An in vitro determination of antibacterial effect of silver nanoparticles gel as an intracanal medicament in combination with other medicaments against Enterococcus fecalis

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Abstract

**Aim:** The main aim of the study is to compare the antibacterial effect of Silver nanoparticle gel alone and combination of silver nanoparticle gel with various medicaments.

**Materials and Methods:** Intracanal dressings: Group 1 – Silver Nanocure gel, Group 2 - Silver Nanocure gel + Cavisept gel(1:1), Group 3 - Silver Nanocure gel + Aveu-Cal gel(1:1) , Group 4 – Silver Nanocure gel + Cavisept gel + Aveu-Cal gel(1:1:1) were taken on a culture plate inoculated with E. faecalis. Antibacterial activity was assessed using Agar diffusion test and results were noted as diameter of growth inhibition zone.

**Statistical Analysis:** Student t –test was used to analyse results.

**Results:** The diameter of combination of Silver nanocure gel+Cavisept + Aveu-Cal gel(1:1:1) was highest in comparison to other medicaments tested.

**Conclusion:** Intracanal dressing with a combination of all the three {Silver nanocure gel+Cavisept + Aveu-Cal gel(1:1:1) } is the best treatment for elimination of highly resistant Enterococcus faecalis in root canals.

**Keywords:** Agar diffusion test, inhibition zone, medicaments

INTRODUCTION

Bacteria and their by-products are responsible for various pulpal and periapical diseases.¹ ² Endodontic infections can be classified as biofilm-mediated infections. Enterococcus faecalis is the most dominant biofilm forming bacteria found in teeth with periradicular pathologies.³ It has an intrinsic resistance to irrigant solutions, intracanal medicaments, several antibiotics, and highly alkaline pH. It has the ability to tolerate starvation, harsh environment, and invade deeply into the dentinal tubules.³ ⁴ To achieve complete success in root canal treatment, suppression of the biofilm is necessary. Cleaning and shaping of the root canal along with irrigation protocol have been shown to effectively decrease the number of microorganisms in the root canal. However, these procedures are unable to completely eliminate bacteria from lateral canals, isthmuses, and apical deltas. The augmentation of the above antibacterial protocol is achieved by utilizing intracanal medicaments.⁴ ⁶

Calcium hydroxide and chlorhexidine have been used since a long time for the removal of E. faecalis. Recently, nanoparticles have come into limelight. Nanoparticles are a class of newer medicaments which are hypothesized to have antibacterial effect. They cause disruption of

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the biofilm due to their nano size and structure. The nano size provides increased surface area which can absorb other medicaments and exert antimicrobial effect.\textsuperscript{[7,8]} Silver nanoparticles are commonly used as they show strong bactericidal potential against Gram-positive, Gram-negative, and multidrug-resistant bacteria.\textsuperscript{[9,10]} Silver has an ability to interact with bacterial cell wall leading to structural changes and then damaging the tissue protein.

In this study, we compared and evaluated the antibacterial effect of silver nanoparticles alone and combination of silver nanoparticles with calcium hydroxide and chlorhexidine against \textit{E. faecalis}. The study will help us evaluate and find the medicament which is highly efficacious in the removal of \textit{E. faecalis} and hence will lessen the number of endodontic failures.

MATERIALS AND METHODS

\textbf{Culturing enterococcus faecalis}

Pure culture of \textit{E. faecalis} (ATCC 29212) was used as the test microorganism. Bacterial colonies were isolated after 24 h of incubation and then suspended in 5 ml of brain heart infusion broth. They were incubated at 37°C for 4 h. After culturing \textit{E. faecalis} in the culture media, 0.5 McFarland standard of the bacterial suspension was prepared and cultured on Mueller–Hinton agar culture medium by a sterile swab in all directions. Then, in each culture plate, 5 wells measuring 6 mm in diameter and 2 mm in depth were created with a sterile pipette for placement of the material samples.

\textbf{Division into groups}

Four groups were made according to the medicament used:

- Group 1 – Silver nanocure gel
- Group 2 – Silver nanocure gel + Cavisept gel (1:1)
- Group 3 – Silver nanocure gel + Aveu-Cal gel (1:1)
- Group 4 – Silver Nanocure gel + Cavisept gel + Aveu-Cal gel (1:1:1).

\textbf{Microbiologic testing}

The wells were then filled with respective medicament from each group. The process was repeated five times and all microbial tests were performed under aseptic conditions. The plates were then incubated at 37°C for 1 week, and the diameter of the growth inhibition zone was evaluated at 24 h, 48 h, and 1 week after culture. The mean of the three values was calculated and reported as the diameter of zone of inhibition. The results were analyzed using student’s \textit{t}-test.

\textbf{RESULTS}

Antibacterial activity of all the three medicaments in different combinations is shown in Table 1. The results obtained revealed statistically significant difference in the antibacterial effect of combination of silver nanocure gel with various medicaments in comparison to silver nanocure gel alone. Silver nanocure gel in combination with calcium hydroxide (Group 2) was more efficacious as compared to silver nanocure gel alone (Group 1) \((P < 0.00001)\). Antibacterial activity of silver nanocure gel in combination with chlorhexidine (Group 3) was also significantly higher than silver nanocure gel alone \((P < 0.00001)\). When silver nanocure gel was combined with both calcium hydroxide and chlorhexidine (Group 4) the antibacterial activity was again higher in comparison to silver nanocure gel used alone \((P < 0.00001)\). The zone of inhibition for combination of all the three medicaments was maximum [Figure 1].

\textbf{DISCUSSION}

Root canal disinfection is the mainstay of successful endodontic treatment.\textsuperscript{[11]} Major challenges involved in achieving the above-mentioned goal are the microorganism-induced biofilms and the limitations associated with the currently used intracanal medicaments and irrigants in eliminating the same. Hence, in the present study, the effectiveness of four medicaments was studied and their efficacy against \textit{E. faecalis} was estimated. The nanoparticles and their various combinations have been used as intracanal medicaments and their effect on \textit{E. faecalis} is measured by observing the diameter of zone of inhibition in the agar diffusion test.

\textit{E. faecalis} was used as a test organism in the study as it has a major role in the etiology of persistent endodontic infections. It is majorly responsible for root
When silver nanocure gel (Group 1) was used alone, it showed slight antibacterial activity against *E. faecalis*. This can be explained by the fact that silver ions released due to oxidation of nanoparticles bind to the cell membrane and penetrate inside the bacteria and react with specific proteins. This alters the bacterial metabolism and inhibits vital enzymatic systems, such as respiratory process and cellular division, resulting in cell death. The results in the present study are in agreement with the study done by Kim et al.[15]

In Group 2, where silver nanocure gel was used in combination with Cavisept gel showed a more significant antibacterial activity in comparison to silver nanocure gel alone, but it was significantly lesser than the other combinations tested (*P* < 0.0001). Chlorhexidine has a unique property of substantivity.[16-19] However, recent studies have shown that biofilm formation by *E. faecalis* is a major hindrance in the working of chlorhexidine.[20] To increase the efficacy of cavisept gel, silver nanoparticles have been added to it. Its scientific basis is that silver gets ionized and destroys the cell membrane allowing the penetration of chlorhexidine leading to cell death.[21] This helps in increased removal of *E. faecalis* from the root canals and its efficient cleaning.

In Group 3, silver nanocure gel in combination with Aveu-cal gel was used and this also proved significantly more effective against *E. faecalis*. This finding is consistent with other studies.[22-23] This finding was in agreement with the findings of other studies which suggested combination of silver nanoparticles and calcium hydroxide to be better in comparison to silver nanoparticle gel and calcium hydroxide gel used alone.[23] It has been observed that silver nanoparticles from silver nanocure gel create pits in the cell wall of microorganism which leads to disruption of the biofilm.[23,24] The other mechanism of action is increased amount of calcium hydroxide delivery due to changes in bacterial permeability. The release of hydroxyl ions in the root canal environment kills bacteria by damaging the cytoplasmic membrane, denaturation of proteins, and damaging DNA.[24,25] Hence, the above combination helps in effective elimination of *E. faecalis*. The Group 4 where silver nanocure gel was used in combination with Cavisept gel and Aveu-cal gel, showed most significant reduction in the *E. faecalis* activity. All the three medicaments combined have been shown to have a synergistic effect and hence have been able to cause maximum eradication of *E. faecalis* from the microbial flora of root canals. This was observed in our study. As per our knowledge, this is the first study done so far in the English literature that has tested all three medicaments in combination.

**Limitations**
The limitation of the study is its *in vitro* nature. The above findings need to be tested *in vivo*. The strength of the study is the efficacy of all the three intracanal medicaments studied with latest scientific approach that is in combination with nanotechnology, which is the future of dentistry. We are hopeful of a bright future of these small particles.

**CONCLUSION**

In this *in vitro* study, it may be concluded that silver nanocure gel, cavisept gel, and Aveu-cal gel combination is the best among for elimination of *E. faecalis* from the root canal.

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**Conflicts of interest**
There are no conflicts of interest.

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