Effects of Silver Nanoparticle Coating on Surface Alterations, Particle Characteristics, and Bacterial Adhesion to the PMMA Surface

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Abstract

In this study, we aimed to compare the effects of different silver nanoparticle (AgNP)-coating protocols on the surface alterations, particle characteristics, and bacterial adhesion to polymethylmethacrylate (PMMA) surfaces.

Three AgNP-coating protocols, including simple immersion, ultrasound irradiation, and microwave irradiation, were compared. PMMA disks were prepared for each method; thereafter, pre- and post-coating surface roughness values (n = 6) were compared. AgNP characteristics were also evaluated using field-emission scanning electron microscopy. Additionally, PMMA disks were divided into five groups, i.e., negative control, positive control, simple immersion, ultrasound irradiation, and microwave irradiation (n = 10). The specimens were incubated in a Staphylococcus aureus suspension at 37 °C for 3 h, following which bacterial adhesion was analyzed by counting colony-forming units (CFUs). Differences in surface roughness were observed between pre- and post-coating conditions as well as between methods. The simple immersion method generated small AgNPs (size, 10 nm; regularly distributed nanospheres). In comparison, AgNPs generated by ultrasound and microwave methods were agglomerated nanospheres with sizes between 10–400 nm and 100–1,000 nm, respectively.

Hence, differences in bacterial adhesion resulted from differing AgNP-coating methods, compared to the control. Based on CFUs, simple immersion caused a superior reduction in bacterial adhesion compared with ultrasound and microwave irradiation (P-values = 0.03 and 0.00, respectively). However, no differences were observed in bacterial adhesion between the ultrasound and microwave methods. In summary, the findings herein indicate that different AgNP-coating methods affect surface roughness and AgNP characteristics, leading to differences in bacterial adhesion. Simple immersion resulted in the most significant reduction in bacterial adhesion. Development of denture base material via AgNP coating might help reduce microbiological complications and enhance longevity for improving the quality of life of denture wearers.

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Introduction

Polymethylmethacrylate (PMMA) is an acrylic resin that is preferentially used as a denture base material due to its low cost and excellent physical properties;[1] however, it has certain inherent structural limitations.[2] These limitations result in fluid absorption that deteriorates denture durability and causes dimensional instability.[3, 4] A combination of fluid absorption, microporosity, and abraded rough surfaces of aged PMMA bases promotes bacterial adhesion and biofilm formation.[5] Removing microorganisms and biofilms from the denture base is vital for reducing microbiological complications and enhancing its longevity.[1]

Silver nanoparticles (AgNPs) have been reported to reduce the accumulation of intraoral microbial plaque on PMMA surfaces;[2, 6, 7] yet, knowledge regarding nano-synthesis and mixing of an appropriate AgNP dose into a denture polymer is lacking. However, despite the different mixing methods, an AgNP coating solution developed by either ultrasound[8] or microwave irradiation[9] can have significant practical clinical applications.[10] Additionally, the combination of AgNPs with denture base material can alter the surface and intrinsic properties of PMMA.[11-13] Specifically, nano layering techniques that produce smaller nanoplate-shaped particles with regular distribution of silver exert a superior antimicrobial effect.[14-16] However, the interrelationship between nanoparticles on the PMMA surface and potential microbial plaque inhibition efficacy appears to be associated with PMMA surface improvement.[7]

Considering that the bacterial adhesion efficiency and characteristics of different AgNPs, created by various coating methods, are not well described, the current study aimed to develop antimicrobial PMMA surface coatings with optimized AgNP geometry and denture base materials with minor surface alterations. The null hypothesis was that different AgNP-coating methods would not differ with respect to the PMMA surface, AgNP characteristics, and adhesion of bacteria on PMMA surfaces.

Materials and methods

2.1 Preparation of self-curing PMMA disks

A self-curing acrylic resin used to prepare the denture base mixture (SR Triplex Cold; Ivoclar Vivadent, Liechtenstein) was poured into a cylindrical putty silicone mold (Amcoflex; Amcorp, San Antonio, USA), placed on the first glass slide, forming a disk-like specimen with a diameter of 5 mm and thickness of 2 mm at a mixing ratio of 23.4 g:10 mL. The excess was removed, the uncured surface was covered with a second glass slide and pressed using a 40 kg weight for 15 min. Sixty-eight acrylic resin disks with smooth surfaces were prepared (Fig. 1).

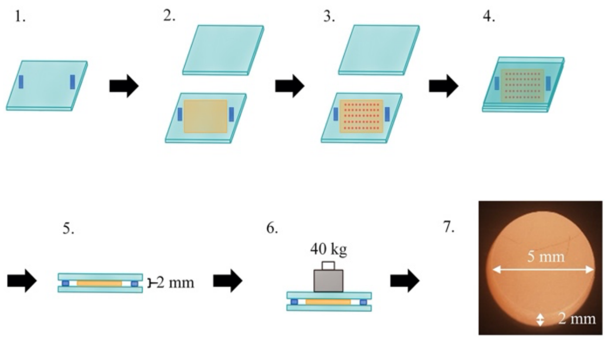

Figure 1. Schematic of putty silicone molds and self-curing acrylic (polymethylmethacrylate (PMMA)) disk preparation. (1)-(3) Preparation of 5-mm round symmetrical holes on 2-mm flat silicone. (4) Filling of the molds with hand-mixed self-curing PMMA. (5)-(6) Repressing via a second glass plate, loaded with 40 kg weight for 15 min. (7) Archived 5-mm acrylic resin disk with flat smooth surfaces and controlled 2 mm thickness.

2.2 Preparation of AgNP coating solution (AgNP solution)

The solution mixture[9] contained silver nitrate 0.85 mL (Merck KGaA, Darmstadt, Germany), 50% polyethylene glycol (PEG) 10 mL (Merck KGaA), 28% aqueous ammonia 0.7 mL (Merck KGaA), 70% ethanol 10 mL, and sterile water 30 mL. Every step was performed in a controlled environment following the laboratory safety guidelines of the Naresuan University Institutional Biosafety Committee.

2.3 AgNP coating on the surface of acrylic disks

Forty-eight prepared PMMA disks were divided into three experimental groups using the following coating methods: (1) simple immersion - the acrylic resin disk was immersed in the AgNP solution for 2 h; (2) ultrasound irradiation - the acrylic resin disk was immersed in the AgNP solution and subjected to ultrasound irradiation for 2 h (CPX130; Cole-Parmer, IL, USA, 30 kHz); (3) microwave irradiation - the acrylic resin disk was immersed in the AgNP solution and subjected to microwave irradiation for 5 min (MS2022D; LG, Thailand, 200 watts); the disk was subsequently left in the solution for 2 h.

2.4 Analysis of PMMA surface alteration and characterization of AgNPs

The average surface roughness (Ra) of PMMA was evaluated using six randomly selected specimens (n = 6) before and after

AgNP coating via atomic force microscopy (FlexAFM5; Nanosurf, Liestal, Switzerland). Pre-coated surface roughness was also used as the baseline to compare the degrees of alteration induced by simple immersion, ultrasound irradiation, and microwave irradiation, together with a comparison of the average achieved Ra values between each pair group of the coating protocols.

Additionally, AgNPs were characterized using two randomly selected specimens from each of the other groups via field-emission scanning electron microscopy (SEM, Apreo2S; Thermo Scientific, Massachusetts, USA) followed by elemental analysis using energy-dispersive X-ray spectroscopy (EDS, Ultim Max; Oxford, High Wycombe, UK), which compared the size, shape, and distribution patterns, while confirming the presence of silver in each coating method.

2.5 Analysis of bacterial adhesion on PMMA surface

2.5.1 Preparation of bacterial culture solution

The Staphylococcus aureus (ATCC25923) suspension solution was prepared in brain heart infusion (BHI) culture media (Difco, New Jersey USA) and adjusted to a Mcfarland factor of 0.5 (absorbance at 625 nm).

2.5.2 Bacterial adhesion assay and analysis

Before the adhesion test, specimens were rinsed with sterile water, dried at room temperature (25–27 °C), packaged in aluminum foil, and autoclaved (SA-300VF; Sturdy, Taipei, Taiwan) at 121 °C for 15 min.

Five groups of 50 PMMA disks (n = 10) were prepared as described in Fig. 2. Bacterial adhesion was achieved by placing each positive control and experimental specimen in a 5 mL tube containing 1 mL of S. aureus suspension (10^6 cells/mL) and incubating (1535-2E; Shellab, Oregon, USA) at 37 °C for 3 h. Specimens were then removed from the S. aureus suspension, rinsed with phosphate-buffered saline (PBS) to remove non-adherent S. aureus, and transferred to new, sterile test tubes, each containing 1 mL of PBS. Adherent S. aureus was subjected to ultrasonic vibration (Sonorex Super 10 P; Bandelin, Berlin, Germany) for 10 min, followed by vortexing (VM 300; Gemmy, Taipei, Taiwan) for 1 min.

A 100-μL solution from each test tube was inoculated on BHI agar via the spread plate method and incubated at 37 ℃ for 24 h. S. aureus adhesion to the PMMA surface was quantified by counting colony-forming units.

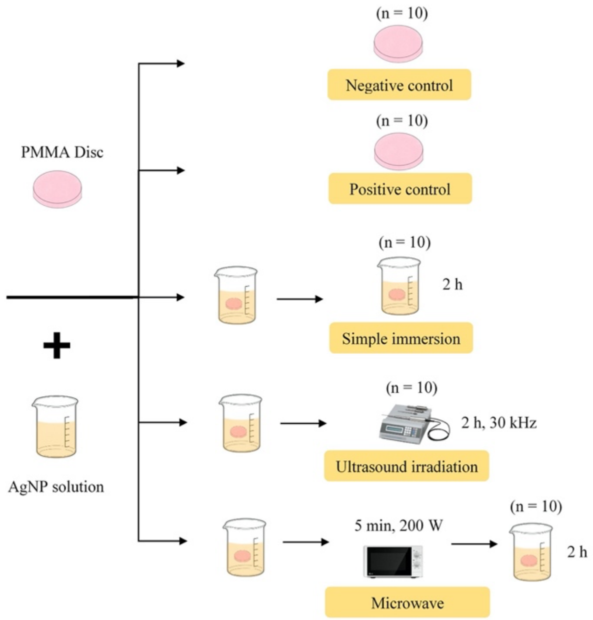

Figure 2. Grouping of the control and experimental groups for analysis of bacterial adhesion on the polymethylmethacrylate surface. AgNP; silver nanoparticle.

2.6 Statistical analysis

The sample size was calculated using the G*power program (Preface by Russell V. Lenth, Version 3.1.9.4, Iowa, USA). Means and standard deviations were retrieved from the literature for the statistical analysis of surface roughness[17] and bacterial adhesion.[7] Based on the formula of the paired t-test and t-test with a significance level of 5% and a power of 95%, a sample size of at least three and five specimens per group was required to detect a difference in PMMA surface roughness and bacterial adhesion, respectively. Thus, the sample size in this study was set at six specimens per group for investigating surface roughness and ten specimens for investigating bacterial adhesion.

Statistical calculations were performed using SPSS (IBM SPSS Statistics for Windows, Version 23.0. IBM Corp, Armonk, NY). The means and standard deviations were calculated for all parameters. Shapiro–Wilk and Levene's tests were used to determine normality and homogeneity of variances, respectively. Statistical significance was set at 0.05. The paired t-test was utilized to compare surface roughness before and after AgNP coating, for each group. Furthermore, the simple t-test was used to compare achieved surface roughness and bacterial adhesion between each pair of the coating protocols.

Results

3.1 Surface roughness

Descriptive statistics of surface roughness are shown in Table 1. From the paired t-test, it was found that the simple immersion method produced a smoother surface than the pre-coated group (P-value = 0.02), while ultrasound and microwave irradiation methods provided a rougher surface than the pre-coated groups (P-values = 0.00 and 0.01, respectively) (Table 1).

Coating protocol	Group	Sample size (n)	Mean (nm)	Standard deviation (nm)	P-value	95% Confidence interval	
						Lower bound	Upper bound
Simple Immersion	Pre-coated	6	22.45	5.69	0.02*	1.7490	4.6243
	Coated	6	19.26	5.29			
Ultrasound irradiation	Pre-coated	6	22.07	7.76	0.00*	−10.0171	−6.0162
	Coated	6	30.09	6.91			
Microwave irradiation	Pre-coated	6	22.34	6.89	0.01*	−21.3526	−10.2807
	Coated	6	38.16	5.69			

Table 1. Comparison of the surface roughness between before and after silver nanoparticle coating. *Significantly different with the paired *t*-test (P-value <0.05).

Coating protocol		P-value	95% Confidence interval	
			Lower bound	Upper bound
Coated simple immersion	Coated ultrasound irradiation	0.01*	−18.7443	−2.9157
	Coated microwave irradiation	0.00*	−25.9637	−11.8330
Coated ultrasound irradiation	Coated microwave irradiation	0.00*	−17.1512	−4.1121

Table 2. Comparison of the surface roughness after silver nanoparticle coating between each pair of the coating protocols. *Significantly different with the simple *t*-test (P-value <0.05)

Among the three coated groups, the simple t-test indicated that the achieved Ra values of the coated surfaces by simple immersion, ultrasound irradiation, and microwave irradiation were significantly different (P-values = 0.01, 0.00, and 0.00, respectively) (Table 2). The results confirmed that the PMMA surface was altered by coating and rejected this study's null hypothesis.

3.2 Characterization of AgNPs

The three different coating methods created coated nanosurfaces with different AgNP characteristics. SEM images combined with elemental analysis via EDS of the control (pre-coated) PMMA surface (Fig. 3a and 3b), showed the surface geometry and smoothness of the polished PMMA surface and the absence of silver. Simple immersion groups displayed fine nanospheres (~10 nm) that were regularly deposited on the PMMA surface, as well as silver (47 wt%; Fig. 3c and 3d). Under ultrasound irradiation, AgNPs deposited on the PMMA surface mixed with small silver nanospheres (~10 nm) to form approximately 400 nm agglomerates, yielding a higher weight percentage of silver compared to simple immersion (62.4 wt%; Fig. 3e and 3f). Under microwave irradiation, fine, densely distributed AgNPs ranging from ~100 nm to ~1000 nm were regularly deposited on the PMMA surface, yielding the highest amount of silver (67.5 wt%; Fig. 3g and 3h). The results confirmed that the AgNP characteristics differed by coating methods and rejected this study's null hypothesis.

3.3 Bacterial adhesion

Descriptive statistics of bacterial adhesion are shown in Table 3. The simple t-test indicated significant differences between the bacterial adhesion levels of the positive control group and experimental groups (Table 4). All three coating methods significantly reduced bacterial adhesion (Fig. 4).

Group	Sample size (n)	Mean (CFUs)	Standard Deviation (CFUs)
Negative control	10	.00	.00
Positive control	10	56.00	11.86
Simple immersion	10	3.60	.90
Ultrasound irradiation	10	7.30	1.34
Microwave irradiation	10	11.80	2.38

Table 3. Descriptive statistics of bacterial adhesion by counting colony-forming units (CFUs).

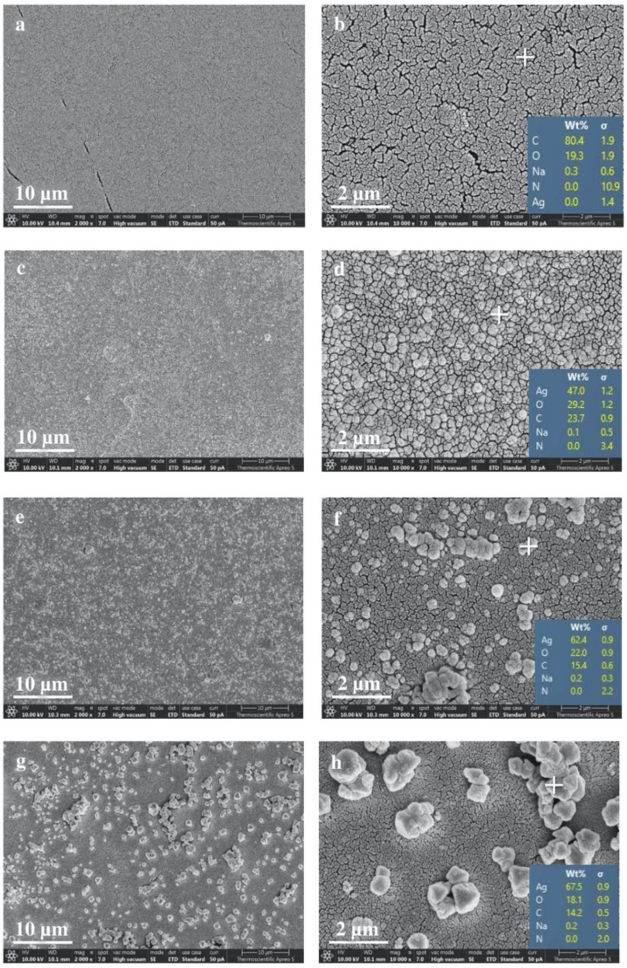

Figure 3. Scanning electron microscopy images at low and high magnification and the combination of the elemental analysis from the energy-dispersive X-ray spectrum show characteristics of the three disks developed using the silver nanosurface coating methods. (A, B) Surface of uncoated polymethylmethacrylate observed at 2000X and 10,000X; (C, D) simple immersion, (E, F) ultrasound irradiation, and (G, H) microwave irradiation.

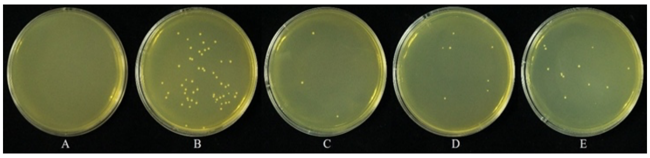

Figure 4. Macroscopic observation of *S. aureus* on brain heart infusion agar after incubation at 37 ℃ for 24 h. (A) Negative control shows the absence of *S. aureus* colonies; (B) Positive control; (C) Simple immersion; (D) Ultrasound irradiation. (E) Presence of *S. aureus* colonies in the microwave irradiation experimental group.

There were significant differences between bacterial adhesion levels corresponding to simple immersion compared with ultrasound irradiation and microwave irradiation (P-values = 0.03 and 0.00, respectively). In contrast, no significant differences were observed between the ultrasound and microwave irradiation groups (P-values = 0.12). These results partially rejected the null hypothesis regarding the adhesion of bacteria on PMMA surfaces.

Group		P-value	95% Confidence interval	
			Lower bound	Upper bound
Positive control	Simple immersion	0.00*	27.400	77.400
	Ultrasound irradiation	0.00*	23.614	73.786
	Microwave irradiation	0.00*	18.778	69.622
Simple immersion	Ultrasound irradiation	0.03*	−7.106	−0.294
	Microwave irradiation	0.00*	−13.543	−2.857
Ultrasound irradiation	Microwave irradiation	0.12	−10.231	1.231

Table 4. Comparison of the bacterial adhesion (colony-forming units) between each pair of the coating protocols and the positive control. *Significantly different with the simple *t*-test (P-value <0.05).

Discussion

This study compared surface antimicrobial activity associated with different silver nanosurface properties of acrylic denture base material developed using simple immersion following three AgNP-coating protocols. These different techniques appear to have affected the surface roughness of coated PMMA (~3.19–15.82 nm). However, such differences were within a nanoscale range and were not clinically relevant in plaque accumulation or denture base adaptation. Reportedly, Ra values above 200 nm significantly increase plaque formation.[18-20]

The characteristics of coated AgNPs, such as small size (approximately 10 to 500 nm), and regular distribution, resulted in a superior antimicrobial effect due to the larger surface area available for contact.[14, 15, 21, 22] Comparatively, microwave irradiation produced a particle size, which was not only outside the range recommended[14, 22] for effective antimicrobial activity but also responsible for the roughest surface coating. Thus, in terms of acceptable particle size and minor surface alteration, the simple immersion method may theoretically be the most suitable for developing a PMMA contact surface for intraoral use.

However, in this study, we prepared the coating solution following Irzh's study[9] and used

PEG instead of ethylene glycol (EG) as a stabilizing agent because the higher viscosity of PEG could delay the growth of AgNPs and produce a smaller particle size than that produced by EG. Temperature shifts caused by differences in microwave power may be an additional factor affecting the size of the resulting AgNPs as well as the agglomeration of these particles.[23]

Bacterial adhesion is a complex process that can be affected by material properties, including surface roughness, hydrophobicity, and the charge of the material surface also affect biofilm formation.[24-27] We found that different AgNP-coating methods resulted in a different nanosurface roughness of PMMA, producing a surface roughness change from −13.45% for simple immersion to +70% for microwave irradiation compared to the precoating condition.

For bacterial adhesion assessment, percent microbial reduction relating to the coated silver nanosurface characteristics was calculated. The percentage of microbial reduction in the current present study for the three coating methods ranged from 78.8%–93.57%. Alternatively, previous studies using AgNPs mixed in PMMA reported the percentage of microbial reduction to be in the range of 40.74%–83.33%.[11, 28, 29] However, one of these previous studies also reported that the adverse mechanical consequences, resulting from the mixing method with more than 1% wt of AgNPs, significantly reduced flexural strength.[11] Therefore, the surface coating techniques developed in the current study could be more effective as surface disinfectants.

The current study was beset by certain limitations. First, only one type of PMMA was used, while other PMMA suppliers may use more complex chemical compositions and various additives to produce a wide range of polymer-based products. This chemical dissimilarity may alter the charge on the pre-coated surface and directly affect the binding efficacy of AgNPs.

Moreover, surface roughness was the only factor controlled and investigated here. The investigation of other surface parameters, such as hydrophobicity, charge, and energy, requires special devices and techniques far beyond our scope. To better understand and correctly measure complex bacterial adhesion, all factors related to bacterial and material surface properties need to be standardized and controlled in further studies.

However, the simple immersion method proved to have the highest potential for use in the development of innovative antimicrobial dental prostheses with minor surface alterations and consistent fine particles, which effectively reduce bacterial adhesion. Further studies should expectedly focus on evaluating the biocompatibility, toxicity, and coating solution stability, as chemical reactivity may decrease over time, particularly after cleaning with solvents or mechanical cleaning devices.

Conclusions

Within certain limitations, the following conclusions can be drawn from the findings of the current study: (1) the PMMA surface and the size, shape, and distribution of the AgNPs on a PMMA surface may differ, depending on the coating method used; (2) bacterial adhesion to the PMMA surface corresponding to the selected AgNP-coating methods differed from that of the controls; (3) the simple immersion method resulted in the most significant bacterial reduction. Therefore, in the future, denture base material development by AgNP coating might be a good option to reduce microbiological complications and enhance longevity for improving the quality of life of denture wearers.

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Declaration of Interest

The authors report no conflict of interest.

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