

ORIGINAL ARTICLE

Microbicidal Power of Silver Nano Particles and its Benefit in Soft Liner Obturator Prosthesis

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ABSTRACT

Key words:

Silver; Silver Nanoparticles; Microbicides; Antimicrobial power

Background: Indeed growth of some microorganisms, such as *Candida* and bacteria like *Staphylococcus*, *Streptococcus*, and *Klebsiella* on the soft liner obturator prosthesis has been demonstrated. Microbial colonisation on acrylic appliances represents a challenge for the scientific community to develop new bioactive compounds. **Objectives:** To achieve this aim, one solution is application of silver nano particles (SNPs) and detects its antimicrobial activity. Silver release from acrylic resins containing silver nano particles is more effective than using silver in micrometer dimensions. **Methodology:** Purification of bacterial culture and preparation of inoculums (10^5 cfu/ml) for seven clinical human isolates: *E. coli*, *K. pneumoniae*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus mutans*, *Enterococcus faecalis* and finally *Candida albicans*. Solutions of SNPs and five antimicrobials (penicillin G, Tetracyclin, Cefoperazone, Ofloxacin and amphotricin B) were prepared in eleven different ppms concentrations as the following; 30 ppm, 15 ppm, 10 ppm, 7.5 ppm, 5 ppm, 3.75 ppm, 2.5 ppm, 1.875 ppm, 1.25 ppm, 0.625 and 0.313. The MICs and the MBCs were detected for all the antimicrobials and compared with those of SNPs. Also antimicrobial susceptibility testing was performed by the disk diffusion method using impregnated disks with 10ppm of SNPs solution which were placed on inoculated agar with *E. coli* and *S. aureus* and the inhibition zones were measured and reported. Antimicrobial effect of silver inside the soft relining acrylic was tested by preparing acrostone soft relining acrylic with silver (12ppm) and another silver free preparation was used as a control. **Results:** The MIC and MBC of the silver were less than those of most antibiotics except those for Ofloxacin which showed less MIC and MBC for both gram positive and gram negative. Gram positive organisms were more resistant to silver than gram negative. The growth inhibition zones by the disk diffusion method were 8 and 6 mm for *S. aureus* and *E. coli*, respectively. The free silver preparation showed microbial growth after 24 hours of incubation unlike the silver preparation. The silver protected the soft relining acrylic from bacterial and fungal growths till the 10th week and produced a safety zone of inhibition around it. **Conclusions:** SNPs showed a respectable antimicrobial effect with low MIC and MBC and it seems that using SNPs in acrylic resins protects the preparation from bacterial and fungal growth inside, adhesion to its surface, and made a safety zone around it and If we put in consider the mechanical hygiene of the mouth we expect a better results.

INTRODUCTION

The medical properties of silver have been known for over 2,000 years. Nanoparticles used for numerous physical, biological, and pharmaceutical applications. Silver Nanoparticles (SNPs) were used as antimicrobial agents in many public places, and showed good antimicrobial effect¹. Antimicrobial properties of silver are mainly due to its effect on cell enzymes and proteins

that are important for respiration, proliferation and transport of substance across the cell membrane and within the cell^{2,3}.

Silver is an agent with low toxicity to human cells^{4,5}, and has been proven to exert a bactericidal effect against a broad range of microorganisms⁶. The exact mechanism which SNPs can cause antimicrobial effect is not clearly known; but there are various theories on its action on microbes to cause the microbicidal effect. Accumulation of the nanoparticles on the cell surface then penetration, takes part in catalytic oxidation reactions, interacts with many vital enzymes then inactivates them and makes change in the structure is one of the theories^{7,8,9}, also formation and

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release of silver ions by the SNPs may be considered to be another mechanism by which the microbial cells die^{10,11,12}.

Silver is a soft acid, and there is a natural tendency of a soft acid to react with a soft base¹³. The cells contain phosphorus in many parts which are soft bases, the DNA has phosphorus as a major component, the nanoparticles can act on these soft bases which lead to problems in the DNA replication and thus terminate the microbes¹⁴.

The antimicrobial property of silver is related to the amount of silver and its released rate. Silver is generally used in the nitrate form to induce antimicrobial effect, but when SNPs are used, there is a huge increase in the surface area available for the microbe to be exposed to it. The ionized silver is highly reactive, silver salts are effective at providing a large quantity of silver at once. But, it has been proposed that the thiol-containing compounds can absorb the silver ions and neutralize their antibacterial activity^{15,16}.

For this reason, prolonged antimicrobial activity from silver is best by continuously releasing a moderate amount of silver. Dental treatment is necessary when bacteria or fungi colonize the prosthetic materials. Restorative materials that inhibit bacterial and fungal growth around the restoration would be desirable. The increase in antibiotic-resistant microorganisms has prompted interest in the use of silver as an antimicrobial agent. Silver can protect the inner and outer surfaces of devices against wide range of microorganisms¹⁷.

This study was done to test the antimicrobial power of SNPs against fungi, Gram positive, and Gram negative organisms. Comparing between the MIC and MBC for SNPs solution and different commonly used antimicrobial drugs. Test the antimicrobial power of silver in dental biomaterials and evaluate this activity by time.

METHODOLOGY

All tests were performed according to CLSI (Clinical and Laboratory Standard Institute) guidelines 2010¹⁸.

SilverSol® 30 ppm and 10 ppm (ABL, USA): contains metallic silver (20-30 nm) in water. The stock solutions were used to prepare different final concentrations; 30 ppm, 15 ppm, 10 ppm, 7.5 ppm, 5 ppm, 3.75 ppm, 2.5 ppm, 1.875 ppm, 1.25 ppm, 0.625 and 0.313 by serial two-fold dilutions (30, 15, 7.5, 3.75, 1.875 and 10, 5, 2.5, 1.25, 0.625, 0.313 ppm).

Purification of bacterial culture and preparation of inoculums: The bacterial species tested (seven human isolates) and the agar mediums used were: *Escherichia Coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* on nutrient agar (NA) (Oxoid, UK), *Streptococcus pyogenes*, *Streptococcus mutans* and *Enterococcus faecalis* on blood agar (Oxoid, UK)

and finally *Candida albicans* on Sabaroud's agar (Oxoid, UK). A single colony was picked with a sterile loop and transferred into fluid broth medium (Oxoid, UK), then incubated at 37°C. The density of the organism suspensions were adjusted by spectrophotometer to contain 10⁸ cfu/ml then part of it was diluted one thousand fold to reach the final inoculums for MIC test (10⁵ cfu/ml), and the remaining part were used for susceptibility testing by the disk diffusion method.

The minimal inhibitory concentration (MIC): were determined by a broth macro-dilution method. Different ppm concentrations were prepared from five antimicrobial powders (Oxoid) in same concentrations (ppm) as were done for the SNPs solutions (penicillin G, Tetracyclin, Cefoperazone, and Ofloxacin). Each antimicrobial and Nano-silver set (the set have eleven different ppm for each tested antimicrobial) were tested against the six bacterial isolates (*candid albicans* was tested against amphotricin B (oxoid) and SNPs). Sets of sterile tubes were arranged in rows for each type of microbes and one mL of the final inoculums (10⁵ cfu/mL) of each organism was added to the test tubes. One mL volumes of each concentration of tested antibiotics or silver were transferred to the labeled corresponding tubes. The contents in the tubes were mixed thoroughly and incubated at 37°C for overnight. The MIC endpoint is the lowest concentration of antimicrobial or SNPs those inhibit the growth when compared with negative control (inoculums free antimicrobial and SNPs).

The minimal bactericidal concentration (MBC): The MBCs were detected by culturing 10 µl from non-turbid tubes on corresponding agar, and then plates were incubated at 37°C. The minimal concentrations of antimicrobial or SNPs that can kill the microorganisms were reported as MBC (no growth was observed).

Antimicrobial susceptibility testing: Antimicrobial testing was performed by the disk diffusion method according to Kirby-Bauer method [19]. Broth cultures of *E.Coli*, *S. aureus* (10⁸ cfu/ml) were cultured on Mueller-Hinton agar media (Oxoid). Blank disks with 6 mm diameter sterilized by autoclaving 15 min at 120 °C were impregnated with 10ppm of SNPs solution and were placed on. The plates were then incubated at 37°C. Inhibition zone were measured after 18 hours with a ruler and the diameter were reported.

Acrylic silver antibacterial effect testing: Acrostone soft relining acrylic with silver (12ppm) added to the powder in one preparation and the other preparation is silver free for comparing. The test was performed against a mixture of *strept. viridans*, *S. aureus*, *klebsiella pnemoni* and *candida albicans* human isolates that were isolated from oral, throat, and nasal samples of 8 patients. Antimicrobial effect by silver inside the soft relining acrylic was tested by implanting

the preparation on the surface of blood agar after immersing the preparation in the former mix. This experiment was periodically tested at 0-3 months for the same preparation by moving it every week to a new agar and re-immersing it before new implanting.

RESULTS

Table 1: MIC and MBC comparison between different antibacterial groups and SNPs against gram positive and gram negative

Tested organisms	Penicillin G		Tetracyclin		Cefoperazone		Ofloxacin		SNPs	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	Ppm	ppm
<i>E.coli</i>	10	15	1.25	5	1.25	10	0.625	1.25	2.5	3.75
<i>K. pneumoniae</i>	10	15	0.625	5	7.5	15	0.625	1.25	2.5	3.75
<i>S. aureus</i>	5	7.5	1.25	10	5	7.5	0.625	1.25	5	7.5
<i>Strept. pyogenes</i>	5	15	0.625	10	0.625	1.25	1.25	2.5	5	7.5
<i>Strept. Mutans</i>	3.75	7.5	0.625	10	2.5	7.5	2.5	7.5	7.5	10
<i>E.fecalis</i>	5	15	0.313	10	10	15	5	7.5	7.5	10

ppm; parts per million, MIC; Minimum Inhibitory Concentration, MBC; Minimum Bactericidal Concentration

Table 2: MIC and MBC comparison between Amphotericin B and SNPs against *C. albicans*

Tested antimicrobial	MIC	MFC
Amphotericin B	2.5 PPM	3.75 PPM
SNPs	1.25 PPM	1.875 PPM

ppm; parts per million, MIC; Minimum Inhibitory Concentration, MFC; Minimum Fngicidal Concentration

Minimum Inhibitory Concentration and Minimum bactericidal Concentration

The MIC and MBC for SNPs is less than of most antibiotics except that for Ofloxacin which shows less MIC and MBC for both gram positive and gram negative bacteria, as showed in table1. Also this table shows that gram positive organisms are more resistant to SNPs than gram negative one.

Antimicrobial susceptibility

In this study, the antibacterial activity of SNPs was tested by the disc diffusion method for *S. aureus* and *E. coli*. The growth inhibition zone of *S. aureus* and *E. coli* was 8 and 6 mm, respectively.

Acrylic silver antibacterial effect testing:

The antimicrobial activity of silver inside the soft relining acrylic protect the preparation from bacterial adhesion to its surface till the 10th week and also form a safety zone of inhibition around it as in figure 1.

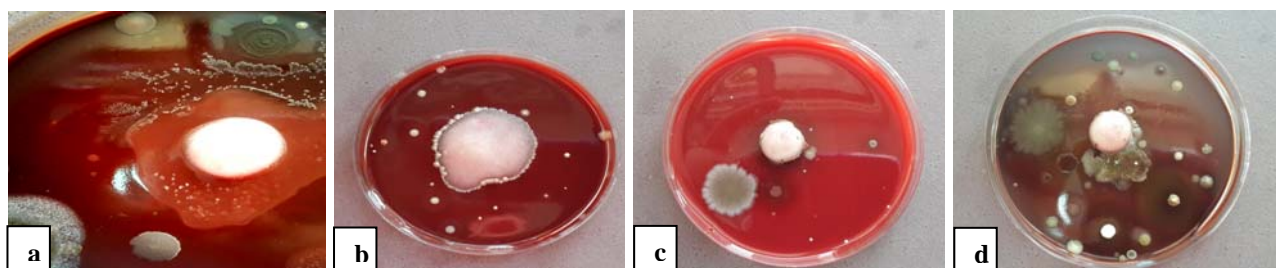


Figure 1: Time related growth results on blood agar plate after aerobic incubation at 37°C of acrylic preparations with or without silver. A: One week after incubation of acrylic preparation with silver. B: 24 hours after incubation of acrylic preparation without silver. C: Acrylic silver preparation was re-contaminated by the microbial mix every week and transferred to a new blood agar after dryness of the used one. Microbial colonies start to grow on the acrylic surface and near to it at the 11th week. D: Acrylic silver preparation at 13th week incubation.

DISCUSSION

In this study the antimicrobial power tested for SNPs Solution showed that the MICs were ranged from 1.25 to 7 ppm for all the tested microorganisms and the MBC were in the range of 1.875 to 10 ppm for all, so 10 ppm can consider as the killing concentration for SNPs against a wide range of microorganisms. Nearly similar

results were reported by Revelly in 2011²⁰. In comparison, this study results shows a low MIC and MBC concentrations when compared to Petrus et al.²¹ who's reported that the MIC values for his silver preparation were in the range of 7 to 25 ppm whereas MBC values exceeded 100 ppm. Among the suspected causes of this variability several factors could be responsible; the most important of all might be

heterogeneity of the tested microbes, and different silver preparations that may lead to different and incomparable results. Nanotechnologies helps in increase the effectiveness of the silver particles and have advantages over the other form of particles as a result of its properties as selectivity, size, shape, and biocompatibility. Properties such as these allow for nanoparticles to affect the human body differently than traditional therapies²².

In the present study, *candida albicans* was the most sensitive organism to SNPs and the gram-positive bacteria were less susceptible to SNPs than the gram-negative bacteria. Kawahara et al.²³ reported that Gram-positive bacteria is less sensitive to silver than gram negative, and he suggested that is due to that gram positive bacteria have thick cell wall that may make the entrance of the silver particles more difficult than in gram-negative bacteria, also it contains more negatively charged peptidoglycan layers, and silver ions are positively charged, so silver may get trapped by peptidoglycan in gram-positive bacteria²³. In this study in spite of that we used a SNPs preparation in the metallic form not in the ionic one we had got the same result and this can support the first reason.

In the current study, Ofloxacin was the real competitor for the SNPs antibacterial effect for both gram positive and gram negative by showing a lower figure in MIC ranging from 0.625 to 5 and MBC from 1.25 to 7.5, while silver MIC range was from 2.5 to 7.5 and MBC from 3.75 to 10. Toxic effects of many silver nanoparticles forms have been reported in mammalian cells, but in this work we tested the metallic form of nano silver (20 nm). Unlike colloidal or ionic silver, metallic silver particles are not metabolized by the body and that make it more stable and not toxic, so if we include the safe preparation in this competition SNPs can be in paralism with Ofloxacin. In the same spirit Merck manual diagnosis and therapy doesn't list metallic silver as a heavy metal that can cause poisoning or as a metal that cause nephrotoxicity²⁴. Also it was cited that oral dose of silver was tested by amount equal to 5g/kg in male and female rat with no evidence of toxicity by the Federal Hazardous Substance Act²¹.

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