Original Article

Evaluation of Antimicrobial Efficacy of Nanosilver Solution, Sodium Hypochlorite and Normal Saline in Root Canal Irrigation of Primary Teeth

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Abstract

Background: Bacteria are the main etiological factors in the development of dentinal caries and its progression to pulpal and periapical disease. *Enterococcus faecalis* is the bacterial species most frequently recovered from the root-filled teeth. **Aim:** This study aimed to evaluate the antimicrobial effectiveness of nanosilver (NS) solution as an endodontic irrigation solution of primary teeth against *E. faecalis*. **Settings and Design:** Thirty-six canals of primary teeth were selected for this *ex vivo* study. **Methods and Materials:** Thirty-six canals of primary teeth were prepared up to the file #35 and all of the specimens were sterilized. Then, root canals were inoculated with a suspension containing *E. faecalis* bacteria. The teeth were then randomly divided into three groups. Antimicrobial effectiveness was evaluated immediately after dividing into groups by counting colony-forming units on brain heart infusion broth plates. **Statistical Analysis:** Data were analyzed using Kolmogorov–Smirnov, Welch, and Dunnett's T3 tests. **Results:** Sodium hypochlorite showed the highest antimicrobial effectiveness against *E. faecalis* and showed significant differences compared with normal saline and NS solution ($P < 0.001$). **Conclusion:** Based on the results of this study, solution of NS particles can be used as an alternative to other root canal irrigating solutions.

Keywords: *Chemical irrigation, nanosilver, primary molar root canals, sodium hypochlorite*

Introduction

Endodontic therapy is a debridement procedure that requires a removal of the irritants from the canal and periapical tissues if the treatment is to be successful.[1] It is well established that bacteria are the main etiological factors in the development of dentinal caries and its progression to pulpal and periapical disease.[2] *Enterococcus faecalis* is the bacterial species most frequently recovered from the root-filled teeth. Studies have shown that *E. faecalis* can withstand a high alkaline environment such as the one generated by calcium hydroxide.[3]

The resistance appears to be related to a cell proton-pump that is necessary for survival of the bacterium at high pH.[4] The current methods available for bacterial reduction in endodontic therapies include mechanical instrumentation to clean and widen the root canal space, and chemical disinfection by irrigation and intracanal medication, known as an antimicrobial dressing.[5] The use of irrigants in conjunction with mechanical instrumentation is essential for loosening and helping to remove debris and bacteria. It is important that the irrigating solution should also provide antibacterial effects which may include the killing of bacteria in the root canal system and provide disinfection in areas of the canal that are inaccessible to mechanical instrumentation. Canal irrigation solutions should possess characteristics such as low toxicity, low surface tension, lubrication, and odorless. Chlorhexidine, sodium hypochlorite (NaOCl), and tetracycline isomers are among the commonly used root canal irrigation solutions.[6] Numerous irrigation solutions have been recommended for clinical endodontic use.[7,8] NaOCl is the most widely used and has aided canal preparation for many years.[9] NaOCl has a wide range of antimicrobial activities and can 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,[10] and risk of physically changing the structure of dentinal canal walls. The antimicrobial properties of nanosilver (NS) particles have also been previously studied.[11] Decreasing the size

How to cite this article: Moradi F, Haghgoo R. Evaluation of antimicrobial efficacy of nanosilver solution, sodium hypochlorite and normal saline in root canal irrigation of primary teeth. Contemp Clin Dent 2018;9:S227-32.

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Access this article online

Website: www.contempclindent.org

DOI: 10.4103/ccd.ccd_95_18

Quick Response Code:

of silver particles to nanoscale increases their antimicrobial effects, biocompatibility, and effective surface area. A previous study on the antimicrobial effect of NS on *E. faecalis* showed that the effect of low concentrations of NS on *E. faecalis* was equal to 5.25% NaOCl, and these materials both yielded the same diameter of the growth inhibition zone.

The nanosize results in specific physicochemical features that may differ from those of the bulk silver of larger size. Due to these special features, the use of substances in nanosize may have advantages over the use of bulk materials. NS, silver nanoparticles composed of bunches of silver atoms that range in diameter from 1 to 100 nm, has recently been identified as anti-inflammatory and as accelerating wound healing. The biomedical application of NS is an emerging field of research, with a variety of commercially available products being used clinically, such as cardiovascular implants, neurosurgical and central venous catheters, bone cement, and wound dressings.[12]

In addition to bacteria, NS has bactericidal effects on a wide range of fungi, protozoa, and even viruses.[13] The aim of the present study is to evaluate the antimicrobial efficacy of NS solution against *E. faecalis*.

Materials and Methods

Study design

This was an experimental and *ex vivo* study which was carried out on extracted primary molar teeth.

Study setting

This experimental and *ex vivo* study was done in the Department of Pediatric Dentistry, Faculty of Dentistry, Shahed University, between February and June 2017.

The study protocol was approved by the Ethics Committee of Shahed University, Tehran, Iran (reference number: 42/457612).

Inclusion criteria

The root was healthy.

Exclusion criteria

The teeth had not previously had pathologic root resorption involving more than one-third of the root.

Randomization method

Randomization was done using a table of random numbers by an individual blinded to the group.

Grouping

- Group A: NS (10 teeth)
- Group B: 2.5% NaOCl (10 teeth)
- Group C: Saline (10 teeth).

At first, calculus and tissue tags were removed using hand scaling. The teeth were soaked in 5% NaOCl for 24 h to remove any remaining residual loose tissue and debris from the root surface. Then, the teeth were stored sterile in normal saline at room temperature to prevent dehydration. All the teeth were sectioned from cementoenamel junction perpendicular to the long axis with a long cylindrical carbide bur and turbine under excessive irrigation. An ISO #15 K file was used to determine the working length. The root apices were sealed with cyanoacrylate glue to prevent bacterial leakage and then mounted in acrylic blocks for ease of instrumentation. All root canals were instrumented using the step-back technique and the circumferential filing motion up to K file #35. During cleaning and shaping, 2 ml sterile distilled water was used after each instrument size. Finally, the canals were flushed with 5 mL of distilled water to remove any debris. Each tooth was sterilized in steam autoclave for 20 min under 15 psi pressures at 121°C. The bacterial strains used in this study are a known resistant strain of *E. faecalis* (ATCC19433). To create a standard infection in all samples, the primary culture was raised by inoculating *E. faecalis* (ATCC19433) in the brain heart infusion (BHI) broth after incubation at 37°C for 24 h. The canals of the experimental teeth were cautiously inoculated using a sterile 20 µL insulin syringe of the freshly prepared suspension of the organisms with a concentration of 1 McFarland (colony-forming unit [CFU]/ml 3×10^8). The teeth were then incubated at 37°C for 72 h.

After incubation, thirty contaminated roots were randomly divided into three groups ($n = 10$) according to the irrigation regimen used, and three roots were considered as a positive control group and three roots as a negative control group.

- Group A: NS (10 teeth)
- Group B: 2.5% NaOCl (10 teeth)
- Group C: Saline (10 teeth).

All the teeth were handled with sterile gloves and sterile tweezers to prevent contamination. A sterile 20 cc syringe with 28 gauge needle was used to deliver 2 ml of irrigant into the canal. After 15 min, all experimental teeth were then flushed with distilled water to prevent potential carryover of irrigants. A 30 number paper point was then placed into the canals at the working length for 60 s each and also used to soak up the canal contents.

Paper points were transferred to the same tubes containing 1 ml saline and agitated in vortex for 1 min. Aliquots of 500 µl of the appropriate dilutions were cultured into BHI agar plates. All plates were cultivated at 37°C for 24 h. The colonies were identified on the basis of their morphology and counted using a stereomicroscope. Microbial counts were expressed as CFU/ml of sample. The laboratory staff and clinicians evaluating the culture plates were blinded to the group assignment.

Results

The results indicated that large numbers of bacteria were present in the tooth canals [Table 1]. Paired Dunnett's T3

Table 1: Colony-forming unit in each sample in different groups against *Enterococcus faecalis*

Group	*n*	CFU
Group A	1	4.3×10^5
Group A	2	4.82×10^5
Group A	3	4.96×10^5
Group A	4	5.06×10^5
Group A	5	4.76×10^5
Group A	6	5.26×10^5
Group A	7	4.8×10^5
Group A	8	5.02×10^5
Group A	9	4.96×10^5
Group A	10	5.04×10^5
Group B	1	0.4×10^5
Group B	2	0.08×10^5
Group B	3	0.1×10^5
Group B	4	0.06×10^5
Group B	5	0.1×10^5
Group B	6	0.12×10^5
Group B	7	0.08×10^5
Group B	8	0.1×10^5
Group B	9	0.08×10^5
Group B	10	0
Group C	1	1.4×10^5
Group C	2	1.98×10^5
Group C	3	2.6×10^5
Group C	4	2.11×10^5
Group C	5	1.62×10^5
Group C	6	1.7×10^5
Group C	7	2.08×10^5
Group C	8	1.72×10^5
Group C	9	1.96×10^5
Group C	10	2.2×10^5

CFU: Colony-forming unit

test showed a significant difference within the groups, as is shown in Table 2. Significance difference was observed in bacteria reduction following irrigation with NaOCl and NS solution [Table 3]. Based on the results of multiple Dunnett's T3 test, NS solution of 80 ppm had lower antimicrobial properties than 2.5% NaOCl.

Discussion

The use of antimicrobial agents has been recommended as an adjunct to mechanical instrumentation to reduce the number of microorganisms, especially in primary teeth.[14-17]

This study aimed to evaluate the antimicrobial efficacy of NS on *E. faecalis* compared with NaOCl in the endodontic therapies of primary molars, and to determine whether it can be suggested as a new irrigation agent.

The results of this study show that NS is an effective agent for eradication of *E. faecalis*.

Infections in the primary root canal are associated with a broad variety of microorganisms. Cogulu *et al*.[18] found that the most prevalent species of bacteria in deciduous root canals are *E. faecalis, Porphyromonas gingivalis,* and *Treponema denticola*. In this study, *E. faecalis* was selected because it is a Gram-positive facultative anaerobic coccus, which is a well-known endodontic pathogen, for the infection of the root canals. Another reason for choosing *E. faecalis* was the ability of this bacterium to tolerate harsh conditions with scant nutrients and its ability to remain viable in treated root canals for a long time.[19,20] Furthermore, it is capable of surviving at very high pH acidity and hot circumstances. Most studies have shown that there is a high prevalence of *Enterococci* species in persistent root canal infections.[21] In this study, the apices of all sampled teeth were sealed with cyanoacrylate glue to prevent any contamination from the outer tooth surface during the sampling procedure. To eliminate the variable effects of mechanical instrumentation and smear layer removal in reducing bacterial count, both were accomplished before sterilization and inoculation of the sample.

The use of NaOCl has been advocated in concentrations ranging from 0.5% to 5.25%, but there has been no agreement on the optimal concentration. It has been confirmed that 2.5% NaOCl is extremely effective in removing vital pulp tissue from dentinal walls.[22] Although NaOCl is recommended and used by the majority of dentists, severe irritations have been reported when such concentrated solutions have been inadvertently forced into the periapical tissues during irrigation or have leaked through the rubber dam.[23] Furthermore, a 5.25% solution significantly decreases the elastic modulus and flexural strength of human dentin compared with physiologic saline, while a 0.5% solution does not.[24] This has led researchers to evaluate various antimicrobial properties.

Nontoxicity to periapical tissues is an important requirement of endodontic irrigants, especially in pediatric patients. In primary teeth, overflow of irrigating solution through the apical region because of possible resorption areas could damage the underlying permanent tooth. The cytotoxicity of 5.25% NaOCl toward periapical tissues has been stated in the case reports;[25] hence, 2.5% NaOCI was chosen as an appropriate concentration for primary teeth root canal treatment.

Chan EL, *et al*.[26] reported that the novel NS particle irrigant was not cytotoxic to either primary human periodontal ligament stem cells (hPDLSCs) or mouse fibroblast National Institutes of Health 3T3 (NIH 3T3s), both in direct contact and in indirect contact assays. The survival response of primary hPDLSCs and mouse fibroblast NIH 3T3 was similar in exposure to NaOCl and a novel NS particle solution.

Nanotechnology deals with processes that take place on the nanometer scale, that is, from approximately 1–100 nm. It is

Table 2: Descriptive indicators of bacteria in three groups

	n	Mean	SD	SE	95% CI for mean		Minimum	Maximum
					Lower bound	Upper bound		
Nanosilver	10	193,700.00	34,215.169	10,819.786	169,223.94	218,176.06	140,000	260,000
NaOCl	8	9000.00	1851.640	654.654	7451.99	10,548.01	6000	12,000
Saline	10	489,800.00	25,654.976	8112.816	471,447.54	508,152.46	430,000	526,000
Total	28	246,678.57	200,698.789	37,928.506	168,855.71	324,501.44	6000	526,000

SD: Standard deviation; SE: Standard error; CI: Confidence interval; NaOCl: Sodium hypochlorite

Table 3: Multiple comparisons

(I) group	(J) group	Mean difference (I−J)	SE	Significant	95% CI	
					Lower bound	Upper bound
Nanosilver	NaOCl	184,700.000*	10,839.573	0.000	153,470.27	215,929.73
	Saline	−296,100.000*	13,523.519	0.000	−331,779.47	−260,420.53
NaOCl	Nanosilver	−184,700.000*	10,839.573	0.000	−215,929.73	−153,470.27
	Saline	−480,800.000*	8139.186	0.000	−504,223.79	−457,376.21
Saline	Nanosilver	296,100.000*	13,523.519	0.000	260,420.53	331,779.47
	NaOCl	480,800.000*	8139.186	0.000	457,376.21	504,223.79

*The mean difference is significant at the 0.05 level. SE: Standard error; CI: Confidence interval; NaOCl: Sodium hypochlorite

believed that due to their large surface areas, nanoparticles have more power in penetration into microorganisms.

Nanoparticles of silver possess fascinating features which theoretically could be ideal in root canal irrigation. Silver and NS in aqueous solution release silver ions, which are biologically active and actually mediate the bactericidal effect.

One of the important aspects in biomedical applications of silver is the subject of toxicity. Despite the toxicity effect of silver for human cells which have long-lasting exposure to silver, nanotechnology has facilitated the production of smaller silver particles with lower toxicity to human cells,[27] but with greater efficacy against microorganisms. Silver ions interact with three main components of the bacterial cell to produce[28] the bactericidal effect: the peptidoglycan cell wall and the plasma membrane, cytoplasmic DNA, and bacterial proteins.[29,30] Through these mechanisms, this irrigation solution will exert its effects on root canal-infecting microorganisms. The exact mechanism employed by NS particles in causing antimicrobial effects is not clearly known. There are, however, various theories of the action of silver nanoparticles on microbes in causing the microbicidal effect. NS particles can anchor to the bacterial cell wall and subsequently penetrate it, thereby causing structural changes in the cell membrane like the permeability of the cell membrane and death of the cell. There was a formation of "pits" on the cell surface, and there was an accumulation of the nanoparticles on the cell surface.[31] Electron spin resonance spectroscopy studies have suggested that there is a formation of free radicals by the NS particles when in contact with the bacteria, and these free radicals have the ability to damage the cell membrane and make it porous, which can ultimately lead to cell death.[32] It has also been proposed that there can be a release of silver ions by the nanoparticles[33] and these ions can interact with the thiol groups of many vital enzymes and inactivate them.[34]

The bacterial cells in contact with silver take in silver ions, which inhibit several functions in the cell and damage the cells. Then, there is the generation of reactive oxygen species, which are produced possibly through the inhibition of a respiratory enzyme by silver ions and which attack the cell itself. Silver is a soft acid, and there is a natural tendency of an acid to react with a base, in this case, a soft acid reacting with a soft base.[35] The cells are majorly made up of sulfur and phosphorus, which are soft bases. The action of these nanoparticles on the cell can cause the reaction to take place and subsequently lead to cell death. Another fact is that the DNA has sulfur and phosphorus as its major components; the nanoparticles can act on these soft bases and destroy the DNA, which will definitely lead to cell death.[36]

Based on the above-mentioned study results, NS particles have bactericidal properties at low concentrations.[37] Since the eukaryotic cells are much larger than the prokaryotic cells, they possess more complex structural and functional appendages compared with prokaryotic cells. Thus, to have a toxic effect on eukaryotic cells, higher concentrations of silver ions will be required.[38] Therefore, it seems that NS particles at the low concentrations at which they are effective against microorganisms 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 NS 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. Furthermore, the temperature in oral cavity is different from the temperature in an incubator. The presence of blood in the environment and variable oxidation and reduction potential at different areas of the oral cavity can also affect the results.[39] 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 the oral cavity.

According to these results, it appears that NS root canal irrigation solution is an effective agent for eradication of *E. faecalis*. There was a significant difference between NaOCl and the NS irrigant in this *in vitro* study. However, beyond antimicrobial activity, many other properties must also be investigated before making the final choice of an irrigation solution for clinical use. Our goal was to evaluate if NS particles during endodontic treatment resulted in a cleaner root canal. In this study, we used bacterial sampling to indicate the presence of infection in the canal. All the teeth treated with nanoparticles showed a positive reduction in bacterial growth. In the present study, grapheme silver composite nanoparticles showed antimicrobial potential as a root canal irrigation solution with less cytotoxicity effect to the bone or soft tissues.

Considering the lower toxicity of NS on soft tissue and bone, the strength of this study is that its results show the antimicrobial effect of NS on *E. faecalis.*

It is recommended that further investigations are performed to specify the conditions of size, concentration, and ideal morphology of nanoparticles in general to optimize their antimicrobial effect mainly by acting against the resistant root canal microorganisms such as *E. faecalis.* Furthermore, additional studies are needed to evaluate antibacterial effectiveness of endodontic irrigation solution, especially in primary root canals, in different clinical situations.

This study evaluated the antimicrobial effectiveness of NS solution against *E. faecalis* in primary teeth.

One of the limitations of this study was to find appropriate samples, the teeth that have not had pathologic root resorption involving more than one-third of the root.

Conclusion

Based on the results of this study, NS particle solution can be used as an alternative to other root canal irrigating solutions.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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