The Effect of Silver Nano Particles on Candida Albicans and Streptococcus Mutans in Denture Acrylic Resins

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Abstract

Introduction: Denture stomatitis is a common complication in patients wearing dentures and preventing the proliferation of related microorganisms and their induced infections is noteworthy. The aim of the present study was to assess the antimicrobial effect of acrylic resins containing various concentrations of silver nanoparticles, on *Candida albicans* and *Streptococcus mutans*. **Materials and Methods:** In this in vitro study, the effect of four different concentrations of silver nanoparticles in 160 acrylic samples (10mm *4mm) on standard and hospital isolated strains of two different microorganisms were investigated. The samples were soaked (containing silver nanoparticles and control) in bacterial suspension and the antimicrobial tests were performed after 0, 1, 6 and 24 hours. Mean and standard deviation were used to describe the data and one-way variance analysis test was performed to compare groups. **Results:** Results have shown that in concentration of 2.5% the highest mean difference for standard *S. mutans,* after 24h of exposure was 540.0 ± 14.4 (P<0.001), while in 5% ,this value for standard *C.albicans* after 6h of exposure was 953.0 ± 87.0 (P<0.001). However in concentration of 10% the highest mean difference of 1000.0 ± 24.9 in standard *C.albicans* after an exposure period of 24h (p<0.001) was observed, which was indicative of the significant effect of the highest concentration of silver nanoparticles. **Conclusion:** 1. in acrylic resins, increase in silver nanoparticle concentration leads to a greater antimicrobial effect. 2. Acrylic resins containing silver nanoparticles have a stronger antimicrobial property on standard *C.albicans* and generally less effect on hospital isolated strains. 3. The longer time elapsed after exposure of bacterial suspension with acrylic resins containing silver nanoparticles, the less the residual antimicrobial effect.

Key words: Silver nanoparticles, Acrylic resins, Antimicrobial effect.

Introduction

Silver nanoparticles are particles of silver ranging in size from 1 to 100 nm. The peculiar characteristic of silver ions and compounds is their toxic impact on microorganisms. This fact persuades researchers to use them for medical purposes (1). Nitrate form of silver is generally used for inducing antimicrobial effects; however silver nanoparticles increase the available surface area to microbes (1, 2). In Kim's study (3), nanosilver were used to inhibit yeast, E.coli and S.aureus proliferation and they were successful in preventing yeast and E.coli growth in low concentrations. Although silver nanoparticles are used in various antibacterial applications, the mechanism of action is not well recognized. It has been hypothesized that silver nanoparticles can cause cell lysis or inhibit cell transduction (1). Kim indicated that nanoparticles of silver could affect cell membrane and cause an antifungal effect, a mechanism similar to amphotericin B, without similar levels of hemolytic effect (4). In another study, silver nanoparticles presented more antifungal effects on C. albicans compared with

Amphotericin B and fluconazole (5). Regardless of the details of silver nanoparticles used and the targeted microorganisms, it has been used in previous studies to control the colonization of certain microorganisms around medical or dental appliances such as denture bases, resin composites, etc. (1, 6, 7).

Polymethyl Methacrylate acrylic resin is a choice for applying Candida the antimicrobial purposes of silver nanoparticles. Growth of the microorganisms under acrylic denture bases, the main material to replace the lost tissue in treatment of edentulous patients, is common. Denture related oral ulcers like denture stomatitis can be the most prevalent lesion in these patients (73% of prosthodontic patients) in which Candida albicans is responsible for about 70% of cases, Common treatment plans for these infections such as Nystatin, fluconazole, and Amphotericin B therapy are short-term, so we tried to produce a denture with self-antimicrobial features (8).

In several studies such as Kassaee's experiment (9), different concentrations of Nano silver particles in acrylic resin have been examined. In this experiment, strong antibacterial effect of 0.5% silver nanoparticles on E.coli has been demonstrated. Furthermore, Casemiro (8), showed that addition of 5% and 2.5% silver nanoparticles to acrylic resin brings about a high antimicrobial effect on C.albicans. Another study illustrated that the addition of 10% β-AgVO3 (β- Silver Vanadium Oxide) to acrylic resin promotes antimicrobial activity against P. aeruginosa, S. aureus, C. albicans, and S. mutans (6). Torres et al. found that the antifungal effect of PMMA-silver nanoparticle disk is related to the reduction of C. albicans adherence with no effect on metabolism or proliferation of cells (10). Moreover, Zhe Li's study demonstrated an antifungal activity, resultant inhibitory effect on adhesion and biofilm formation on denture base resin containing nano-silver ,especially at higher concentrations (11).

The aim of this study is to examine whether acrylic resins containing silver nanoparticles, release silver ions and generate long-term antimicrobial effect.

Materials and Methods

Silver nanoparticle solution preparation: Colloidal silver nanoparticles (fig 1) were prepared via chemical process of reduction. 0.001 M of silver nitrate (Ghataran Shimi T. Co. Iran) was dissolved in water and heated up to 80 ℃. Next, 5 ml of 1% trisodium citrate was added to the solution drop by drop while it was being shaken severely. Warming process continued until the color of the solution changed to brown. Finally, the solution was cooled in room temperature, while being shaken.

The shape of nanoparticles shown under an electronic microscope was spherical (fig 2). A nanosizer (nano- sz, Malvern Instruments Ltd, England) device was also used to measure the size. The average size of the particles was about 22 nm. In the end, a potential zeta device was utilized to specify the ionic charge of the silver nanoparticles, the result of which was a -2 surface charge.

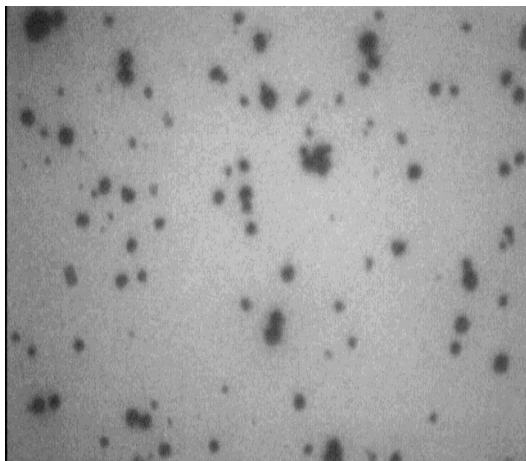Preparing acrylic samples without silver nanoparticles (control samples).

Cylindrical pieces with 10 mm diameter and 4 mm thickness were used to prepare the samples (fig 3).

Plaster and water were mixed using manufacturer instructions and poured in the inferior part of the flask leaving 10 mm free space. After plaster setting was completed, 10 mm of silicon (putty) was placed in it. Then, 10 metallic cylinders were submerged completely into the unset putty.

Next, the superior part of the flask was placed on the inferior part, plaster poured in it and pressed under a hydraulic pressure device. Metallic cylinders were extracted after three hours and the acrylic paste was prepared considering manufacturer liquid/powder ratio. While the mixture was going through its doughy phase, we placed it in the empty spaces. Finally, the flask was pressed under a hydraulic pressure device andthen placed into a hot water tub (72 degrees C) for six hours. After 24 hours, the flask was opened and samples were extracted from the plaster. After the finishing procedures were carried out using acrylic burs, they were placed in normal saline solution. (Fig. 4).
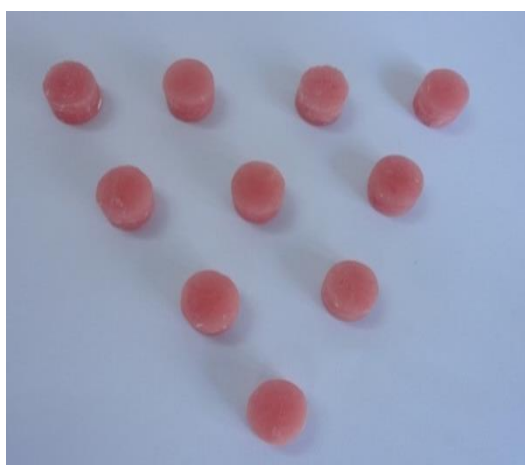

Figure 1. Colloidal silver nanoparticles

Figure 2. Electronic microscopic image of silver nanoparticles (×8000)

Figure 3. Cylindrical pieces

Figure 4. Acrylic samples (without nanosilver)

Preparing acrylic samples containing different concentrations of silver nanoparticles:

We poured about 25cc of colloid into a special bulb and set the device temperature at 85°C and speed at 200 rpm until colloids were concentrated to a volume of 2 cc. The weight of 1.5 cc silver nanoparticle colloid was 2 gr after concentration.

Then, the volume of silver nanoparticle colloids for sample preparation was evaluated considering weight percentage of silver nanoparticles in the acrylic samples.

Fifty samples were prepared without silver nanoparticles to estimate the weight of each sample. The average weight of each sample was 0.4gr.

The required weight of silver nanoparticle colloid for preparation of an acrylic sample containing 2.5% silver nanoparticles: Weight of each acrylic sample (0.4 gr) × 2.5% (weight of silver nanoparticles (gr))/ (weight of each acrylic sample (gr)) = 0.01gr

The required weight of silver nanoparticle colloid for preparation of 40 acrylic samples containing 2.5% silver nanoparticles: 0.01gr × 40 = 0.4 gr

The required volume of colloid for preparation of 40 acrylic samples containing 2.5% silver nanoparticles:

0.4 gr silver nanoparticles × (1.5 cc of silver nanoparticle colloids)/ (2 gr of silver nanoparticle colloids) = 0.3cc

regarding the above-mentioned equations, the required volume of colloid for preparing 40 blocks of 0.01 gr acrylic samples in 2.5, 5 and 10 w/w % (mass fraction) is 0.3 cc, 0.6 cc and 1.2 cc, respectively.

We mixed 10 cc of acrylic liquid and 20 cc of acrylic powder. Then, the whole paste was cured and its weight was measured after it was cooled down. The resulting 6 gr was equivalent to 15 samples.

The required volume of powder for preparing 40 acrylic samples:

(Number of samples (15))/ (number of samples (40)) = (required volume (10 cc))/ (required volume (x cc)) x = 26.6 cc

We had to mix .3 cc, 0.6 cc and 1.2 cc of silver nanoparticle colloids with 53.3 cc of acrylic powder and 26.6 cc of acrylic liquid to prepare 40 acrylic samples containing silver nanoparticles concentrated at 2.5, 5 and 10 w/w%, respectively.

The prepared mixture was mixed up properly until it became completely homogenous, then it was poured into a flask designed empty space and other procedures were followed just like samples without silver nanoparticles.

Antimicrobial effects
Studied Microorganisms comprised:
1). Candida albicans ATCC 10231
2). Streptococcus mutans ATCC 35668
3). Streptococcus mutans hospital isolated
4). Candida albicans hospital isolated

Sample 1 and 2 were taken from Industrial and Scientific Research Organization of Iran and 3 and 4 were taken from a hospital (Ghaem Hospital, Mashhad, Iran).
Acrylic samples were prepared in four different concentrations:
1). Acrylic specimens without silver nanoparticles as control group (n=40)
2). 2.5% silver nanoparticles (n=40)
3). 5% silver nanoparticles (n=40)
4). 10% silver nanoparticles (n=40)

Each group samples were divided into 4 subgroups depending on the microorganisms.

Samples were kept in physiologic serum after preparation until they were placed in separate isolated laboratory tubes and were sterilized in 120 degrees centigrade for 15 minutes.

Brain Heart Broth culture environment was prepared and sterilized according to the manufacturer's instructions.

Blood Agar culture environment was prepared as usual. In this process, Muller Hinton Broth environment was prepared and after being sterilized in autoclave and cooling in room temperature to 10 percent of the volume, Blood sheep was added to the solution and the solution was poured in 8centimeter sterilized plates. Bacterial strains were solved in Brain Heart Broth and incubated for 24 hours. Then, linear culture was applied on the sterilized plates to grow the bacteria. After the growth of the bacteria, bacterial suspension was provided with concentration of 0.5 Mc Farland (equals 1.5×10^8cfu) in comparison with Mc Farland standard. In the next stage, this suspension was diluted to 1.5×10^5cfu.

To prepare 1.5×10^5 CFU bacterial suspension, 1 cc of suspension with 1.5×10^8 CFU was mixed with 9 cc of the culture environment and a suspension with 1.5×10^7 CFU was obtained. The same process was repeated twice unitl a suspension with the concentration of 1.5×10^5 was provided.

One milliliter of the suspension was poured in laboratory tubes with sampler, and an acrylic sample was placed in each tube. Then, 10 µl of every suspension was isolated via sampler, which was cultured linearly, during the primary time of exposure to the bacteria.

In the next stage, laboratory tube was placed in the incubator shaker for 24 hours in 37 ℃. 10 µl of the suspension was taken after 1, 6, and 24 hours and cultured.Each plate was incubated for 24 hours post cultureand bacterial colonies were counted afterward.

Statistical Analysis
Mean and standard deviation were employed to describe the data. One-way ANOVA was used for comparing the amount of bacteria in silver nanoparticle.

Results
Obtained results after analysis of the gathered data were shown in the tables 1,2 and 3.

Mean and standard deviation of the reduction of bacterial suspension in different concentrations of silver nanoparticles were shown in table 1. The results indicated a significant difference among all groups in 2.5% concentration after 0, 1, and 24 hours of exposure (P<0.001). The maximum effect occurred after 24hours against standard S. mutans .

Significant difference was observed among all groups of 5% concentration (P<0.001). The maximum effect on Standard Candida occurred at 6 hours after exposure Candida .

There was a significant difference among all groups in concentration of 10% (P<0.001). The maximum effect occurred on Standard Candida at 24 hours post exposure Candida. The anti-microbial property after 6h of exposure was approximately equal to 24h post exposure .

Analyses illustrated that the maximum effect occurred at 24 hours post exposure in concentration of 2.5% in standard S.mutans. However, in standard Candida, the maximum effect occurred at 24 hours post exposure in concentration of 10%. In hospital Candida and hospital S.mutans, there was a maximum effect at 24 hours post exposure in concentration of 10%.

Table 1. Mean and standard deviation of the reduction of bacterial suspension in different concentrations of silver nanoparticles considering type of bacteria, concentration, and exposure time

Silver nanoparticles density	Bacteria	CO-TO SD±Mean	C1-T1 SD±Mean	C6-T6 SD±Mean	C24-T24 SD±Mean
2.5 %	Standard mutans	212.0 ± 48.9	176.8 ± 26.8	150 ± 31.2	540.0 ± 41.4
	Standard Candida	29.0 ± 24.5	35.0 ± 11.1	432.0 ± 45.3	501.0 ± 23.1
	Hospital Candida	10.0 ± 90.7	150.0 ± 47.5	320.0 ± 29.1	400 ± 35.7
	Hospital mutans	106.9 ± 29.9	212.6 ± 23.2	291.2 ± 15.2	350.0 ± 17.1
5%	Standard mutans	187.7 ± 26.2	241.5 ± 26.3	250.0 ± 24.1	310.0 ± 15.6
	Standard Candida	61.5 ± 42.0	59.5 ± 8.5	953.0 ± 87.0	860.0 ± 34.3
	Hospital Candida	132.8 ± 38.7	358.3 ± 21.4	320.0 ± 19.7	380.0 ± 23.2
	Hospital mutans	84.4 ± 58.7	166.8 ± 39.5	222.5 ± 26.8	358.5 ± 3.8
10%	Standard mutans	247.2 ± 33.4	187.1 ± 32.3	360.0 ± 5.2	400.0 ± 21.7
	Standard Candida	72.9 ± 60.6	162.0 ± 53.2	963.0 ± 52.0	1000.0 ± 24.9
	Hospital Candida	279.5 ± 6.2	232.8 ± 6.8	360.0 ± 12.5	840.0 ± 13.1
	Hospital mutans	190.2 ± 25.7	262.2 ± 22.1	360.0 ± 21.6	385.0 ± 29.4

C = average bacteria in 10 test tubes of the initial control sample (C0), an hour later (C1), six hours later (C6), and 24 hours later (C24)

T = average bacteria in 10 test tubes of silver nanoparticles in initial time (T0), an hour later (T1), six hours later (T6), and 24 hours later (T24)

Table 2. The results of one way variation ANOVA categorized by a bacterium and separated by concentration and time

Silver nanopartilcles density	Bacteria	CO-TO SD±Mean	C1-T1 SD±Mean	C6-T6 SD±Mean	C24-T24 SD±Mean
2.5 %	Standard mutans Standard Candida Hospital Candida Hospital mutans	F = 38.2 P < 0.001	F = 65.3 P = 0.55	F = 0.7 P=0.55	F = 18.3 P < 0.001
5%	Standard mutans Standard Candida Hospital Candida Hospital mutans	F = 14.2 P < 0.001	F = 123.1 P <0001	F = 518.1 P < 0.001	F = 27.8 P < 0.001
10%	Standard mutans Standard Candida Hospital Candida Hospital mutans	F = 60.0 P < 0.001	F = 18.2 P < 0.001	F = 1340.6 P < 0.001	F = 1822.7 P < 0.001

Table 3. Results of one way variation analysis categorized by concentration and separated by bacterium and time

Bacteria	concentration	CO-TO	C1-T1	C6-T6	C24-T24
Standard mutans	2.5% 5% 10%	F = 5.55 P = 0.01	F = 12.4 P < 0.001	F = 14.2 P < 0.001	F = 7.8 P = 0.007
Standard Candida	2.5% 5% 10%	F = 15.4 P < 0.001	F = 30.4 P < 0.001	F = 12.6 P < 0.001	F = 25.7 P < 0.001
Hospital Candida	2.5% 5% 10%	F = 54.6 P < 0.001	F = 140.4 P < 0.001	F = 12.4 P < 0.001	F = 25.7 P < 0.001
Hospital mutans	2.5% 5% 10%	F = 18.6 P < 0.001	F 26.2 P < 0.001	F = 148.4 P < 0.001	F = 689.4 P < 0.001

Discussion

Complete or partial edentulous patients are the most frequent clients of dentistry. Infection of the mucosa under prosthesis is a common problem in these patients. Denture-related Stomatitis is the most frequent problem in patients with complete denture,(especially in maxilla) which is caused by an ill-fitting denture, trauma during mastication, poor oral hygiene and presence of opportunistic organisms such as Candida albicans in oral cavity (12). Old people, who suffer from special systemic diseases such as Parkinson and Alzheimer that decrease their ability to observe oral hygiene are common users of dentures.

Current local treatments of Candidiasis including Nystatin or Fluconazole therapy and in more serious situations, administring Amphotericin B, cause complications such as drug resistance which must be considered, as well. Considering all these issues, an antimicrobial substance added to the acrylic prosthesis might help reduce the above-mentioned problems. The best applicable substance in this area is "silver nanoparticle", with none of the complications of other drugs, but with properties such as increasing the physical strength of dentures bases as shown in Kassaee's article(9). Therefore, in this paper we mixed silver nanoparticles and acrylic material in denture prostheses to add an antimicrobial property to them. Gradual release of silver nanoparticles results in imposing the anti-microbial effect and decrease cavities and denture-related stomatitis.

The antimicrobial property of silver has been utilized both in researches and medical purposes such as irrigations. Dressing of wounds, surgical equipments and bone prosthesis, either coated or mixed with silver particles, are common medicinal applications of this element. The nature of these particles is related to their size and crystal type. The increased surface area in nano-sized particles causes more effective contact with microorganisms with subsequent improved antimicrobial property (1). Silver nanoparticles attach to cell membrane or penetrate into bacteria and react with sulfur or phosphor containing molecules such as cell membrane proteins and DNA. Furthermore, these nanoparticles attack respiratory chain and cause death of bacteria. Release of Ag ions increases the antibacterial ability of silver nanoparticles (13, 14).

Our experiment was done on C.albicans,as the most prevalent strain in denture stomatitis, and on S. mutans, which is the leading etiological factor in dental carries and frequently found in oral cavity (12, 15). Samples were soaked in bacterial suspension. The similarity between this method and the process occurring in oral cavity is the greatest positive point achieved .

Our study indicated that "over time, the number of the four types of microorganisms studied decreased through the silver nanoparticles effect." This effect was time related and proportional to the concentration of silver nanoparticles. The maximum decrease in each group was as follows:

a) After 24 hours in 2.5% silver nanoparticles (Standard S.mutans)

b) After 24 hours in 10% silver nanoparticles (Hospitalized S.mutans)

c) After 6 hours in 5% silver nanoparticles and after 24hours in 10% silver nanoparticles (Standard C.albicans)

d)   After 24 hours in 10% silver nanoparticles (Hospitalized C.albicans)

These findings are similar to Sondi's results (16). In that study, antibacterial effect of silver nanoparticles was dependent on both the bacterial (E.coli) and solution concentrations. It is worth mentioning that, in our experiment the concentration of bacterial suspensions in different groups were similar to simplify comparison. However, the species studied are different.The same result were also found in Casemiro's study (8) which added 2.5% and 5% silver nanoparticles to IrgaguardB5000 and Qc20 lucit acryl, respectively. Their findings revealed a great antimicrobial effect on C.albicans. The other factor (particles' size) was evaluated in Espinosa's study and outcomes presented an increased antimicrobial effect by decreasing particles' size (2). As the sizes of silver nanoparticles were equal in our study this factor was not considered in the analyses.

Two important criteria of dentures are biocompatibility and esthetics. Silver does not have a considerable toxicity compared with other heavy metals (4, 5). However, it reduces translucency of polymethyl methacrylate which is proportional to the concentration of silver suspension (8). This fact was indicated in our study, as well. Hence, more studies are needed to omit this negative effect .

In our study, the antimicrobial agents in the superficial layers were released and produced a short time effects. Clearly, mixing nanoparticles in a suspension and exposing them to the bacteria makes more durable effects than solid acrylic samples. Literatures have shown that saturation of polymers with silver nanoparticles presented better antimicrobial effect than a superficial layer of silver on acrylic resin. The possible reason can be the inactivation of silver particles by anionic proteins.(17, 18). Therefore, in long-term, nanoparticles are needed to be released from deep layers, which is a complicated procedure. In our study, silver nanoparticles produced antimicrobial effects for 4 weeks; however, it does not necessarily prove long-term effects. Matsuura and colleagues (19) showed that tissue conditioners with silver-zeolite induce an antimicrobial effect against C.albicans for 4 weeks. Long term antimicrobial effect is needed due to the condition of oral cavity. Therefore, more experiments are needed to increment and improve antimicrobial properties of acrylic denture bases.

Conclusion

1, In acrylic resins, an increase in concentration of silver nanoparticles result in greater antimicrobial effects.
2, Acrylic resins containing silver nanoparticles have stronger antimicrobial rather than antifungal properties

(such as their effect on C.albicans) and generally have less effect on hospital isolated strains.
The longer the acrylic resins containing silver nanoparticles is worn and in contact with bacterial suspension, the less the residual antimicrobial effect.

References

1.   Prabhu S, Poulose EK. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. International Nano Letters. 2012; 2(1):1-10.

2.   Espinosa-Cristobal L, Martinez-Castanon G, Martinez-Martinez R, Loyola-Rodriguez J, Patino-Marin N, Reyes-Macias J, et al. Antibacterial effect of silver nanoparticles against Streptococcus mutans. Materials Letters. 2009; 63(29):2603-6.

3.   Kim JS, Kuk E, Yu KN, Kim J-H, Park SJ, Lee HJ, et al. Antimicrobial effects of silver nanoparticles. Nanomedicine: Nanotechnology, Biology and Medicine. 2007; 3(1):95-101.

4.   Kim K-J, Sung WS, Suh BK, Moon S-K, Choi J-S, Kim JG, et al. Antifungal activity and mode of action of silver nano-particles on Candida albicans. Biometals. 2009; 22(2):235-42.

5.   Nasrollahi A, Pourshamsian K, Mansourkiaee P. Antifungal activity of silver nanoparticles on some of fungi. International Journal of Nano Dimension. 2011; 1(3):233-9.

6.   CASTRO DTd, Holtz RD, Alves OL, Watanabe E, VALENTE MLdC, SILVA CHLd. Development of a novel resin with antimicrobial properties for dental application. Journal of Applied Oral Science. 2014; 22(5):442-9.

7.   Han Z, Zhu B, Chen R, Huang Z, Zhu C, Zhang X. Effect of silver-supported materials on the mechanical and antibacterial properties of reinforced acrylic resin composites. Materials & Design. 2015; 65(0):1245-52.

8.   Casemiro LA, Martins CHG, Pires-de-Souza FdC, Panzeri H. Antimicrobial and mechanical properties of acrylic resins with incorporated silver–zinc zeolite–part I. Gerodontology. 2008; 25(3):187-94.

9. Kassaee M, Akhavan A, Sheikh N, Sodagar A. Antibacterial effects of a new dental acrylic resin containing silver nanoparticles. Journal of applied polymer science. 2008; 110(3):1699-703.

10. Acosta-Torres LS, Mendieta I, Nuñez-Anita RE, Cajero-Juárez M, Castaño VM. Cytocompatible antifungal acrylic resin containing silver nanoparticles for dentures. International Journal of Nanomedicine. 2012; 7:4777-86.

11. Li Z, Sun J, Lan J, Qi Q. Effect of a denture base acrylic resin containing silver nanoparticles on Candida albicans adhesion and biofilm formation. Gerodontology. 2014: n/a-n/a.

12. Zarb GA, Hobkirk J, Eckert S, Jacob R. Prosthodontic treatment for edentulous patients: complete dentures and implant-supported prostheses: Elsevier Health Sciences; 2013.

13. Panáček A, Kvitek L, Prucek R, Kolar M, Vecerova R, Pizurova N, et al. Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity. The Journal of Physical Chemistry B. 2006; 110(33):16248-53.

14. Damm C, Münstedt H, Rösch A. The antimicrobial efficacy of polyamide 6/silver-nano-and microcomposites. Materials Chemistry and Physics. 2008; 108(1):61-6.

15. Greenberg MS, Glick M. Burket's oral medicine: diagnosis and treatment: Philadelphia; 2003.

16. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. Journal of colloid and interface science. 2004; 275(1):177-82.

17. Stobie N, Duffy B, McCormack DE, Colreavy J, Hidalgo M, McHale P, et al. Prevention of Staphylococcus epidermidis biofilm formation using a low-temperature processed silver-doped phenyltriethoxysilane sol–gel coating. Biomaterials. 2008; 29(8):963-9.

18. Melaiye A, Youngs WJ. Silver and its application as an antimicrobial agent. Expert Opinion on Therapeutic Patents. 2005; 15(2):125-30.

19. Matsuura T, Abe Y, Sato Y, Okamoto K, Ueshige M, Akagawa Y. Prolonged antimicrobial effect of tissue conditioners containing silver-zeolite. Journal of dentistry. 1997; 25(5):373-7.

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