Original Article

Antibacterial efficacy of nitrofurantoin impregnated with silver nanoparticles as an intracanal medicament

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Abstract

Aim: This study aimed to evaluate and compare the antibacterial efficacy of nitrofurantoin (Nit) and calcium hydroxide (CH) coated with silver nanoparticle (AgNP) against *Enterococcus faecalis*-infected root canals using flow cytometry.

Materials and Methods: Seventy-eight extracted noncarious human single-rooted teeth were decoronated and chemomechanical preparation was done. The decoronated roots were inoculated with *E. Faecalis* for 21 days. The roots were then grouped depending on the types of intracanal medicaments used, namely, Group 1: Nit paste; Group 2: Nit with 0.02% AgNP suspension; Group 3: CH paste (Ultracal); Group 4: CH paste with 0.02% AgNP suspension; Group 5: 0.02% AgNP suspension; and Group 6: normal saline (0.9%) was taken as control. After 14 days of incubation, dentin chips from root canals were retrieved and transferred into Eppendorf tubes containing distilled water, and flow cytometry analysis was done. The statistical analysis was done using ANOVA.

Results: Flow cytometry analysis revealed that antibacterial activity of intracanal medicament was highest for Nit + AgNp. The least activity was observed for AgNP.

Conclusion: Both Nit and Nit with 0.02% AgNP can be used as potential intracanal medicaments against *E. faecalis*.

Keywords: Calcium hydroxide; *Enterococcus faecalis*; nitrofurantoin; silver nanoparticle

INTRODUCTION

Continuous presence of microbial infection in the root canal system is the main reason behind endodontic failure.[1] There is widespread agreement that intraradicular infection is a significant contributing factor to posttreatment apical periodontitis and a major cause of primary infections.[2] Due to the complexity and unpredictability of the root canal anatomy, as well as the multispecies biofilms, chemomecchanical preparation of root canal is insufficient to reduce the bacterial load.[3] Hence, a combination of instrumentation, irrigation, and intracanal medication is important. *Enterococcus faecalis*, a facultative anaerobe found as a part of the polymicrobial flora inside the canal, is resistant to many of the intracanal medicaments and reason behind failed endodontic therapy.

Various intracanal medicaments such as calcium hydroxide (CH), antibiotics, and steroids have been tried, with CH-based intracanal medicaments being the gold standard. Due to the production of hydroxyl ions, $Ca(OH)_2$ has antibacterial characteristics and produces an environment that is very alkaline with a pH of roughly

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12.5.[4] The microbiology of root canals in retreatment cases, where CH tolerant microorganisms were commonly isolated, was studied, and it was proposed that new drugs may be needed to treat the infection and promote healing.[5]

Local application of antibiotics as intracanal medicament has been an option in endodontics to combat endodontic infections. It improves a number of outcomes including complete or nearly complete bacterial eradication, greater local drug concentrations, and fewer systemic adverse effects.[6] However, the development of bacterial resistance may present a challenge for this approach. The main causes of the development of resistant microbes, which in turn cause the generation of resistance genes, are the improper use of antibiotics.[7] Nitrofurantoin (Nit), a known antibiotic, is frequently used for urinary tract infections.[8] Numerous research suggested that Nit is effective against *E. faecalis*.[6]

Because of its broad-spectrum antimicrobial activity and ease of fabrication, many studies have been conducted for silver nanoparticle (AgNP) against *E. faecalis*.[9] NPs are implicated in the destruction of cell walls and the inhibition of a number of enzymes, including DNA gyrase and DNA-dependent RNA polymerase. In addition, the mechanical, optical, chemical, and electrical properties of NPs can be changed to maximize their advantages.

Therefore, this study aimed to compare and assess the antibacterial efficacy of several intracanal medicaments, such as Nit and CH alone and in combination with AgNPs, against *E. faecalis* using flow cytometry.

MATERIALS AND METHODS

This study was approved by the Ethical Committee of Kalinga Institute of Medical Sciences, KIIT Deemed to be University.

Preparation of intracanal medicaments

AgNP (0.02%) was prepared using green synthesis method by reducing and stabilizing silver nitrate (AgNO3) using *Calotropis gigantea* floral extract. Ultraviolet-visible spectroscopy and dynamic light scattering (DLS) were used to assess the physiochemical characteristics of green synthesized AgNP.

Nit paste was prepared by mixing pure form of Nit (25 mg; Panchsheel Organic Limited, India) with distil water (1 ml). CH paste (Ultracal; Ultradent's product) was used. Both Nit and CH paste were mixed with 0.02% AgNP at 1:1 ratio to prepare the combination product.

Tooth preparation

Seventy-eight extracted noncarious human single-root teeth were collected and stored in saline. Decoronation of each tooth was done below the cementoenamel junction to standardize the length of 14 mm with a diamond disc. To prepare the root canals, Protaper files (Dentsply Maillefer, Switzerland) up to size F3 were used. Sodium hypochlorite, followed by 17% EDTA, was used as the final irrigant. All the prepared teeth were autoclaved at 121°C.

Inoculation of bacterial suspension

In a microbiological safety cabinet, using a sterile endodontic needle, 24 h cultured broth of *E. faecalis* was inoculated in each root canal. The inoculated samples were then kept in an Eppendorf tube and aseptically cultured at 37°C for 21 days. To ensure bacterial viability, the canals were reinoculated every 3 days with fresh bacterial samples.

Placement of intracanal medicament

The canal contents were cleaned with 5 ml of saline after 21 days of incubation and then dried with sterile paper points. After retrieving contents from canal, the roots were divided randomly into six groups ($n = 13$), depending on the medicaments placement – Group 1: Nit paste; Group 2: Nit paste + 0.02% AgNP suspension; Group 3: CH paste (Ultracal); Group 4: CH + 0.02% AgNP suspension; Group 5: 0.02% AgNP suspension; and Group 6: normal saline (0.9%) was taken as control. Using 27-gauge needles, the prepared medicaments were injected into the root canals. Similar to how the medication was given, saline solution was injected into the control group's roots. The canal orifices were covered with a sterile cotton pellet and temporarily filled to seal it. The samples were kept in the incubator for the second time for 14 days at 37°C. The intracanal medicaments were removed from the canals after 14 days by irrigating them with 10 mL of distilled water. Dentinal chips were extracted from whole length of the root canals with a GG drills no 4 and 5 and transferred into Eppendorf tubes containing distilled water.

Flow cytometry analysis

The suspensions in the Eppendorf tubes were stained using the Live/Dead BacLight Bacterial Viability and Counting Kit (Molecular Probes, Invitrogen, USA). Flow cytometry analyses were performed using Cytomics FC 500 (Beckman Coulter, CA, USA).

Statistical analysis

All the data were evaluated using IBM SPSS version 25. The number of live and dead bacteria was presented as mean ± standard deviation and compared among the six groups using ANOVA. $P < 0.05$ was considered statistically significant.

RESULTS

Characterization of silver nanoparticle

As shown in Figure 1, the AgNP showed a specific surface plasmon resonance (SPR) peak at 438 nm. Previous studies

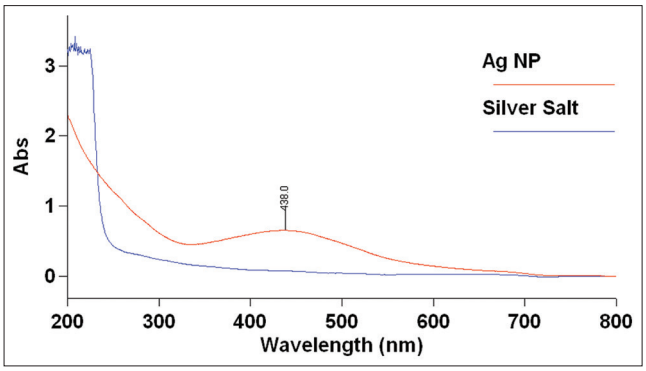

Figure 1: Ultraviolet-visible spectrum of silver nanoparticle showing surface plasmon resonance peak at 438 nm

have attributed SPR peak of 400–450 nm to AgNP.[10] The presence of this peak revealed the presence of AgNP.

DLS assessed the size and zeta potential assessed the surface charge, stability, and surface interaction of nanoparticles with other molecules. As shown in Figure 2, the zeta potential of AgNP was − 18.6 ± 6.08.

Flow cytometry analysis

As shown in Figure 3, the highest antibacterial activity against *E. faecalis* was observed for Nit + AgNp (96.3% dead cells). The least activity was observed for AgNP (80.1% dead cells). There was a statistically significant difference among all the groups ($P < 0.05$).

The mean ± standard deviation values of live bacteria [Table 1] were significantly greater within the negative control group, followed by AgNp, $Ca(OH_2)$, CH + AgNp, Nit, and Nit + AgNP.

The mean ± standard deviation values of the dead bacteria [Table 2] were significantly greater within the Nit + AgNP group when compared to the control. This was followed by Nit, CH + AgNP, CH, and AgNP.

DISCUSSION

Eliminating bacteria from the root canal system and eliminating reinfection are prerequisites for endodontic treatment to be effective. Root canal treatment involves multiple steps, such as irrigation and chemomechanical instrumentation, with the goal of making root canals free of bacteria by 50%–70%.[11] Therefore, 30%–50% of nonbacteria-free root canals may develop intracanal infection and, as a result, periapical infection, which may result in failure of root canal treatment. Henceforth, intracanal medications seem to be a vital step toward complete bacterial eradication. *E. faecalis* recovered from failed root canal cases retains factors that are accountable for its high pathogenicity and persistence inside the root canals.[12] They are significant due to their resistance

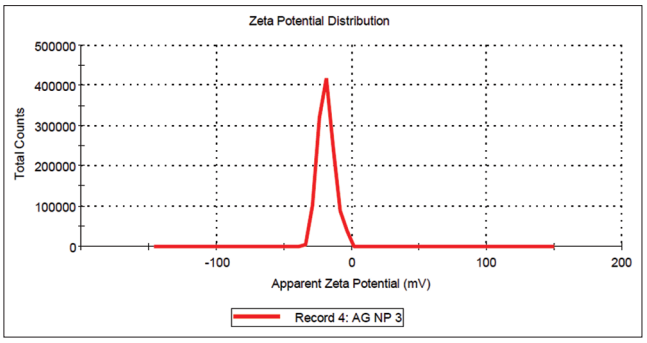

Figure 2: Zeta potential of silver nanoparticle showing the stability of the nanoparticles

Table 1: Intergroup comparison of number of live bacteria among the six groups using ANOVA

Group	Mean±SD	*P*
1. Nitrofurantoin	491.54±7.195	<0.001
2. Nitrofurantoin + AgNP	373.46±6.105	
3. CH	1006.69±4.768	
4. CH + AgNP	889.31±8.750	
5. AgNP	1664.54±5.967	
6. Negative control	9456.62±4.664	

SD: Standard deviation, AgNP: Silver nanoparticle, CH: Calcium hydroxide

Table 2: Intergroup comparison of number of dead bacteria among the six groups using ANOVA

Group	Mean±SD	*P*
Nitrofurantoin	9508.46±7.195	<0.001
Nitrofurantoin + AgNP	9626.54±6.105	
CH	8993.31±4.768	
CH + AgNP	9114.85±16.082	
AgNP	8315.00±92.983	
Negative control	574.15±108.492	

SD: Standard deviation, AgNP: Silver nanoparticle, CH: Calcium hydroxide

to a variety of antimicrobials. As evidence of *E. faecalis* resistance to commonly used intracanal medicament grows, more effort is being made to acquire materials that could eliminate *E. faecalis* completely from the root canals.[13]

$Ca(OH)_2$ has been utilized as an medicament in endodontics since Herman introduced it in 1920.[4] Its antibacterial effectiveness decreases over time and is reliant on coming into contact with microorganisms. In addition, it is not believed that CH is highly efficient at removing microorganisms from the dentinal tubule.[14] Because of its small size, *E. faecalis* can easily enter and survive inside dentinal tubules. However, *E. faecalis* in the dentinal tubules is immune to CH intracanal dressing.[15]

AgNPs have attracted attention due to their unique biological and physical characteristics. When compared to CH, AgNP + $Ca(OH)_2$ demonstrated superior antibacterial efficacy against *E. faecalis*.[16] 0.02% AgNP gel in combination with $Ca(OH)_2$ was more efficient when compared to CH in eliminating *E. faecalis* with minimal to no residual cells.[17]

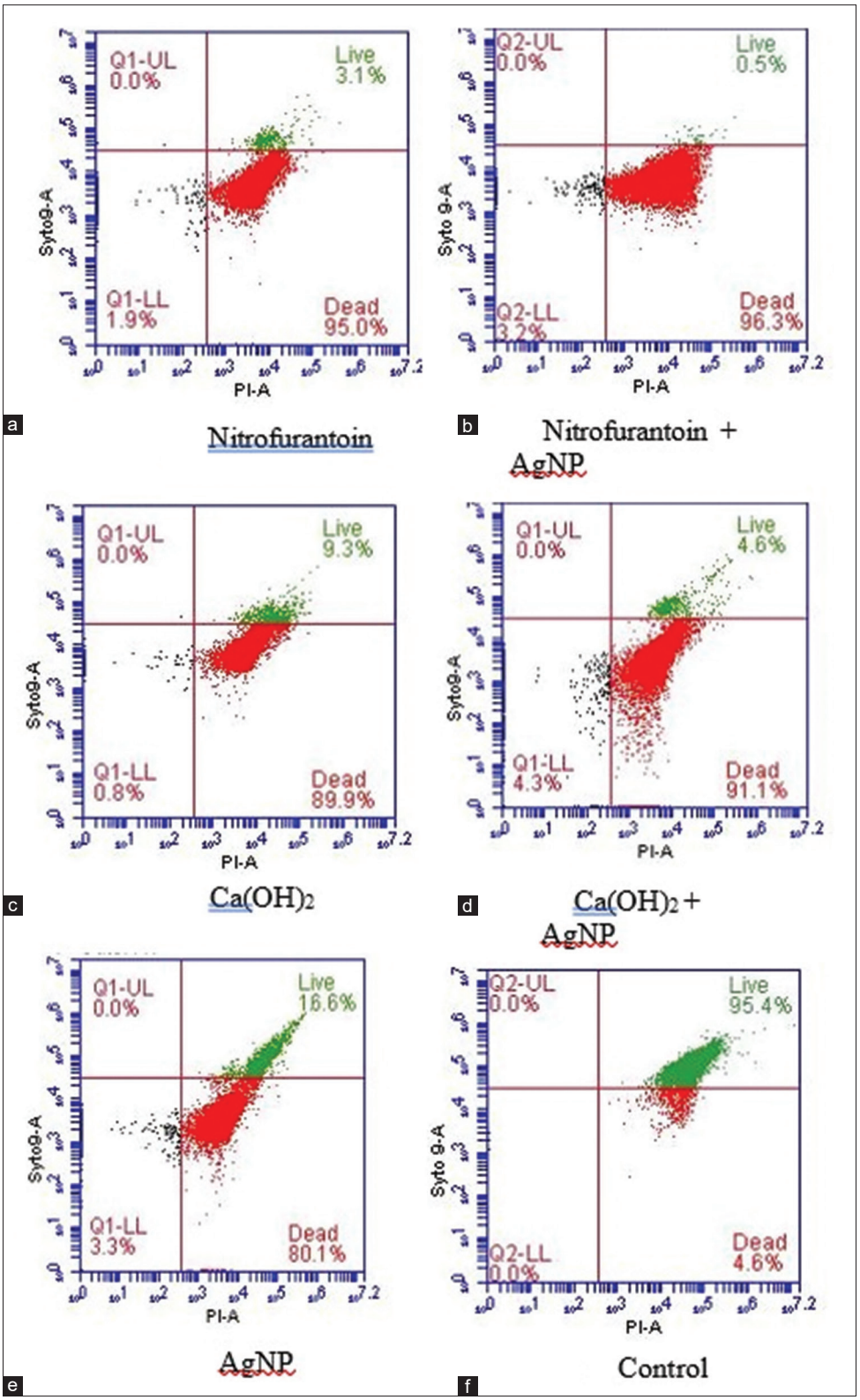

Figure 3: Images presenting plots showing bacterial cells incubated with (a) nitrofurantoin (Nit), (b) Nit + silver nanoparticle (AgNp), (c) calcium hydroxide (CH), (d) CH + AgNp, (e) AgNP, (f) negative control

In the present study, superior antibacterial activity through flow cytometry was exhibited by Nit + AgNP, followed by Nit, CH + AgNP, CH, and the least for AgNP.

Our findings are consistent with those of Alrahman *et al*. They concluded that Nit paste was not only efficacious in completely eradicating *E. Faecalis* but also biocompatible

with rat subcutaneous connective tissue. After being reduced by bacterial flavoproteins, Nit denatures bacterial ribosomal proteins; this phenomenon occurs again with other bacterial macromolecules. Therefore, many essential processes within bacteria will be suppressed, including aerobic metabolism, cell wall synthesis, and DNA and RNA synthesis. Because of the vast array of suppression mechanisms, the development of bacterial resistance to Nit is extremely rare.[6] Nit's biocompatibility can be attributed to its neutral pH of (7.1).[6,18] However, Silva *et al*. proposed in one of their studies that antimicrobial efficacy of Nit against *E. faecalis* is less as compared to doxycycline.[19]

There have been no studies on the combination of Nit and AgNP to date. Vazquez-Muñoz hypothesized the mode of action of combination AgNP-antibiotic treatments in which AgNP simultaneously boost antibiotic internalization in the cell, destabilize bacterial cell membranes, and improve microbicidal activity. Antibiotics that work inside the cell may have a synergistic or additive effect as AgNP facilitates and promotes the antibiotic access to their target.[20] The high antimicrobial efficacy of Nit along with AgNP could be attributed to this synergistic effect.

The present study is in agreement with those of Balto *et al*.; they concluded that CH + AgNP had a larger proportion of dead cells than samples treated with CH and AgNP alone.[21] Afkhami *et al*. found that 1-week exposure to CH + AgNP had a notable alteration in the number of colonies.[16] According to Zhang *et al*., CH + AgNP suppresses *E. faecalis* biofilms at day 1 and 7 than AgNP and CH alone.[22]

Our findings also support the use of multimodal approach which is consistent with the previous finding, in which CH was combined with the AgNP, and chlorhexidine, as the most effective intracanal medicament regimen for eradicating *E. faecalis*.[23,24] The NPs result in the formation of a cavity in the bacterial cell wall, which leads to a decrease in adhesion of bacteria, and eventually, the biofilm formation is prevented. NPs also help reduce bacterial invasion of dentin. Thus, CH and AgNP combination not only improves antibacterial activity but also increases CH residual antibacterial activity as a root canal medicament. CH showed limited antibacterial efficacy against *E. faecalis* as the high pH (which destroy *E. faecalis*) of CH (>11.5) is rare to be achieved inside dentinal tubules, where *E. faecalis* can penetrate deeply because of the buffering effect of dentine.[25] Hence, a combination of CH with AgNP outperformed $Ca(OH)_2$ alone in terms of antibacterial properties. However, when compared to other medications, AgNPs demonstrated the least antibiofilm efficacy in this study. This is in accordance with a study by Balto *et al*.,[21] but contradicts with Wu *et al*.[17] As a result, AgNP can be used as an appropriate vehicle for intracanal medications to eradicate *E. faecalis* bacteria.

CONCLUSION

All experimental groups have antimicrobial activity against *E. faecalis*. However, Nit + AgNP has the highest antimicrobial activity against *E. faecalis*. Moreover, AgNP has shown the least antimicrobial efficacy among all groups against *E. faecalis*.

The use of these novel formulations as intracanal dressings may hold promise for improving the success of endodontic therapy in persistent infections.

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Conflicts of interest
There are no conflicts of interest.

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