Evaluation of the antimicrobial activity of heat-cure denture base resin materials incorporated with silver nanoparticles

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INFORMATION

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ABSTRACT

Background: Poly (Methyl methacrylic acid) based materials are the most widely used for the fabrication of removable complete and partial dentures. Certain microorganisms adhere to the tissue surface of a denture base, especially on palatal region, often leading to Denture stomatitis. Numerous attempts were made to treat the denture stomatitis with various antifungal agents showing variable success rates. This may be attributed to the loss of the drug rapidly into the saliva, inhomogeneous distribution of the drug and the development of resistance to antifungal therapy.

Aim: This study was done to evaluate the effect of incorporating various concentrations of silver nanoparticles on the antimicrobial activity of heat-cure denture base resin materials.

Materials and methods: Silver nanoparticles were incorporated at various concentrations (0.5, 1.0, 2.0 and 5.0 wt%) into three heat-cure denture base materials. A total of 300 disc-shaped specimens (10 × 2 mm) of heat-cure acrylic resin were made using compression molding technique which comprises 100 specimens with each denture base material. Fifty specimens from each denture base materials were allocated to each microorganism used in the study which comprises into five groups with ten specimens (n=10) for each concentration such as control, 0.5wt%, 1.0wt%, 2.0wt% and 5.0wt% concentrations of silver nanoparticles. Antimicrobial activity of control and modified specimens were evaluated using direct contact method against *C albicans,* and *S Mutans* by counting the number of colony-forming units. The data were subjected to One way ANOVA and Tukey HSD tests for statistical analyses.

Results: Significant($P<0.05$) differences were observed in the antimicrobial activity against *C albicans* and *S Mutans* between the control and modified groups of heat-cure denture base resin materials.

Conclusion: Silver nanoparticles are the favourable materials to incorporate into denture base materials as they exhibit superior antimicrobial activity.

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1. Introduction

Dentistry witnessed the evolution of denture base materials from bone, wood and ivory to cellulose plastics and Poly (methyl methacrylate) in time. Due to favourable working characteristics, accurate fit, stability in the oral environment, superior aesthetics, and ease of processing with inexpensive equipment, PMMA has been the material of choice since 1939. Despite its advantages, PMMA exhibits frequent fracture of dentures because of fatigue and chemical degradation of the base material, low thermal conductivity [1-4], and ease of microbial adherence to the intaglio surface [6,7]. Reinforcing the denture base materials with various metallic fillers, fibres [3] and grafting PMMA with styrene rubber [3] may have compensated the strength issues but the problem of microbial adhesion remains critical for PMMA often leading to Denture stomatitis (DS) [6-8].

Denture stomatitis is the most common consequence of prolonged denture wearing, improper cleaning practices, ill-fitting dentures and biofilm formation on the prosthetic surface. Though the condition has not been considered severe, it may result in chronic inflammation and infection in the elderly or immune-compromised patients [6-8]. The treatments for this Candidal infection include removal of the source of irritation and applying antifungal agents orally in the form of drops, lozenges, cream, pastille, lacquer, gel or mouthwashes [6-8]. However, such attempts have mostly been unsuccessful due to the loss of the drug rapidly into the saliva, inhomogeneous distribution of the drug and the development of resistance to antifungal therapy. Therefore, alternative method to hinder the candidal adherence like addition of antimicrobial additives to conventional PMMA formulation has been attempted. Limited research is available exploring the possibility of formulating a suitable denture base material with potent antifungal activity. In this regard, nanoparticles of silver, titanium dioxide, and silver vanadate are essential precursors for the development of newer functional devices as they improve physical properties by increasing surface area yet efficiently preventing the growth of several microorganisms including Candida, Salmonella, Staphylococcus, and Escherichia coli. Because of their proven antimicrobial activity, experimental incorporation of these nanoparticles into various materials for biomedical applications has been studied[9-12]. The purpose of this study is to evaluate the antimicrobial effect of denture base resin modified with the addition of silver nanoparticles.

2. Materials and methods

2.1 Materials

Silver nanoparticles (nanolabs, India) with an average particle size of 80-100 nm were incorporated at various concentrations (0.5, 1.0, 2.0 and 5.0 wt%) in to three heat-cure denture base materials such as Trevlon (Dentsply), Lucitone (Dentsply) and TriplexHot (Ivoclaur Vivadent) were used in the study. A total of 300 specimens (10 × 2 mm) of heat-cure acrylic resin were made which comprises 100 specimens with each denture base material. The specimens from each denture base materials were divided into 50 specimens for each microorganism used in the study. The 50 specimens were again divided in to five groups with 10 specimens (n=10) for each concentration such as control, 0.5wt%, 1.0wt%, 2.0wt% and 5.0wt% concentrations of silver nanoparticles.

2.2 Methods

2.2.1 Making of acrylic specimens

Acrylic specimens were fabricated by investing the rectangular metal discs in a dental flask. The lower half of the dental flask was filled with a freshly mixed dental stone. Subsequently, the metal disc was carefully seated at the centre of the mix before it's initial set. On reaching its initial set, the stone was coated with a cold mold seal to prevent the stone mix that would be poured into the upper half of the flask from adhering to that in the lower half. After complete setting of the stone mix, the middle member of the dental flask was placed and filled with a stone mix. The lid was placed and secured in a dental clamp and allowed the stone mix to set. Metal strips were carefully removed after the investment medium was set. A thin layer of separating medium was applied in the mould space and allowed to dry. The mould was then ready to be used for the preparation of acrylic specimen.

The acrylic specimens were made by mixing PMMA resin powder with the MMA liquid in the ratio of 3:1 respectively. Control group test specimens were made with conventional heat polymerized PMMA resin polymer and monomer mixed and allowed to reach dough

consistency. For the reinforced specimens, the silver nanoparticles with the concentration of 0.5%, 1%, 2% or 5% by weight were mixed with monomer liquid prior to mixing it with the acrylic powder. Dough thus formed was kneaded and then packed into the mould, flask is closed and bench cured for 30 minutes under pressure in a hydraulic press apparatus. Then the flask was tightly secured in a clamp and transferred into a thermostatically controlled water bath/ acrylizer. The packed acrylic resins were cured according to the manufacturer's instructions. After the curing process is completed, the specimens were carefully removed from the investment mold and the excess material was trimmed followed by finishing and polishing with pumice slurry and rouge respectively.

2.2.2 Evaluation of antimicrobial activity

Antimicrobial activity of the unmodified and modified denture base materials was evaluated against *Streptococcus mutans* (*S. mutans*) and *Candida albicans* (*C. albicans*). American type culture collection (ATCC) approved *S. mutans* strain 25175 and American type culture collection (ATCC) approved *C. albicans* strain 90028 in brain-heart infusion (BHI) broth with concentration of 0.5McFarland were prepared (1 mL of this solution contains approximately 1×10^8 bacteria). Subsequently, the solution was diluted to achieve a concentration of 1×10^5 bacteria in 1 mL. The acrylic resin discs were sterilized in an autoclave. A sampler was used to place 0.01 mL of the bacterial suspension on the surface of the disc samples. Then the samples containing bacterial suspension were incubated in an incubator for 2 hours at 37^0 C to vaporize the water. The samples were placed in test tubes containing sterile 0.5 mL BHI broth and incubated in an incubator at 37^0 C for overnight (around 12 hours). After 12 hours of incubation, a sterile sampler was used to retrieve 0.01 mL from each liquid culture media to uniformly spread on a blood agar plate. The blood agar plates were incubated for 48 hours at 37^0 C and then the numbers of bacterial colonies (Colony Forming Unit, CFU) were visually counted.

2.2.3 Scanning electron microscopy

Acrylic specimens were vacuum dried in a desiccator containing silica gel until a constant weight is obtained. The dried specimens were gold sputtered and were subjected to scanning electron microscopy at 10 kV.

The data was subjected to One way ANOVA and TukeyHSD tests for statistical analyses using SPSS for windows, Version 21.0., SPSS Inc.

3. Results

Figures 1 to 6 show the antimicrobial activity of denture base materials incorporated with AgNPs. The mean colony forming units along with standard deviation observed during the study are presented in Table 1. Control groups of denture base materials showed no antifungal activity against *C. albicans* and *S. Mutans*. However, denture base materials modified with various concentrations of silver nanoparticles showed significant decrease in colony forming units and exhibited superior antimicrobial activity against *C. albicans* (Figures 1-3) and *S. Mutans* (Figures 4-6). Relatively similar anti-candidial activity has been observed among the modified groups at various concentrations of AgNPs. Significant differences (p=0.000) were observed between unmodified and modified denture base resins against *C. Albicans* and *S. Mutans*. However, the anti-candidal activity observed with denture base materials at different concentrations of AgNPs was relatively similar hence considered insignificant. An increase in colony forming units was observed as the concentration of AgNPs increased among the modified against *S. Mutans*. However, modified TriplexHot denture base material showed more antimicrobial activity against *S. Mutans* than the other two denture base materials even at higher concentrations. Denture base materials modified with 5.0wt% of AgNPs showed significant differences (p=0.015) among them. Scanning electron microscopic analysis showed agglomeration of nanoparticles as their concentration increases (Figure 7).

4. Discussion

Nanotechnology, a field of science concerning the nano -sized matter, has brought alteration of properties and characteristics of materials at atomic and molecular levels possible [13,14]. This field has revolutionised various scientific specialities, including dentistry [13-15]. Incorporating antimicrobial efficacy to dental biomaterials using nanoparticles seemed a feasible solution to prevent denture stomatitis by barricading microbial accumulation on PMMA. One such element which had potent bactericidal efficiency in spite of bei-

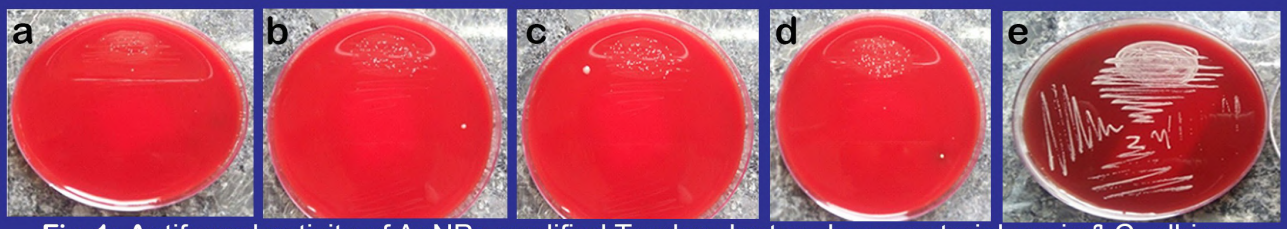

Fig.1: Antifungal activity of AgNPs modified Trevlon denture base material against C. albicans

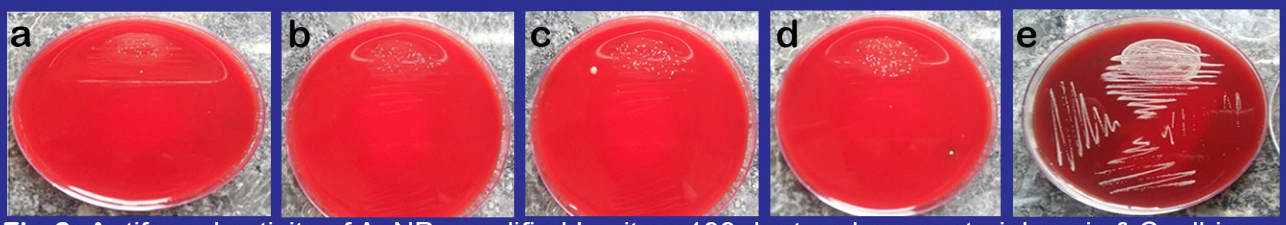

Fig.2: Antifungal activity of AgNPs modified Lucitone199 denture base material against C. albicans

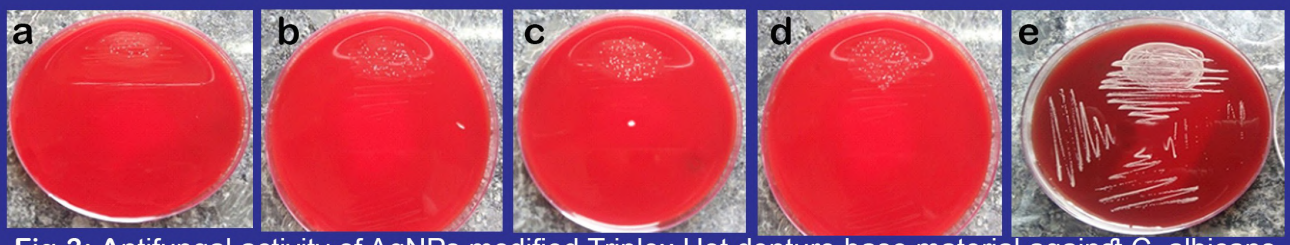

Fig.3: Antifungal activity of AgNPs modified Triplex Hot denture base material against C. albicans

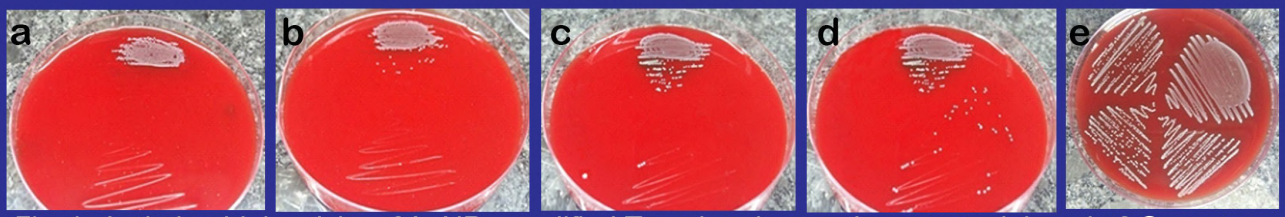

Fig.4: Antimicrobial activity of AgNPs modified Trevalon denture base material against S. mutans

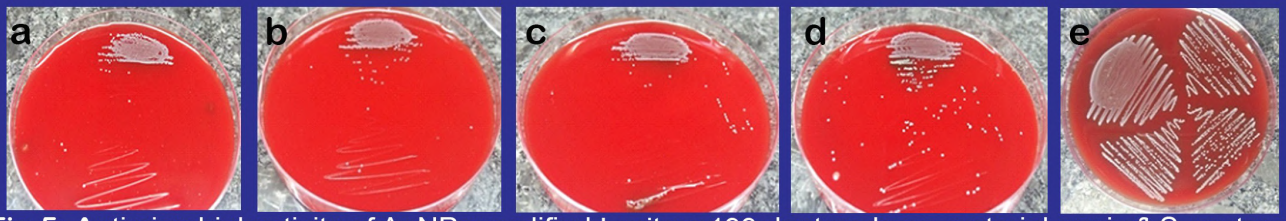

Fig.5: Antimicrobial activity of AgNPs modified Lucitone199 denture base material against S. mutans

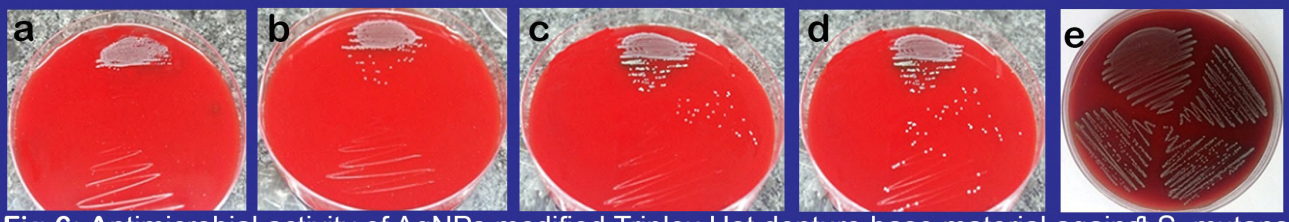

Fig.6: Antimicrobial activity of AgNPs modified Triplex Hot denture base material against S. mutans

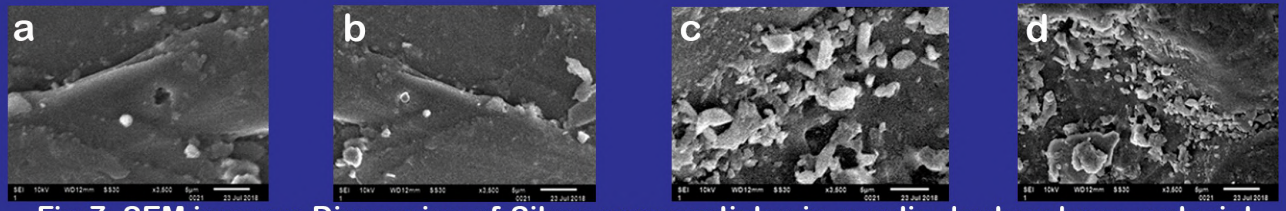

Fig.7: SEM images: Dispersion of Silver nanoparticles in acrylic denture base material

* a. 0.5wt% AgNPs b. 1wt% AgNPs c. 2wt% AgNPs d. 5wt% AgNPs e. Control

ing non-toxic to human cells was silver [16-18]. The present study thus attempted to evaluate the antimicrobial nature of denture base resins incorporating silver nanoparticles which had proven antibacterial effect in the field of medicine. The micro-organisms targeted were Streptococcus mutans and Candida Albicans due to their significant role in DS and dental caries.

Many researchers have proposed the Agar disc diffusion technique to evaluate the antibacterial properties of cured acrylic resins [15,19]. However, inhibitory halo has not been formed around the cured PMMA/ NPs composite resin discs; indicating no release of antibacterial agents into the medium, thereby negating the use of this method[19-22]. Therefore, antimicrobial activity of denture base materials was evaluated using direct contact technique and BHI broth culture media in the present study. As this liquid medium contains both live and dead bacteria, 0.001 ml from each bacterial suspension tube was cultured on blood agar medium to trace and count the live and active colonies.

The results of the present study showed that the number of colonies of viable bacteria in the control group was significantly higher than that in the nanoparticle modified groups, demonstrating the antimicrobial activity of acrylic resins containing AgNPs, against *S. mutans*, and *C. albicans*. Studies show antimicrobial activity mainly depends on the efficacy, particle size and distribution of nanoparticles in the resin matrix along with the composition of denture base acrylic resins [23,24]. Results also suggest incorporation of silver nanoparticles suppress the growth of *S. mutans* in a dose-dependent manner, unlike that seen with *C. albicans*.

The results of this study are similar to the findings of Acosta-Torres *et al.* (2012) [25] and Issa *et al.* (2015) who reported acrylic resins and soft relining materials incorporated with AgNPs exhibited higher antifungal property. These studies also suggested the disk diffusion method to be unsuitable for resin materials as they could not release any antimicrobial agents [26]. Wady AF *et al.* also found no inhibitory effect on *C. albicans* adherence and biofilm formation with incorporation of AgNPs into denture base resin [27]. However, Kamikawa *et al. (*2014) [28] coated AgNPs on acrylic denture base resin discs and reported its inhibitory effect on adhesion of Candida to the denture surface thereby preventing Oral Candidosis. Therefore, AgNPs effectively inhibited fungal adherence to acrylic resins but did not exhibit potent antifungal capability [27].

Concentration of AgNPs	*C. albicans*			*S. mutans*		
	Trevlon	Lucitone 199	TriplexHot	Trevlon	Lucitone 199	TriplexHot
Control	4.0000 ± 0.000	4.0000±0.000	4.0000±0.000	4.0000± 0.000	4.0000±0.000	4.0000±0.000
0.5wt%	0.5200 ± 0.252	0.5200 ± 0.252	0.4600 ± 0.189	0.4600 ± 0.189	0.4600 ± 0.189	0.4600 ± 0.189
1.0wt%	0.5200 ± 0.252	0.5200 ± 0.252	0.5800 ± 0.289	0.8800 ± 0.252	0.9400 ± 0.189	0.8800 ± 0.252
2.0wt%	0.5800 ± 0.289	0.5200 ± 0.252	0.5200 ± 0.252	2.0000 ± 0.000	1.8000 ± 0.421	1.6000 ± 0.516
5.0wt%	0.5800 ± 0.289	0.5200 ± 0.252	0.5800 ± 0.289	2.9000 ± 0.316	2.7000 ± 0.483	2.3000 ± 0.483

Table 1. Antimicrobial activity, Colony forming units, of denture base materials incorporated with silver nanoparticles (mean x 10^2 ± SD).

The antifungal activity of NPs can be due to the increased ROS and radical hydroxyl production on exposure to *C. albicans*. As a result, mitochondrial dysfunction and mutation in CDC48 or expression of mammalian Bax occur leading to apoptosis[29]. So, generation of oxygen radicals is an essential event in the ancestral apoptotic pathway of yeast [30].

S. mutans is a known etiological factor; playing a vital role in dental plaque formation and thereby in dental caries and periodontal problems [31]. In the present study, incorporation of 0.5wt% AgNPs exhibited superior antimicrobial activity against *S. mutans* which decreased slightly on increasing the nanoparticle concentration to more than 0.5wt%. A possible reason for this observation could be due to the addition of NPs in the powder form which might result in particle-particle aggregation at higher concentrations. Such an aggregate may reduce the active surface area available for the antimicrobial action [32,33]. In this study, particle aggregation was evident in the scanning electron microscopic images of the NPs modified acrylic specimens (Figure 7). Similarly, Azarsina *et al.* (2013) [34] also reported an insignificant decrease in the antibacterial activity against *S. mutans* as the concentration of AgNPs increased in the resin composites from 0.5wt% to a maximum of 1.0wt%. In contrast to this, previous investigations using a colloidal solution of AgNPs may have led to their uniform distribution reducing the chances of aggregation and therefore, the superior antimicrobial activity even at higher concentrations [35-39].

Conclusion

Within the limitations of the present in vitro study, it can be concluded that the incorporation of silver nanoparticles reported significant antimicrobial activity to the denture base materials tested. Silver nanoparticles showed superior resistance to the *C albicans* at all concentrations used in the study. However, a decrease in antimicrobial activity was observed against *S mutans* as the concentration of silver nanoparticles increased.

Conflicts of interest: *None*

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References

1. Anusavice KJ. Denture Base Materials (in) Science of Dental Materials. 11th edition, Saunders, India, 2010: 721-758.
2. Alla RK. Denture Base Materials (in) Dental Materials Science. 1st edition, Jaypee Medical Publishers Pvt. Ltd., India, 2013; 248-284.
3. Alla RK, Suresh Sajjan MC, Ramaraju AV, Ginjupalli K, Upadhya NP. Influence of fiber reinforcement on the properties of denture base resins. J Biomater Nanobiotech. 2013; 4(1):91-97.
4. Alla RK, Raghavendra Swamy KN, Ritu Vyas, Konakanchi A. Conventional and Contemporary polymers for the fabrication of denture prosthesis: part I – Overview, composition and properties. Int J App Dent Sci. 2015;1(4):82-89.
5. Rama Krishna Alla, K. N. Raghavendra Swamy, Ritu Vyas, Anusha Konakanchi, Vineeth Guduri, Praveen Gadde. Influence of Silver Nanoparticles Incorporation on Flexural Strength of Heat-cure Acrylic Denture Base Resin Materials. Annual Research & Review in Biology. 2017;17(4):1-8.
6. Najla S. Dar-Odeh, Mohammad Al-Beyari, Osama A. Abu-Hammad, The role of antifungal drugs in the management of denture-associated stomatitis, The International Arabic Journal of Antimicrobial Agents 2(1); 2012:1-5.
7. KN Raghavendra Swamy, Rama Krishna Alla, Shammas Mohammed, Anusha Konakanchi. The Role of Antifungal Agents in Treating Denture Stomatitis. Research Journal of Pharmacy and Technology. 2018;11(4):1365-1369.
8. Pachava KR, Shenoy KK, Nadendla LK, Reddy MR, Denture Stomatitis – A Review, Ind J Dent Adv 5 (1);2013: 1107-1112.
9. Nagy A, Harrison A, Sabbani S, Munson RS, Dutta PK, Waldman WJ. Silver nanoparticles embedded in zeolite membranes: release of silver ions and mechanism of antibacterial action. Int J Nanomedicine 2011;6:1833–52.
10. Stoimenov P, Klinger R, Marchin G, Klabunde K. Metal Oxide Nanoparticles as Bactericidal Agents. Langmuir 2002;18:6679–86.
11. Nomiya K, Yoshizawa A, Tsukagoshi K, Kasuga NC, Hirakawa S, Watanabe J. Synthesis and structural characterization of silver(I), aluminium (III) and cobalt (II) complexes with 4-isopropyltropolone (hinokitiol) showing noteworthy biological activities. Action of silver(I)-oxygen bonding complexes on the antimicrobial activities. J Inorg Biochem.

2004;98:46–60.
12. Marambio-Jones C, Hoek EMV. A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. J Nanoparticle Res. 2010;12:1531–51.
13. Sahoo SK, Parveen S, Panda JJ. The present and future of nanotechnology in human health care. Nanomedicine Nanotechnol Biol Med. 2007;3:20–31.
14. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv 2009;27:76–83.
15. Kumar R, Münstedt H. Silver ion release from antimicrobial polyamide/silver composites. Biomaterials 2005;26:2081–8.
16. Svanberg M, Mjör IA, Orstavik D. Mutans streptococci in plaque from margins of amalgam, composite, and glass-ionomer restorations. J Dent Res 1990;69:861–4.
17. Bürgers R, Eidt A, Frankenberger R, Rosentritt M, Schweikl H, Handel G, et al. The anti-adherence activity and bactericidal effect of microparticulate silver additives in composite resin materials. Arch Oral Biol 2009;54:595–601.
18. Li LH, Deng JC, Deng HR, Liu ZL, Xin L. Synthesis and characterization of chitosan/ZnO nanoparticle composite membranes. Carbohydr Res 2010;345:994–8.
19. Imazato S. Antibacterial properties of resin composites and dentin bonding systems. Dent Mater 2003;19:449–57.
20. Bundy KJ, Butler MF, Hochman RF. An investigation of the bacteriostatic properties of pure metals. J Biomed Mater Res 1980;14:653–63.
21. Azarsina M, Kasraei S, Yousefi-Mashouf R, Dehghani N, Shirinzad M. The Antibacterial Properties of Composite Resin Containing Nanosilver against Streptococcus mutans and Lactobacillus. Patil SG, editor. J Contemp Dent Pract 2013; 14: 1014–8.
22. Kasraei S, Sami L, Hendi S, AliKhani M-Y, Rezaei-Soufi L, Khamverdi Z. Antibacterial properties of composite resins incorporating silver and zinc oxide nanoparticles on Streptococcus mutans and Lactobacillus. Restor Dent Endod 2014;39:109–14.
23. Yoshida K, Tanagawa M, Matsumoto S, Yamada T, Atsuta M. Antibacterial activity of resin composites with silver-containing materials. Eur J Oral Sci 1999;107:290–6.
24. Spencer CG, Campbell PM, Buschang PH, Cai J, Honeyman AL. Antimicrobial effects of zinc oxide in an orthodontic bonding agent. Angle Orthod 2009;79:317–22.
25. Acosta-Torres LS, Mendieta I, Nuñez-Anita RE, Cajero-Juárez M, Castaño VM. Cytocompatible antifungal acrylic resin containing silver nanoparticles for dentures. Int J Nanomedicine 2012;7:4777–86.
26. Issa MI, Abdul-Fattah N. Evaluating the effect of silver nanoparticles incorporation on antifungal activity and some properties of soft denture lining material. J Baghdad Coll Dent 2015;27:17–23.
27. Wady AF, Machado AL, Zucolotto V, Zamperini CA, Berni E, Vergani CE. Evaluation of Candida albicans adhesion and biofilm formation on a denture base acrylic resin containing silver nanoparticles: Antifungal activity of silver nanoparticles. J Appl Microbiol 2012;112:1163–72.
28. Kamikawa Y, Hirabayashi D, Nagayama T, Fujisaki J, Hamada T, Sakamoto R, et al. In Vitro Antifungal Activity against Oral Candida Species Using a Denture Base Coated with Silver Nanoparticles. J Nanomater 2014;2014:1–6.
29. Hwang I, Lee J, Hwang JH, Kim K-J, Lee DG. Silver nanoparticles induce apoptotic cell death in Candida albicans through the increase of hydroxyl radicals. FEBS J 2012;279:1327–38.
30. Madeo F, Fröhlich E, Ligr M, Grey M, Sigrist SJ, Wolf DH, et al. Oxygen stress: a regulator of apoptosis in yeast. J Cell Biol 1999;145:757–67.
31. Michalek SM, Hirasawa M, Kiyono H, Ochiai K, McGhee JR. Oral ecology and virulence of Lactobacillus casei and Streptococcus mutans in gnotobiotic rats. Infect Immun 1981;33:690–6.
32. Lara HH, Garza-Treviño EN, Ixtepan-Turrent L, Singh DK. Silver nanoparticles are broad-spectrum bactericidal and virucidal compounds. J Nanobiotechnology 2011;9:30.
33. Ginjupalli K, Shaw T, Tellapragada C, Alla R, Gupta L, Perampalli NU. Does the size matter? Evaluation of effect of incorporation of silver nanoparticles of varying particle size on the antimicrobial activity and properties of irreversible hydrocolloid impression material. Dent Mater 2018;34:e158–65.
34. Azarsina M, Kasraei S, Yousefi-Mashouf R, Dehghani N, Shirinzad M. The Antibacterial Properties of Composite Resin Containing Nanosilver against Streptococcus mutans and Lactobacillus. J Contemp Dent Pract 2013; 14: 1014–8.
35. Panacek A, Kvítek L, Prucek R, Kolar M, Vecerova R, Pizúrova N, et al. Silver Colloid Nanoparticles:

Synthesis, Characterization, and Their Antibacterial Activity. J Phys Chem B 2006;110:16248–53.
36. Lkhagvajav N, Ya I. Antimicrobial Activity of Colloidal Silver Nanoparticles Prepared By Sol-Gel MethoD. Dig J Nanomater Biostructures 2011;6: 149–54.
37. Agnihotri S, Mukherji S, Mukherji S. Size-controlled silver nanoparticles synthesized over the range 5–100 nm using the same protocol and their antibacterial efficacy. RSC Adv 2013;4:3974–83.
38. Ahrari F, Eslami N, Rajabi O, Ghazvini K, Barati S. The antimicrobial sensitivity of Streptococcus mutans and Streptococcus sangius to colloidal solutions of different nanoparticles applied as mouthwashes. Dent Res J 2015;12:44–9.
39. Guzman M, Dille J, Godet S. Synthesis and antibacterial activity of silver nanoparticles against gram-positive and gram-negative bacteria. Nanomedicine Nanotechnol Biol Med 2012;8:37–45.