

***In Vitro* Comparison of Antibacterial Efficacy of a New Irrigation Solution Containing Nanosilver with Sodium Hypochlorite and Chlorhexidine**

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Abstract

Objective: Antibacterial properties of silver nanoparticles have recently come into the spotlight in endodontic therapy. This study was conducted aiming at comparing the antimicrobial activity of a new irrigation solution containing nanosilver particles with that of sodium hypochlorite and chlorhexidine against *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Candida albicans* with direct culture technique.

Methods: In this *in vitro* experimental study, Mueller Hinton agar medium was prepared for cultivation of *E. coli*, *C. albicans* and *P. aeruginosa* species and Bile-Esculin agar culture medium was used for *E. faecalis*. Understudy irrigation solutions were chlorhexidine 2%, chlorhexidine 0.2%, sodium hypochlorite 5.25%, sodium hypochlorite 2.5%, sodium hypochlorite 1.125% and nanosilver solutions of 25, 50, 100, 150, 200, 400 and 4000 ppm. After preparation, the bacteria were exposed to these solutions and the culture media were stored in an incubator at 37°C for 24 hours. The diameter of growth inhibition zone was determined for different microbial species and data were analyzed using Kruskal-Wallis and Dunnett's tests.

Results: Significant differences were found between various irrigation solutions based on the diameter of growth inhibition zones for *E. faecalis*, *E. coli*, *C. albicans* and *P. aeruginosa* (P<0.0001). The greatest antimicrobial activity against microbial species belonged to sodium hypochlorite 5.25% and 2.5%. Silver nanoparticle solution had an acceptable antimicrobial activity in comparison to other solutions and its antimicrobial property constantly improved by increased concentration of Ag ions. The nanosilver containing irrigation solution at different concentrations up to 100 ppm did not show a significant difference with sodium hypochlorite 1.25% in terms of antimicrobial efficacy. Furthermore, the greatest antibacterial activity against *P.aeruginosa* was observed at different concentrations of nanosilver up to 100 ppm; whereas, chlorhexidine showed no antimicrobial activity against this microorganism.

Conclusion: Based on the obtained results, nanosilver canal irrigation solution had a lower but acceptable antimicrobial activity against various bacterial species compared to conventional irrigation solutions. Therefore, once other characteristics of nanosilver are approved, further studies can be performed to improve its properties and use it as an alternative to conventional root canal irrigation solutions.

Key words: Antibacterial activity, Chlorhexidine, Nanosilver, Root canal irrigation solutions, Sodium hypochlorite.

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Introduction:

Elimination of microorganisms from the root

canal system plays an important role in achieving a long-term success in endodontic treatments (1). This task is done through canal

preparation and mechanical cleaning and shaping along with irrigation with various antibacterial agents (2, 3). Use of antibacterial intra-canal medicaments in between treatment sessions also helps in removal of bacteria from the root canal system (4). Elimination of infectious materials from the root canals before obturation increases the chances of treatment success. Otherwise, there is a possibility that microorganisms remain in the canal or invade it after obturation and increase the risk of treatment failure (5).

Canal irrigation solutions should possess characteristics such as low toxicity, low surface tension, lubrication, long-lasting antimicrobial effect, easy availability, tolerable odor, and reasonable cost. Chlorhexidine, sodium hypochlorite, EDTA, MTAD or tetracycline isomer, phenol and alcohol derivatives, iodide potassium iodine and formocresol are among the commonly used root canal irrigation solutions (6).

Chlorhexidine is a popular antimicrobial agent in dental treatments. It has a cationic molecular component that attaches to the areas of cell membrane with a negative charge and causes cell lyses. However, it is not capable of dissolving debris or pulp tissue (7). Sodium hypochlorite has a wide range of antimicrobial activity and is able to kill various bacteria. It also has disadvantages such as toxicity and risk of tissue destruction, bad taste, inability to eliminate all the microorganisms present in infectious canals (8) and risk of physically changing the structure of dentinal canal walls.

Silver is a white and shiny metal element with high ductility and electrical and thermal conductivity. Antimicrobial effects of silver have long been recognized. After the introduction of antibiotics, application of silver decreased. However, at present, the ability to produce silver as nanocrystalline structure has greatly enhanced its biological and antimicrobial values (9). Silver nanoparticles provide a greater

contact surface compared to mass silver; which increases its antimicrobial efficacy. Therefore, a tiny amount of silver nanoparticles is required to exert an antimicrobial effect similar to that of mass silver (10-13). Various nanosilver-coated products have been manufactured such as the wound dressings, contraceptive devices, surgical tools and skeletal prosthesis. At the same time, many researchers have assessed the possibility of using nanosilver products in endodontic therapy (14-17).

In addition to bacteria, nanosilver has cidal effects on a wide range of fungi, protozoa, and even viruses (18-20). The present study aimed at comparing the antibacterial efficacy of a new irrigation solution containing nanosilver with that of sodium hypochlorite and chlorhexidine against *E. coli*, *E. faecalis*, *C. albicans* and *P. aeruginosa* with direct culture of bacteria next to antibacterial agents.

Methods:

This in-vitro experimental study evaluated and compared the antimicrobial effects of different canal irrigation solutions on 4 bacterial species. *E. coli* (ATCC 25922), *E. faecalis* (ATCC 29212), *C. albicans* (ATCC 10231) and *P. aeruginosa* (ATCC 27853) species were prepared and used.

Bile Esculin agar medium was used for the culture of *E. faecalis* and Muller Hinton agar medium was used for cultivation of other bacteria.

A 0.5 McFarland suspension was prepared from the respective bacteria and confirmed with spectrophotometer. At 0.5 McFarland, number of bacteria is equal to 1.5×10^8 . The bacterial suspension was applied to the entire surface of the Muller Hinton agar culture plate using a swab. Understudy irrigation solutions and their concentrations were as follows:

- 1- Sodium hypochlorite 5.25%, 2.5% and 1.125%

- 2- Chlorhexidine 2% and 0.2%
- 3- Nanosilver solutions of 4000, 400, 200, 150, 100, 50 and 25 ppm (Lotus Nanochemistry Pasargad)

Preparation and dilution of solutions were done using sterile twice-distilled water.

In the next step, blank discs were soaked with various concentrations of solutions and placed next to the culture media at an appropriate distance. Culture plates were placed in an incubator at 37°C and after 24 hours size of the growth inhibition zone was measured with a caliper and recorded in respective tables.

Bile Esculin agar medium was prepared similar to the Muller Hinton agar medium, poured in plates and *E. faecalis* was cultured on it. *E.*

faecalis causes a black discoloration on this medium which is due to the sedimentation of iron. Diameter of the growth inhibition zones caused by understudy irrigation solutions in bacterial species was compared using non-parametric Kruskal-Wallis test. Dunnett's test was applied for pair-wise comparison of groups.

Results:

Diameter of growth inhibition zone caused by different irrigation solutions in various bacterial cultures after 24 hours is demonstrated in Table 1.

Table 1- Diameter of growth inhibition zone (mm) caused by different irrigation solutions in various bacterial cultures after 24 hours (mean (SD))

Irrigation solution	Bacteria			
	<i>E. faecalis</i>	<i>C. albicans</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
Chlorhexidine 2%	25.0 (1.6)	24.0 (1.6)	24.7 (0.9)	0
Chlorhexidine 0.2%	20.0 (3.2)	20.0 (0.8)	20 (1.6)	0
Sodium hypochlorite 5.25%	20.0 (1.6)	21.5 (1.2)	27.7 (0.5)	8.5 (0.5)
Sodium hypochlorite 2.5%	18.0 (1.6)	20.0 (0.8)	26.5 (1.0)	1.75 (3.5)
Sodium hypochlorite 1.125%	10.0 (0.8)	10.0 (1.6)	16.0 (0.8)	1.75 (3.5)
Nanosilver solution of 4000 ppm	10.5 (1.0)	11.0 (0.8)	17.2 (3.5)	14.7 (1.2)
Nanosilver solution of 400ppm	9.2 (1.2)	9.7 (0.9)	12.0 (0.8)	12.2 (5.1)
Nanosilver solution of 200ppm	8.7 (0.9)	9.0 (0.8)	10.2 (0.9)	11.5 (5.6)
Nanosilver solution of 150ppm	8.0 (0.8)	8.5 (1.2)	10.0 (1.4)	11.0 (5.3)
Nanosilver solution of 100ppm	8.0 (0.8)	8.2 (1.2)	9.5 (1.2)	9.2 (7.0)
Nanosilver solution of 50ppm	6.0 (4.0)	7.7 (0.9)	8.2 (1.2)	5.7 (7.2)
Nanosilver solution of 25ppm	5.5 (3.6)	3.75 (4.3)	5.75 (3.8)	5.5 (6.8)

For *E. faecalis*, the largest growth inhibition zone was caused by chlorhexidine 2% and the smallest by nanosilver solution of 25 ppm. Chlorhexidine 2%, chlorhexidine 0.2%, hypochlorite 5.25% and hypochlorite 2.5% had

the highest efficacy against this microorganism and showed no significant difference in this respect with one another ($p>0.2$). However, other understudy solutions had significantly lower antibacterial effects ($p<0.001$).

Antibacterial properties of nanosilver solution at different concentrations were not significantly different from that of sodium hypochlorite 1.125% ($p>0.2$). The obtained results for *C. albicans* were exactly the same as *E. faecalis*.

For *E. coli*, the largest growth inhibition zone was observed due to sodium hypochlorite 5.25% and the smallest was noted around nanosilver solution of 25 ppm. Sodium hypochlorite 5.25%, sodium hypochlorite 2.5%, chlorhexidine 2% and nanosilver solution of 4000 ppm had the highest efficacy with no significant difference with one another ($p>0.1$). Other antibacterial agents showed no significant difference with each other ($p>0.05$).

For *P. aeruginosa*, the greatest antibacterial activity was observed in nanosilver solution of 4000 ppm and the lowest efficacy belonged to chlorhexidine. Antibacterial effects of nanosilver solutions up to the concentration of 100 ppm were greater than that of sodium hypochlorite. Chlorhexidine had no antibacterial effect on this microorganism.

Discussion:

Based on the present study results, all the understudy canal irrigation solutions had antimicrobial effects on *E. coli*, *E. faecalis*, *C. albicans* and *P. aeruginosa* (except for chlorhexidine 2% and 0.2% that had no antimicrobial activity against *P. aeruginosa*). Sodium hypochlorite 5.25% and 2.5% had the highest and nanosilver solution of 25 and 50 ppm had the lowest antibacterial activity against *E. coli*. By increased concentration of nanosilver solution, its antibacterial activity against these species improved as well.

Chlorhexidine 2%, sodium hypochlorite 5.25%, chlorhexidine 0.2% and sodium hypochlorite 2.5% had the highest and nanosilver solution of 25 and 50 ppm had the lowest antibacterial efficacy against *C. albicans*. By increased concentration of nanosilver solution, its

antibacterial activity against these species improved as well (similar to other species).

In contrast to other species, chlorhexidine 2% and 0.2% had no antimicrobial activity against *P. aeruginosa*. Nanosilver solutions of 4000 and 400 ppm had the highest antimicrobial activity against these species. In general, antibacterial activity of nanosilver solution improved by its increased concentration. Nanosilver exerts its antimicrobial effect through catalytic and ionic mechanisms. In catalytic mechanism or production of reactive oxygen species by silver, particles act like an electrochemical cell, oxidize the oxygen molecule and produce oxygen or OH⁻ ions by hydrolyzing water which are both active valences and are among the most potent antimicrobial agents. In the ionic mechanism, the microorganism is changed through the conversion of -SH bonds to -Sag bonds. Silver nanoparticles radiate Ag ions in time; through a substitution reaction, they convert the SH- bonds in cell membrane of microorganisms to -Sag bonds. This reaction results in death of microorganism.

Antimicrobial effects of silver nanoparticles against different bacterial species have been confirmed in several studies (14, 21, 22). However, it should be noted that silver nanoparticles used in the mentioned studies are different from those used in the present study (18, 23). There is no doubt that nanoproductions are unique based on characteristics such as size, shape and concentration of nanoparticles, type of surfactant and stabilizing factor (24) and these characteristics affect their antimicrobial properties (25).

Hiraishi *et al.* in 2010 evaluated the antimicrobial efficacy of silver diamine fluoride Ag (NH₃)₂F and impact of its sedimentation on root dentin and showed that this solution had adequate antimicrobial properties against *E. faecalis* (26). Sotiriou and Pratsinis (2010) also studied the antibacterial activity of nanosilver ions and particles and reported that the

antibacterial activity of Ag⁺ ions and nanosilver particles were the same (27). Shavandi *et al.* (2010) evaluated the inhibitory effect of colloidal silver nanoparticles on three bacterial strains and calculated the MIC of silver nanoparticle solution as 1.56 ppm for *E. coli*, 1.56 ppm for *S. aureus* and 3.125 ppm for *P. aeruginosa* (28). Sadeghi *et al.* in 2011 assessed the effects of silver nanoparticles against *Actinomyces viscosus* and *Streptococcus sanguinis* present in microbial plaque and revealed that silver nanoparticle solution had good antibacterial activity against these strains and this effect was achieved in lower concentrations of the solution compared to chlorhexidine (29).

Based on the mentioned study results, silver nanoparticles have bactericidal properties at low concentrations (30-32). Since the eukaryotic cells are much larger than the prokaryotic cells, they possess more complex structural and functional appendages compared to prokaryotic cells. Thus, in order to have a toxic effect on eukaryotic cells, higher concentrations of silver ions will be required (23). Therefore, it seems distant that silver nanoparticles at low concentrations-at which they are effective against micro-organisms have any toxic effect on eukaryotic cells. However, this issue is in need of further investigation.

It should be noted that the present study evaluated the antibacterial effects of nanosilver solution under in-vitro conditions and these effects may be different in a clinical setting. Obviously, there are differences between the in-vitro (laboratory environment) and in-vivo (oral cavity) conditions. In the oral cavity, saliva plays a role in changing the pH of the mouth and diluting the substances. Also, the temperature in oral cavity is different from the temperature in an incubator. Presence of blood in the environment and variable oxidation and reduction potential at different areas of the oral cavity can also affect the results (34). Another

point worthy of noting is the fact that in tubes and plates containing culture medium, antimicrobial agent is in constant contact with the microorganism; whereas, in the oral cavity, the antimicrobial agent is usually washed off the mouth within a few seconds and its effects are neutralized by the factors present in oral cavity. Furthermore, root canal environment has a unique structure and is significantly different from in-vitro condition. Presence of dentinal tissue, its effects on different materials, the potential of canal irrigation solutions for penetration into the dentinal tubules and their efficacy are important issues worthy of further investigation in future studies.

Based on the results of the present study, despite the adequate antibacterial effect of chlorhexidine against three bacterial strains, it had no antimicrobial effect on *P. aeruginosa*. However, studies comparing the antibacterial activity of chlorhexidine with other antibacterial agents have shown its superior antimicrobial properties against different microorganisms (35). Haffajee *et al.* in 2008 showed that chlorhexidine had greater antimicrobial effects against 40 oral microorganisms compared to other antibacterial agents.

Conclusion:

This study showed that nanosilver solution has favorable antimicrobial properties and once its other characteristics are proved safe, it can be used as an alternative to other canal irrigation solutions.

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