care declines, a demand for a simple, effective and preventive denture-care system is required³⁶. An agent with a modest antifungal effect, although not biocidal, may even be desirable to decrease the potential side effect of various agents²⁵. Further studies are still required if the cations may precipitate within the oral epithelial cell for a long period of time, possibly causing argyria and disruption of normal microflora^{37,38}.

The results of the present study implicate that Ag-denture acrylic might act as a low-releasing antifungal device and therefore could help geriatric denture wearers who have restricted manual dexterity or cognitive disturbances to improve their oral hygiene status.

Conclusion

Within the limitations of present *in vitro* study, the modified denture base acrylic combined with silver nanoparticles at 20.0 wt% displayed antifungal properties and synthesis of Ag-denture acrylic resulted in appropriate physical characteristics through the thermal and EDX analysis when compared to unmodified denture acrylic. However, the improvement of colour stability is now required for clinical use.

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Correspondence to:

Ki-Young Nam, Department of Dentistry, College of Medicine, Keimyung University,
194 Dongsan-dong, Jung-gu, Daegu, Korea.
Tel.: 82 53 250 7807
Fax: 82 53 250 7802
E-mail: nkyp@dsmc.or.kr