Effect of Silver Nanoparticles on Antimicrobial Property of Acrylic Denture Base

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Abstract

Objective: To investigate the effect of silver nanoparticles on the antimicrobial property of the acrylic denture base with different ratios of silver nanoparticles.

Materials and Methods: Heat polymerizing acrylic resin (Triplex hot Ivoclar vivadent, Liechtenstein) and silver nanoparticles (Zeomic AJ10N Sinanen Zeomic Co., Ltd., Japan) were used. The specimens were divided into four groups according to the concentration of silver nanoparticles incorporated to acrylic resin: 0, 0.25, 0.5 and 1.25% by weight. The antimicrobial activity against Staphylococcus aureus was assessed by Japanese Industrial Standard (JIS Z 2801:2000). The dispersion of silver nanoparticles was evaluated by Scanning Electron Microscope (SEM).

Results: This study showed that only 0.5% and 1.25% silver-nano containing acrylic can against Staphylococcus aureus, assessed by JIS Z 2801:2000. SEM images confirmed the presence of silver-nano embedded to the polymer matrix. Nanoparticles are homogeneously dispersed over the specimen surface.

Conclusions: The addition of silver-nano to acrylic resin at least 0.5% by weight revealed antimicrobial activity.

Keywords: Silver nanoparticles, Acrylic denture base, Antimicrobial property

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Introduction

The major role of denture base is to support tooth and transfer the mastication force to supporting structure such as natural tooth, soft tissue or implant. The crucial property of the denture base is rigidities moreover no distortion or changing shape when using for a long time. Materials which widely used in denture base are acrylic resin, metal and nylon. Nowadays, acrylic resin is mostly used for denture base [1]. Dr. Walter Wright (1937) introduced polymethyl methacrylate (PMMA) as a denture base material which became the major polymer to be used in the next ten years [2]. This material has been divided into two group based on the method of activation; heat-activated PMMA and chemically activated PMMA. Heat-activated PMMA that mostly use for denture base supplied in powder-liquid form. The powder contains PMMA beads along with benzoyl peroxide (Initiator), dibutyl phthalate (plasticizer), pigments and opacifiers. The liquid contains PMMA monomer with hydroquinone (inhibitor), glycol dimethacrylate (cross-linking agent) and plasticizers [3]. The major problems when patients used acrylic dentures for a long time that have the accumulation of *Candida albicans* and *Streptococcus mutans* especially at the border of the dentures [4]. Therefore, one of goals in acrylic dentures used should be to induce anti-microbial capability in these appliances.

Among various anti-microbial agents, silver and silver-nano have been most extensively studied and used for antibacterial appliances especially in medical appliances that use contact with human body including tissue surface. Silver is a safer anti-microbial agents in comparison with some organic anti-microbial agents that have been avoided because of the risk of their harmful effects on the human body. Silver has been described as being “board spectrum” anti-microbial agents because of its therapeutic property has been proven against a broad range of micro-organisms, over 650 disease-causing organisms in the body even at low concentrations [5]. In addition, silver nanoparticles also prevent bio-film formation. Silver nanoparticles developed from silver with nanotechnology that improved the antimicrobial property of silver nanoparticles. Silver nanoparticles have made to increase in number of particles per unit area so that anti-bacterial effects have increased. Furthermore, silver nano-particles are a non-toxic, non-tolerant disinfectant, bio-compatibility, high hydrophilicity and acceptable solubility in water and most organic solvent. Good processability has made it suitable for a wide variety of applications especially in bio-medical fields [6].

Anti-microbial mechanism of silver nanoparticles can be explained by metal ions destroy or pass through the cell membrane and bond to the –SH group of cellular enzymes. The enzymatic activity of micro-organisms is decrease from metabolisms change and inhibits their growth, and then the cell is death [7]. Another anti-microbial mechanism is the metal ions also catalyze the production of oxygen radicals that oxidize molecular structure of bacteria. This mechanism does not need any direct contact between anti-microbial agent and bacteria because the produced active oxygen diffuses from fiber to the surrounding environment. Silver ions can forming R–S–S–R bonds cause respiration blocking and microorganisms cell death [6, 7].

Nano-silver modified surface preparation is sol-gel and coating method. Co-sputtering method is improved from sputtering method that improved isotropic thinner film of silver nanoparticles. Thin films co-sputtering coating method is divided into group co-sputtering and radio-frequency co-sputtering method [8, 9].

Concept of anti-microbial agents developing in dentistry example used with alginate impression material [10], composite resin filling material [11-13] and implant [11-14]. Ferraris et al. (2010) [9] have reported silver nanocluster–silica composite coatings are able to produce a significant inhibition halo towards *Staphylococcus Aureus* stock by method RF co-sputtering glasses and other surfaces by antibacterial thin films. Moreover, Li et al. (2009) [11] stated that the addition of microparticulate silver to a resin composite material increased the surface hydrophobicity and reduced the number of adhering *Streptococci*. Simultaneously it increased the percentage of dead and inactive cells on the composite surface. Thus, silver additives seem to demonstrate anti-adherence activity as well as a bactericidal effect. Chen et al. (2006) have developed hydroxyapatite/silver composite coating that has been designed particularly for reducing bacterial infections (*Staphylococcus epidermidis* and *Staphylococcus aureus*) after implant placement and founded no significant difference on toxic to human cells and surface roughness [12]. Flores et al. (2010) also reported that the modified AgNP- Ti/TiO2 surface shows a good resistance to colonization by Pseudomonas aeruginosa in a model system for biofilm formation [13]. In addition, anti-microbial agents also use in polymer such as poly (styrene-co-acrylic acid). da Silva Paula et al. (2009) have reported the influence of nano-silver introduction into poly(styrene-co-acrylic acid) copolymer on antibacterial activity. They believed that the carboxylic groups of acrylic acid lead to increased ionic mobility in the copolymer responsible for the enhanced antibacterial surface activity of the copolymer [15]. The addition of silver nanoparticles would be beneficial to polymeric denture base materials such as self or heat curing acrylic resin in order to enhance the antimicrobial property.

The aim of this study was to investigate the effect of silver nano on the antimicrobial property of the acrylic denture base (PMMA) with different ratios of silver nanoparticles.

Materials and methods

In this study, heat polymerizing acrylic resin (Triplex hot Ivoclar vivadent, Liechtenstein) and silver nanoparticles (Zeomic AJ10N Sinanen Zeomic Co., Ltd., Japan) were used. The composition of silver nanoparticles is $M_2/n\ O\text{-}Al_2O_3\text{-}2SiO_2\text{-}XH_2O$; M: Ag, Zn, NH_3, Na; n valence of M; X: integral number.

Powder and silver nanoparticles were mixed with simple blending method and placed in stainless steel mold at the dough stage of polymerization in 3 concentrations of 0.25, 0.5 and 1.25% by weight. The specimens prepared for antimicrobial activity test were divided into four groups, group 1; heat polymerizing acrylic resin containing 0% siver-nano (control group), group 2; heat polymerizing acrylic resin containing 0.25% siver-nano, group 3; heat polymerizing acrylic resin containing 0.5% siver-nano and group 4; heat polymerizing acrylic resin containing 1.25% siver-nano.

The antimicrobial efficacy was evaluated by means protocol of the Japanese Industrial Standard method (JIS Z 2801:2000). Mid-log phase culture in Muller Hilton (MH) broth of Staphylococcus aureus ATCC 6538p were centrifuged at 1000g for 5 min and resuspended in phosphate buffered saline (PBS). Incubate the test piece inoculated with the test inoculums at a temperature of 35°±1°C and relative humidity of not less than 90% for 24h±1h. After incubation, the numbers of colonies were counted in a serially diluted petri dish in which 30 to 300 colonies appear. Determination the number of viable cells of bacteria by the counts of colonies according to the formula: N = C x D x V where, N; number of viable cells of bacteria (per test piece), C; number of colonies (average of the number of colonies in two petri dishes adoped), D; dilution ratio (dilution ratio of the diluted solution dispensed into petri dish adopted), V; volume (ml) of the SCDLP broth used for washing out. SEM images were recorded by means of a PHILIPS JSM-5410LV scanning microscope operating at 15 kV. Elemental analysis in each specimens were evaluated by X-ray fluorescence Spectrometer; Phillips PW-2404 with semi-quantitative at temperature 25±5°C and relative humidity 60±10% R.H.

Results

SEM images confirmed the presence of silver-nano attached to the polymer matrix. Nanoparticles are homogeneously dispersed over the specimen surface. The average size is about 1 μm but occasionally both larger (up to 30 μm) and smaller can be found (Fig. 1).

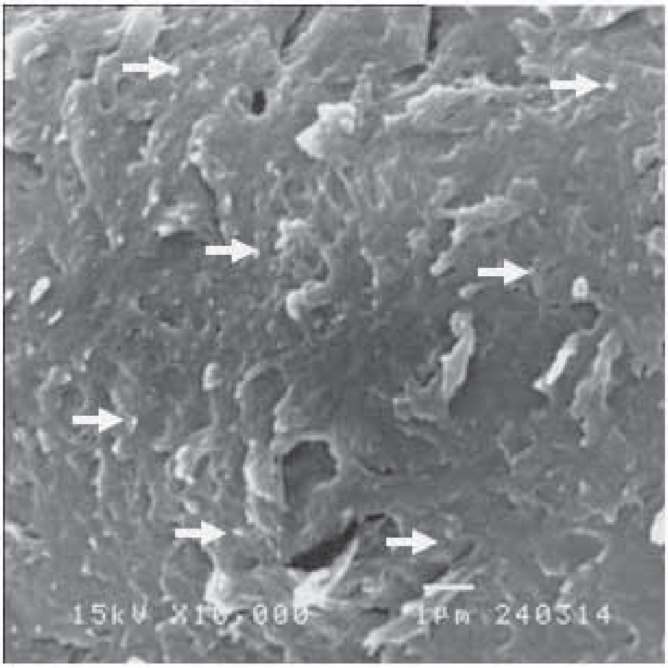

Figure 1. Scanning electron microscope of PMMA-silver nanoparticles showing a homogeneous silver nanoparticles distribution (white arrows) in the PMMA polymer matrix (Bar = 1 μm).

Antimicrobial efficacy tests were carried out with Staphylococcus aureus ATCC 6538p. After 24 h of incubation, the bacteria were detached from the samples and the number of colony forming units (CFU) ml-1 was evaluated. When the test has been effective, calculate the value of antimicrobial activity according to the formula: R = log (B/C) where, R: value of antimicrobial activity, A: average of the number of viable cells of bacteria immediately on the untreated test piece, B: average of the number of viable cells of bacteria on the untreated test piece after 24 h and C: average of the number of viable cells of bacteria on the antimicrobial test piece after 24 h. Antimicrobial activity of the heat polymerizing acrylic resins containing different percentages of silver-nano are shown in Table 1. None of the control groups showed antimicrobial activity. For all specimens, heat polymerizing acrylic resin containing 0.5% siver-nano (group 3) and heat polymerizing acrylic resin containing 1.25% siver-nano (group 4) were enough to express antimicrobial activity against the test strains.

Table 1. Antimicrobial activity of heat polymerising acrylic resins containing different percentages of silver-nano by JIS Z 2801:2000

Specimens	Strains Staphylococcus aureus ATCC6538p
Antimicrobial activity after incubation 24 h	
Heat polymerizing acrylic resin containing 0.25%	-0.1
Heat polymerizing acrylic resin containing 0.5%	3.4*
Heat polymerizing acrylic resin containing 1.25%	3.5*

* The superscripted symbol means positive to antimicrobial effect.

Discussion

Antimicrobial efficacy tests were carried out with Staphylococcus aureus ATCC6538p. evaluated by JIS Z 2801:2000 tests which is widely used in antimicrobial test of medical and dentistry materials [16-19]. After that, the number of colony forming units (CFU) ml-1was evaluated. Antimicrobial activity in group of heat polymerizing acrylic resin containing 0.5% silver-nano and group of heat polymerizing acrylic resin containing 1.25% silver-nano indicated that bacterial strains were effectively inactivated by addition of silver nanoparticles. It should be noticed that the antimicrobial activity of silver-nano is not impaired by their incorporation in the polymer layer. The

0.5% and 1.25% silver-nano should be added to acrylic resin for antimicrobial activity. These finding are similar to the previous studies that assessed the incorporation of silver-nano into resin composite [9] or into poly-(styrene-acrylic acid) polymer matrix [15]. Nevertheless, the antimicrobial activity of silver-nano against other microorganisms involved in denture stomatitis, a common problem of denture wearers [20], should be investigated. Moreover, this study evaluated the short-term (24 h) antimicrobial activity of materials and hence their long-term action should also be evaluated. It seems that addition of silver-nano to heat polymerizing acrylic resin for antimicrobial activity may be advantage; however, there should be evaluation of its effect on the other physical and mechanical properties of acrylic resin.

It is important to confirm that the potential cytotoxicity of dental materials is minimal but the hazardous and toxic effects of silver nanoparticles have not been studied extensively. Acosta Torres et al. (2012) reported that the PMMA-silver nanoparticles showed biocompatible behavior in that they do not influence cell proliferation when observed in the MTT assay including 24 hours and 72 hours of exposure. Moreover, genotoxicity was assessed using human lymphocytes for the comet assay and the results suggested no toxicity to cells. Therefore, PMMA-silver nanoparticles are suitable for producing nontoxic materials with antimicrobial properties [21].

In the clinical application, dentures with antimicrobial properties could improve the oral health of elderly patients and prevent denture stomatitis. Further study may focus on the addition of silver nanoparticles into different materials such as soft liner. Furthermore, the mechanical properties should also be evaluated to ensure the effect of silver nanoparticles on polymeric prosthetic material.

Conclusion

Within the limitation of this study, it can be concluded that heat polymerizing acrylic resin containing 0.5% silver-nano has the most beneficial for addition of silver nanoparticles into acrylic denture base because of antimicrobial effect.

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