Research Article

Evaluation of Antibacterial Effect of Silver and Copper Oxide Nanoparticles in Denture Base Material Against *Streptococcus mutans* and *Escherichia coli*

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Abstract

Objective: Heat cure acrylic is the most common used material for fabrication of dental prosthesis. This *in vitro* study was undertaken to discover the effect of nanoparticles on antibacterial properties of the denture base.

Methods: Nanosized Silver and Copper oxide were impregnated at 1%, 3% and 5% by weight to the monomer of methyl methacrylate with the aid of probe sonicator before mixing it with acrylic powder. Seventy samples were prepared to determine the effect of the nanoparticles on *Streptococcus mutans* and *Escherichia coli*.

Results: The results for *Streptococcus mutans* revealed a statistically significant difference ($p<0.05$) for both nanoparticles at the three concentrations except for 1% silver. The most influential concentration on the tested material was 3% copper oxide, which caused a 49% decline. Regarding *Escherichia coli*, only 3% and 5% of silver showed a statistically significant difference.

Conclusions: Within the limitations of this *in vitro* study, it can be concluded that the addition of Silver nanoparticles into heat-cure acrylic was more effective against *Streptococcus mutans* than *Escherichia coli*. Regarding Copper oxide nanoparticles, it caused a drastic reduction in *Streptococcus mutans* activity but with no significant effect on *Escherichia coli* for all of its concentrations. Since denture stomatitis is caused by both bacterial species, Silver nanoparticles might be considered as a suitable additive for reducing denture induced infections.

Keywords: *nanoparticles, Copper oxide nanoparticles, Streptococcus mutans, Escherichia coli.*

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Introduction

Complete and partial dentures made from polymethylmethacrylate (PMMA)[1] because of its aesthetics, biocompatibility, good tissue response and outstanding toxicity profile[2]. However, the ability to retain plaque and facilitating microbial adhesion to its surface makes it the most common source of oral cavity infection and stomatitis[3].

Several hundred species of microorganisms live in the oral cavity, but only a few them are able to invade tissues[4]. Denture stomatitis is a condition which usually associated with denture wearers which initiated by trauma from dentures with above aerobic, anaerobic bacteria and yeast organisms[5]. Current conservative treatment includes: prescribing an antifungal agent to control yeast and other microorganisms, refitting or remaking the denture or providing a sanitary denture[5].

Acrylic dentures are usually used by geriatric patients, because of inadequate manual dexterity; the denture will most often get neglected, resulting in bad oral health and poor denture hygiene[2]. Thus, there is a need for effective broad-spectrum antimicrobial resin in dentistry[6]. Also, the emergence of pathogenic microorganism that possesses resistance to pharmacological treatments has necessitates the creation of a new class of disinfection system[7]. The use of Nanoparticles (NPs) has overcome this issue because they are less prone to resistance development as the particles will get into direct contact with the bacterial cell wall, without the need to penetrate the cell and this gives hope for preventing bacterial resistance[8]. The addition of NPs into acrylic resin consider as one of the ways to promote antimicrobial characteristics of the material.

Silver (Ag) is known for its broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi, protozoa and certain viruses[9] making it the most promising system to enhance antimicrobial properties of materials[10]. Is a well-known fact that Ag based compounds are highly toxic to microorganisms with long-term antibacterial activity due to a sustained ion release[10,11] that does not contribute to the development of resistant strains[12]. The toxicity of Ag depends on the release of Ag cation (Ag^+) to the pathogenic environment that occurs when metallic Ag changes to active species through interaction with water and dissolved oxygen, resulting in the release of a small amount of Ag^+[13]. Researchers have studied the effect of Ag on different bacterial species. In a study performed by Kong et al.[14] which added AgNPs into PMMA and tested it against both gram-positive and gram-negative bacteria that was *Streptococcus mutans* (*S. aureus*) and *Escherichia coli* (*E. coli*) respectively. Results showed enhancement in antimicrobial efficacy (as a bactericidal agent) of the material against both strains of bacteria. Furthermore, Martinez- Castanon et al.[15] tested AgNPs at three different sizes against *S. aureus* and *E. coli*. The results showed inhibition of bacterial growth that was more effective for *E. coli* than for *S. aureus* for all sizes. Moreover, Ghahremanloo and Movahedzadeh tested *S. mutans* against 2.5%, 5% and 10% AgNPs when added to heat cure acrylic resin. Their analysis illustrated a significant difference in a reduction with various concentrations[16].

Regarding copper oxide (CuO) NPs, considered a better option than Ag because it is more affordable, easily mixed with polymers and relatively stable in terms of both chemical and physical properties[17]. Yoon *et al.*[18] tested the sensitivity of *E. coli* and *Bacillus subtilis* against CuO and AgNPs. It revealed a greater antimicrobial activity for CuO than Ag NPs for both strains of bacteria. Moreover, the effect of the same NPs was studied on *S. mutans* and *E. coli* but with the opposite result as Yoon's[19]. Finally, Amiri and his colleagues examined the effect of CuO NPs on *S. mutans;* it showed superior antimicrobial effect; as a result, CuO can be considered as a suitable candidate for preventing infections[20].

This study was carried out to assess the sensitivity of *S. mutans* and *E. coli* against Ag and CuO NPs when incorporated into heat-polymerized PMMA in hope to minimize denture-induced stomatitis.

Materials and methods

Dispersion of nanoparticles

The NPs were added at 1, 3 and 5% by weight for both Ag and CuO NPs into the monomer of heat cure acrylic with the aid of probe sonicator (Biosafety 900-02) for 1 min at 900w. Spherical NPs with 50nm in diameter were treated with a saline coupling agent. These specifications were selected in particular, according to dos Santos\[12] spherical NPs with 15-50nm in diameter exhibits the maximum antimicrobial properties.

Specimen design

For the antibacterial test, 70 specimens were prepared in total, 35 samples for each tested bacterium. The samples were square shaped with dimensions of 10 x 2 mm[21]**.** After the samples were finished and polished; they were placed in distilled water for 24 hr at 37°C to leach out any residual monomer[22]. Then sterilized under UV-radiation for 30 min[23] using Bioair Top safe. Once the sterilization was complete, they were placed back in the container, and the lid was tightly closed to prevent re-contamination.

Broth preparation

For growing *S. mutans,* Brain Heart Infusion Broth was used[24]. Regarding *E. coli,* Nutrient Broth was selected because it can support the growth of the bacteria to an extended period (4 weeks)[25].

For broth preparation, the powder was dissolved in the desired volume of distilled water and autoclaved for 15 min at 121 °C. After autoclaving, the broth was cooled down using ice. Then 4mL from each broth was placed in 10 mL plastic test tubes. The test tubes with the broth were sterilized for 30 min under UV radiation before placement of microorganism.

Cultures

For microbial tests, American Type Culture Collection (ATCC) strand for *E. coli* (ATCC 25922) was taken from Medya Diagnostic Centre. While for S. *mutans,* a supra-gingival plaque was taken with a sterile explorer from the cervical margin of the tooth and placed in a tube containing sterile normal saline and vertex for the 30s[26]. Afterward, the suspension was cultured on Blood agar, and the plate was grown under anaerobic condition using candle jar to provide CO_2 rich atmosphere[27]. The identity of *S. mutans* was confirmed using VITEK 2 before using it in the experiment.

The *S. mutans* and *E. coli* were chosen as gram-positive and gram-negative bacteria, respectively to check for susceptibility against the tested NPs because they are the predominant cultivable flora in denture-induced stomatitis[28].

Testing procedure

For the entire procedure working was done under Bioair Top safe with continuous air ventilation and Bunsen burner that was turned on near the working area to prevent contamination of the testing components by airborne pollutants. With modification from Clinical and Laboratory Standard Institute (CLSI)[27] and Manual of Antimicrobial Susceptibility Testing[29] bacterial colonies, 3 to 5 in number were taken using a loop from the agar plate into a 4 mL broth. They were mixed using vertex mixer for 15s and then incubated at 35 ± 2 °C for 2-6 hours. After that, they were taken out and mixed again for 5s using vertex. Then 1 mL of the mixture was transferred into a test tube to check for turbidity of the solution with the aid of Densi CHEK Plus (Figure 1).

Turbidity was adjusted using sterilized normal saline until it reaches the turbidity of 0.5 McFarland (McF) which contains 1-2x10^8 colony forming a unit (CFU)/mL[27]. Once reached, the acrylic sample was added (Figure 1) within 30 min to prevent a change in turbidity. The tubes with the sample inside were placed on an orbital shaker at 200 revolutions per minute (rpm) in an incubator for 24 hours at 37 °C[19].

Afterward, the test tubes were taken out, and 1 mL was pipette into a cuvette. In this study, visible growth was examined with the aid of spectrophotometer to check for the change in turbidity of the samples. The optical density indicates cell count; as cell count increases the density will increase in the process[25]. The spectrophotometer was fired at an optical density of 600 nm[25] for both bacteria.

Statistical analysis

Statistical Package for Social Sciences (SPSS, version 23) was used for data analysis and entry. One-way ANOVA with Tukey's Honest Significant Difference (HSD) was performed to determine if there is a statistically significant difference between the mean of each concentration of the NPs when compared to control. For statistical analysis, significant level $\alpha = 0.05$ was considered. The obtained data for both *S. mutans* and *E. coli* were calculated using one-way ANOVA with HSD to discover the bioactivity of Ag and CuO NPs against them, for the three concentrations.

Results

S. mutans showed a statistically significant difference ($p < 0.05$) for all NPs concentrations except for 1% AgNPs concerning control. The 3% and 5% Ag demonstrated 26% and 5% reduction, respectively. Regarding CuO NPs, 3% resulted in a 49% decrease in turbidity, which was the most influential concentration among the nanoparticles on *S. mutans*. While 1% and 5% caused 39% and 44% decline in bacterial activity, respectively when compared to control (Table 1 and Figure 2).

The AgNPs was the most effective against *E. coli* by showing a statistically significant difference at 3% and 5% only. On the contrary, CuO NPs did not display a significant effect on *E. coli* (Table 2 and Figure 3).

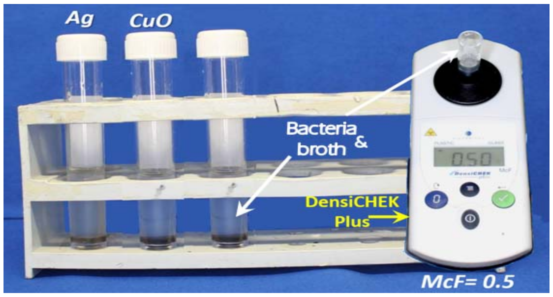

Figure 1: Acrylic samples placed in 0.5 McF solution.

Table 1: Effect of silver and copper oxide nanoparticles on *S. mutans*.

Nanoparticle	Concentration (%)	Mean (nm)	SD	ANOVA P value of concentration with control
Silver	1	0.953	0.004	0.980
	3	0.709	0.055	0.000
	5	0.903	0.008	0.034
Copper oxide	1	0.584	0.018	0.000
	3	0.490	0.024	0.000
	5	0.541	0.019	0.000
Control		0.960	0.004	

Table 2: Effect of silver and copper oxide nanoparticles on *E. coli*.

Nanoparticle	Concentration (%)	Mean (nm)	SD	ANOVA P value of concentration with control
Silver	1	0.485	0.001	0.130
	3	0.445	0.005	0.000
	5	0.454	0.007	0.003
Copper oxide	1	0.490	0.027	0.261
	3	0.483	0.015	0.590
	5	0.487	0.014	0.385
Control		0.469	0.007	

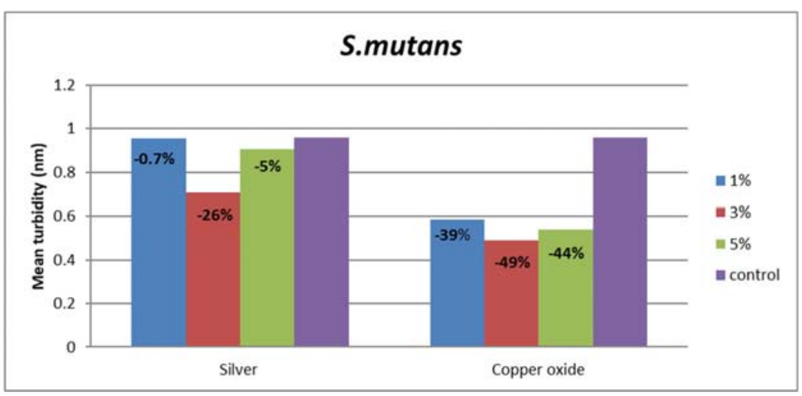

Figure 2: Effect of nanoparticle concentrations on *S. mutans*.

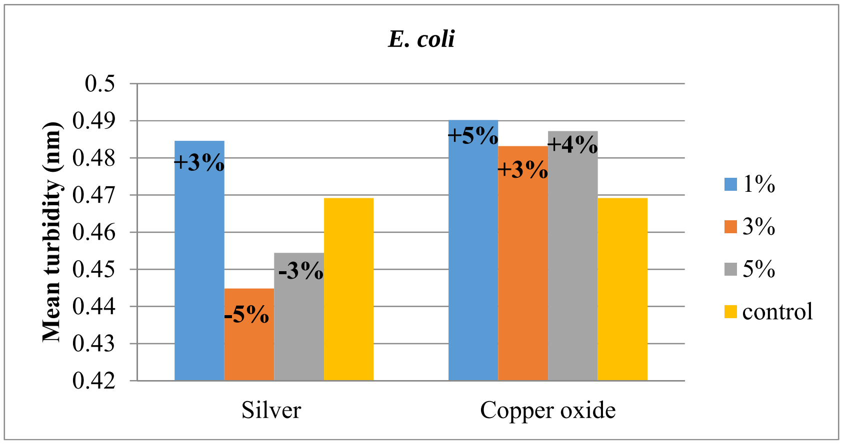

Figure 3: Effect of nanoparticle concentrations on *E. coli*.

Discussion

PMMA remains the most common denture base material for fabrication of removable prosthesis since it was induction in 1937. However, due to it is porous nature and food retention ability, they can stimulate bacterial activity and cause denture stomatitis(30). It has been shown that microorganisms have more potential to adhere to the acrylic surface than the tooth(30) due to this, mechanical and chemical methods have been put to the market to eradicate pathogenic microorganisms, but they did not show the desirable effect(31). In this study, incorporation of antimicrobial agents (NPs) into acrylic resin was done in the hope to enhance its antibacterial properties.

The results for *S. mutans* showed a statistically significant difference for all NPs concentrations except for 1% AgNPs. The AgNPs caused 26% and 5% reduction in turbidity for 3% and 5%, respectively, which agrees with Ghahremanloo *et al.*(16) While for CuO revealed a great reduction for all of it is concentrations; the results were 39%, 49% and 44% decrease for 1%, 3% and 5% NPs, respectively.

E. coli showed resistance against both NPs. The most effective concentration was 3% and 5% AgNPs, which showed a reduction in turbidity by 5% and 3%, respectively, which is an agreement with Kong and Jang(14) research.

The antibacterial efficiency of NPs has been linked to many factors. One of the proposed causes is the antibacterial effect of polymer is related to the diffusion of water molecules into the material and the migration of NPs through the polymer to the aqueous medium. As a result, property of the matrix polymer and its water diffusion ability play a role in ion release process(14,32).

Other factors might be due to the dispersion of NPs that has shown a fairly good role in the antimicrobial effect. Good dispersion within the polymer matrix will result in higher ion release and consequently, higher antimicrobial activity(3,33).

Reports in the literature have shown that electrostatic attraction plays a great role in the bactericidal effect of the material(10). This probably overcomes other factors, such as size and shape of NPs, which can influence bacterial cell death(34). If the NPs have the same charge as the bacteria's cells, this induces repulsion and prevents contact(35). Gram-negative bacteria exhibit only a thin peptidoglycan layer between the cytoplasmic membrane and the outer membrane which is negatively charged; when positively charged NPs interact with bacterial cell wall causes electrostatic attraction(36) and increase its bioactivity(9). Regarding gram-positive bacteria, it is antibacterial effect can be described by the cell membrane which has a loose cell wall, that can be attacked by NPs and can pass through the membrane and binds to the DNA, causing DNA denaturation(14).

Also, the different membrane structure of gram-positive and gram-negative bacteria have displayed a crucial role in distinct antimicrobial effect(15,20) that can be seen in the present results. Many studies have shown that NPs have greater activity against Gram-positive than Gram-negative bacteria because the cell wall of Gram-negative bacteria is composed of Lipopolysaccharide, lipoproteins, and phospholipids that allows the entrance of only macromolecules(8) and provides resistance against NPs(37). In contrast, the cell wall of Gram-positive bacteria has a thin layer of peptidoglycan as well as teichoic acid and abundant pores that allow foreign molecules to penetrate, resulting in cell membrane damage and cell death(8,34).

It has been stated that the composition of nutrient medium plays an important role in influencing the antimicrobial effect of AgNPs. The Brain Heart Infusion broth and Nutrient Broth that was used for the growth of *S. mutans* and *E. coli,* respectively, contain sodium chloride. Sodium chloride will cause precipitation of silver ions as silver chloride leading to inactivation of silver ion(7,13). This trend can be seen on the effect of Ag and CuO NPs on *S. mutans*.

The results obtained for CuO NPs showed a greater vulnerability for *S. mutans* than *E. coli,* this might be due to a greater abundance of amines and carboxyl groups on the cell surface of gram-positive bacteria which copper has a greater affinity to(19). Furthermore, the negatively charged peptidoglycan would also make gram-negative bacteria less susceptible to positively charged NPs(38). Also, extremely high surface areas and unusual crystal morphologies of CuO NPs contribute to its reactivity(38).

NPs can also display poor antibacterial effect in denture base material. This might be linked to a hydrophobic polymer network which compromises water uptake that may not be sufficient for ion release from the polymer samples(13,32). Another reason is the presence of the cross-linking agent in the liquid that might have caused a network of the specimens to be in such configuration that the NPs were probably trapped into the cross-linked polymer structure and their release into the aqueous medium was restricted(32). Is important to point out that the difference in sensitivity of the microorganism tested against the individual NPs is contributed to chemical reactivity and surface energy of individual particles which plays a central role in defining the antibacterial properties(39).

Conclusions

Within the limitations of this *in vitro* study, it can be concluded that the addition of AgNPs to heat-cure acrylic was more effective against *S. mutans* than *E. coli*. Regarding CuO, NPs caused a drastic reduction in *S. mutans* activity, whereas, the non-significant effect on *E. coli* was detected. Since denture stomatitis is caused by both bacterial species, AgNPs might be considered as a suitable method for reducing denture induced infections.

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