

# TO EVALUATE THE ANTIBACTERIAL PROPERTIES OF SILVER NANO PARTICLE BASED IRRIGANT AS ENDODONTIC ROOT CANAL IRRIGANT

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## ABSTRACT:

**Aim:** To evaluate the antibacterial properties of Silver Nano Particle based irrigant as endodontic root canal irrigant.

**Methodology:** The Silver Nano Particle based irrigant was tested with reference bacterial strains of *Enterococcus faecalis* (ATCC: 29212). NaOCl was used as control. 1 ml of organism suspension made equivalent to McFarland 0.5 was contacted with 1 ml of irrigation solution and mixture was removed at interval of 3, 5 & 10 min. After 72 hrs of incubation colony counts were measured using a microscope. Inhibition Zones measured with Presterilised Whatman paper discs soaked with 20 µL of irrigant were plated on bacterial plates. Kruskal Wallis test and Post hoc tests i.e Mann Whitney U test and Duncan's-test of multiple comparisons were done.

**Results:** The number of colony forming units dropped to zero after 3 minutes contact time with Silver Nano Particle based irrigant with highly significance ( $P = 0.000$ ) and showed large inhibition zones as compared to NaOCl 3% group.

**Conclusion:** Silver Nano Particle based irrigant is an effective endodontic irrigant.

**Key words:** Silver Nano, NaOCl, Antimicrobial, Irrigation.

## INTRODUCTION:

Endodontic therapy is done with a primary objective of microbial reduction, which in turn promotes the normal healing process of the periodontal tissues [1]. Anaerobic bacteria, especially black-pigmented gram-negatives, have been linked to the signs and symptoms of pulpless teeth. However, facultative microorganisms such as *Enterococcus faecalis*, *Staphylococcus aureus*, and even

*Candida albicans* are considered by many to be the most resistant species in the oral cavity, and one possible cause of root canal treatment failure.[2] An endodontic irrigant should ideally exhibit powerful antimicrobial activity, dissolve organic tissue remnants, disinfect the root canal space, flush out debris from the instrumented root canals, provide lubrication, and have no cytotoxic effects on the periradicular tissues, among other properties. [3]

Nowadays, Various nano-particles have gained popularity as antimicrobial agents as a result of their broad spectrum of activity and biocompatibility. Recent studies have focused on using nano-particulate materials to disinfect root canals. Nanosilver (NS) shows antibacterial effect and also exhibits novel physicochemical and biological activities. [4]

Importantly, the current concept in endodontic microbiology emphasizes endodontic disease as a biofilm-mediated infection. Consequently, elimination or significant reduction of bacterial biofilms is an essential element for the successful outcomes of endodontic treatment. However, clinical studies have shown that even after meticulous chemomechanical disinfection and obturation of the root canals, bacterial biofilm may still persist in the root canal system. Thus, it is vital to develop advanced endodontic disinfection strategies that are effective in eliminating biofilm bacteria within the root canals. [5]

The most commonly used endodontic irrigant is sodium hypochlorite 3%. Instrumentation and irrigation with sodium hypochlorite could eliminate bacteria in 50 to 75% of infected root canals at the end of the first treatment session. It has solvent activity for both necrotic and vital tissues. Negative findings regarding toxicity prompted recommendations to dilute 5.25% NaOCl to lower concentrations of 3%. It has a cytotoxic effect when injected into the periapical tissues, a foul smell and taste, a tendency to bleach, and corrosive

potential. It is also known to produce allergic reactions. Therefore, an equally effective but safer irrigant is desirable. [6]

## MATERIAL AND METHODS:

Two tests were completed to determine the antibacterial activity of NS. In the first one, the Colony forming unit (CFU) of materials against *E. faecalis* was measured and second the zones of inhibition were calculated.

**Bacterial Strain:** *E. faecalis* was chosen as one of the test microorganisms in this experiment for the following reasons: (I) it is a well-recognized pathogen associated with persistent apical periodontitis in endodontically treated teeth [7]; (II) it is resistant to NaOCl, especially at low concentrations [8]; (III) it readily colonizes in dentinal tubules and can penetrate to the entire width of dentin; and (IV) it is easy to culture and it grows rapidly [9,10].

The aim of the study was to Compare and evaluate the as antibacterial and antifungal action of octenidine hydrochloride and chlorhexidine gluconate as root canal irrigant.

**Irrigants:** The irrigant used in the study is AgNP solution and NaOCl 3% is served as control. The irrigant is composed of three components. The major component is Silver nanoparticle phase and two other components are ethanol and Sodium Hydroxide. The combination of these elements may provide an irrigation solution that provides essential capabilities for an ideal endodontic irrigant.

**Bacterial Inoculation of Specimens:** Three reference bacterial strains, *Enterococcus faecalis* (ATCC: 29212) and *Staphylococcus aureus* (ATCC: 25923) and a fungal strain, *Candida Albicans* (ATCC 10231) were obtained from Institute of Microbial Technology, Chandigarh.

Bacterial strains were inoculated on BHI broth supplemented with 1.5% (wt/vol) agar (Himedia laboratories, Mumbai, India) and incubated anaerobically at 37°C for 24 hours. A single colony of *E. faecalis* from a BHI agar plate was collected and suspended in sterile BHI broth at 37°C. Microbial cells were diluted with distilled water to reach the concentration of  $1.6 \times 10^8$  CFU/ml (adjusted to Mc Farland 0.5).

1 ml of each organism suspension was contacted with 1 ml of irrigation solution and subsequently, one hundred microliters of each mixture was removed in 3 min(t3), 5 min(t5) & 10 min(t10) time interval. Each contact period sample is taken and plated on Brain Heart Infusion agar to determine the number of colony forming unit (CFU) per plate. After 72 hrs of incubation at 37°C colony counts were measured using a microscope. The mean number of CFUs in the 3 areas of bacterial growth on each plate was determined and the number of CFU/mL was calculated for each contact period and analysed stastically.

Zones of inhibition were also measured. BHI agar plates were swabbed with the bacteria suspension. Pre-sterilised Whatman paper discs, 6 mm in diameter, were soaked with 20  $\mu$ L of irrigation solution and NaOCl 3% (control). They

were placed on BHI agar plates and incubated at 37°C for 24 h. Zones of inhibition were measured across the diameter with a transparent ruler and recorded. The tests were repeated three times for all strains.

**STATISTICAL ANALYSIS:** A Kruskal Wallis test was conducted on mean number of colony forming units to evaluate differences among the irrigants. Post hoc tests were conducted to evaluate pairwise differences among the groups by using the Mann Whitney U test and Duncan's-test of multiple comparisons.

## RESULTS:

The medians of CFU mL<sup>-1</sup> of *E. faecalis* after the application of the tested irrigation solutions at different contact times i.e. t3, t5 and t10 are given in Table 1.

The number of CFUs dropped to zero after 3 minutes and remained zero after 5 minutes and 10 minutes contact time with 0.1% AgNP solution. Control i.e. NaOCl 3% showed bacterial growth in 3 minutes, 5 minutes and dropped to zero in 10 minutes . A Kruskal Wallis test was conducted to evaluate differences among the irrigants on mean number of CFU. The test was highly significant (P = 0.000). Post hoc tests were conducted to evaluate pairwise differences among groups using Mann Whitney U test. The results of these tests indicated a significant difference between both the irrigants.

The mean values of the inhibition zone of silver nano particle based irrigant is 14mm

as compared to 6mm of NaOCl 3%. Silver nano particle based irrigant showed large inhibition zones. This indicated a good antimicrobial effect for Silver nano particle in agar diffusion test. Significant difference ( $P < 0.001$ ) between both the groups was also apparent.

## DISCUSSION:

In this study, antibacterial effect of Silver nano particle based irrigant on *E. faecalis* was evaluated and compared with that of NaOCl 3%.

The effectiveness of irrigation solutions is directly related to the concentration as well as the volume. The choice of irrigant should be one that rapidly exerts its antimicrobial activity against resistant microorganisms found in the root canal and dentinal tubules. In the present study, Silver nano particle based irrigant significantly reduced the microorganisms within dentinal tubules in a period of 3 min.

Pulpal and periapical diseases are biofilm-mediated infections, and the elimination of bacterial biofilm from the root canal system remains the primary goal of root canal treatment.<sup>[11]</sup> Antibacterial nanoparticles such as chitosan nanoparticles exhibit significant antibacterial activity in biofilm disinfection of root canal.<sup>[12]</sup> Antibacterial nanoparticles did not provide the bacteria any ability to gain resistance against the antimicrobial.<sup>[13]</sup> The pronounced antibacterial efficacy of cationic nanoparticles might be due to the fact that positively charged nanoparticles

electrostatically interact with the negatively charged bacterial cells, resulting in altered cell permeability, leakage of intracellular components, and killing of bacteria.<sup>[14]</sup> The AgNPs interact with multiple targets in the microbial cell, such as cell membrane, enzymes, and plasmids, simultaneously providing the bacteria least capacity to gain resistance.<sup>[13]</sup>

The biologic effects of silver are believed to be closely related to silver ion.<sup>[15,16]</sup> In an aqueous microenvironment, silver nano-particles continuously release silver ion.<sup>[17]</sup> It is well known that smaller silver nano-particles show stronger and better bactericidal effect than larger particles because they have a larger surface area for interaction.<sup>[18]</sup> Binding to essential cellular structural elements like enzymes and other proteins<sup>[19]</sup>, particularly to their SH-groups and interfering with the integrity of the bacterial cell<sup>[20]</sup> are the main reasons for bactericidal properties of silver ions. With regards to cytotoxicity of NS, Miura and Shinohara<sup>[21]</sup> determined the biologic effects of NS exposure to mammalian cells. In their paper, Hela cells were evaluated by being exposed to different concentrations of NS. They concluded that 80 µg/ml concentration could be harmful for Hela cells. Alt et al.<sup>[22]</sup> showed that 1% NS polymethylmetacrylate bone cement was free of *in vivo* cytotoxicity.

Barber et al showed that 5.25% concentration of NaOCl is the most potent amongst three different concentrations of 0.5%, 2.5%, 5.25%.<sup>[23]</sup> It is obvious that

higher concentration have more irritating effects on apical and periapical tissues. [24]

The two other components of this irrigation solution are ethanol and sodium hydroxide. Ethanol is not only a disinfectant but also effectively reduces the surface tension of the solution, which facilitates the penetration of nanosilver containing solution into accessory canals and dentinal tubules. [25] Overall, the combination of these elements may provide an irrigation solution that meets the essential abilities for an ideal endodontic irrigant.

### CONCLUSION:

The silver nanoparticles with their unique chemical and physical properties are proving as an alternative for the development of new antibacterial agents. The silver nanoparticles have also found diverse applications in the form of wound

dressings, coatings for medical devices, silver nanoparticles impregnated textile fabrics, etc. the advantage of using silver nanoparticles for impregnation is that there is continuous release of silver ions and the devices can be coated by both the outer and inner side hence, enhancing its antimicrobial efficacy. The results of this study indicated that silver nano particle based irrigant was significantly effective on the tested microorganisms. NaOCl 3% took significant time for antibacterial property. Cytotoxic properties of NaOCl 3% has to be considered before to be used as an irrigant. From the results of the present study, it seemed reasonable to assume that silver nano particle based irrigant solution is an effective endodontic irrigant with all properties of an effective endodontic irrigant in lower concentrations.

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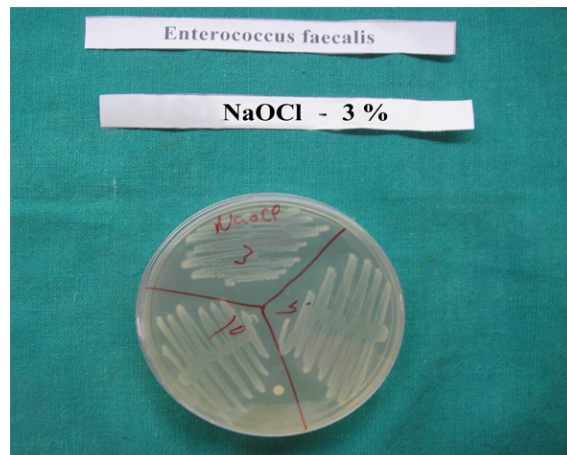
## TABLES:

**Table 1:** The mean of CFU ML<sup>-1</sup> of *E. faecalis* at different time intervals.

All values are in 10<sup>8</sup> CFU/ML. NG is No Growth

	<b>Silver Nano Particle Based Irrigant</b>	<b>NaOCl 3% (CONTROL)</b>
<b>t3</b>	NG	1.6
<b>t5</b>	NG	1.6
<b>t10</b>	NG	1.6

**FIGURES:**



**Figure 1:** Full Growth of E.Facecalis on NaOCl 3%



**Figure 2:** No Growth of E.Facecalis on Silver Nano Particle Based Irrigant



**Figure 3 :** Inhibition zones for Silver Nano Particle Based Irrigant.