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ORIGINAL RESEARCH

Antimicrobial Efficacy of Mixtures of Nanosilver and Zinc Oxide Eugenol against *Enterococcus faecalis*

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ABSTRACT

Aim: This study aimed to assess the antimicrobial efficacy of 0, 0.5, 2, and 5 wt% nanosilver in conjunction with zinc oxide eugenol (ZOE) against *Enterococcus faecalis*.

Materials and methods: Nanosilver in 0.5, 2, and 5 wt% concentrations was added to ZOE and the antibacterial activity of the mixtures on *E. faecalis* was assessed using disk diffusion method, and the results were reported as the diameter of the growth inhibition zone.

Results: The diameters of the growth inhibition zones around 0, 0.5, 2, and 5 wt% concentrations of nanosilver particles were not significantly different at 24 and 48 hours and 1 week; however, the difference with the azithromycin disk was significant.

Conclusion: Considering the lack of a significant increase in the diameter of the growth inhibition zones around 0, 0.5, 2, and 5 wt% ZOE containing nanosilver, it appears that addition of nanosilver up to 5 wt% cannot improve the antibacterial properties of ZOE sealer against *E. faecalis*.

Clinical significance: Microorganisms present in the root canal system of primary teeth are mainly responsible for endodontic infections. *Enterococcus faecalis* is the most important cause of endodontic failure. Application of sealers that decrease the adhesion and colonization of bacteria, as well as susceptibility to bacterial infections can greatly help in this regard. Using these sealers in conjunction with antibacterial agents, such as nanosilver particles may yield higher antibacterial efficacy.

Keywords: Antibacterial agents, *Enterococcus faecalis*, Laboratory research, Nanosilver, Zinc oxide eugenol cement.

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INTRODUCTION

Bacteria present in the root canal of primary teeth are the main cause of endodontic infection.[1] Root canal treatment aims to eliminate the microorganisms and to improve the periapical status.[2] *Enterococcus faecalis* is the most frequently (and sometimes the only) isolated bacterial strain from the endodontically treated root canals and is the most important causes of endodontic treatment failure.[3] It has been isolated from carious lesions and chronic periodontitis and chronic apical periodontitis cases. The role of this microorganism in the pathogenesis of endodontic infection has not been well understood; however, it is often the dominant bacterial strain in both the primary and secondary infections.[4] These microorganisms can be eliminated via mechanical debridement, intracanal irrigation with proper irrigants, and filling the root canal with the root canal filling materials.[5] Pulpectomy of the primary teeth is an appropriate method for saving the infected teeth. High success rate of this method greatly depends on the efficacy of canal debridement, adequate irrigation, and type of material used for canal obturation and sealing.[6] Zinc oxide eugenol (ZOE) sealer has a long history of being used as a root canal filling material. Although ZOE can be irritating for the periapical tissue to some extent, advantages, such as adequate dimensional stability, proper solubility, and high antimicrobial activity have made it a suitable material for pulpectomy. The antibacterial effect of ZOE on the bacteria present in the root canals of primary teeth has been previously documented.[5]

The antimicrobial properties of silver nanoparticles have also been previously studied. Decreasing the size of

silver particles to nanoscale increases their antimicrobial effects, biocompatibility, and effective surface area.[7-9] A previous study on the antimicrobial effect of nanosilver on *E. faecalis* showed that the effect of low concentrations of nanosilver on *E. faecalis* was equal to 5.25% NaOCl, and these materials both yielded the same diameter of the growth inhibition zone.[3] Another study also showed the antibacterial effect of ZOE on *E. faecalis*. However, its efficacy was reported to be significantly lower than that of Diapaste.[10] A study on the antibacterial efficacy of ZOE sealer revealed that ZOE was effective against *Streptococcus mutans* and *Prevotella melaninogenica*.[11] Another study also indicated the antibacterial effect of nanosilver on *E. faecalis*.[12]

Some previous studies evaluated the efficacy of combinations of silver nanoparticles with other materials with antimicrobial properties, such as glass ionomer cements and showed that addition of nanosilver to these materials improved their antimicrobial efficacy. Moreover, the antimicrobial efficacy of nanosilver has been reported to be higher than that of zinc or gold nanoparticles against oral *Streptococci*, which are the main causes of dental caries.[7,13-16] Furthermore, the bactericidal effect of nanosilver on *E. faecalis* has been documented.[17]

Considering the importance of cleaning the primary root canals and the confirmed antibacterial efficacy of nanosilver, this study aimed to assess the antibacterial efficacy of nanosilver added in 0.5, 2, and 5 wt% concentrations to ZOE against *E. faecalis*.

MATERIALS AND METHODS

This *in vitro* study had the following experimental design.

Preparation of ZOE Sealer containing Nanosilver

Silver nanoparticles (Ag nanopowder; Nanoshel LLC, Delaware, USA) with a mean particle size of 20 nm in 0.5, 2, and 5 wt% concentrations were added to ZOE sealer (Dentonics; Master Dent, Monroe, NC, USA). To obtain a homogeneous mixture, it was mixed in a mortar. A sample of ZOE sealer without nanosilver was also prepared.

Disk Diffusion Test

After culture and activation of *E. faecalis* (ATCC 4082) in specific culture media, 0.5 McFarland standard of the bacterial suspension was prepared and cultured on Mueller–Hinton agar culture medium (BIOLAB, Mueller–Hinton Agar; BioLab Inc., Hungary) by a sterile swab in all directions. Next, in each culture plate, 5 wells with 6 mm diameter and 2 mm depth were created with a sterile pipette for placement of the material samples. The ZOE sealer was mixed on a sterile glass slab with a plastic spatula according to the manufacturer's instructions and was then injected into the wells using a sterile syringe. This process was repeated five times and all microbial tests were performed under aseptic conditions. Next, the specimens were incubated at 37°C for 1 week, and the diameter of the growth inhibition zone was evaluated at 24 and 48 hours and 1 week after culture.

The susceptibility of *E. faecalis* (ATCC 4082) to the study materials was tested five times using the disk diffusion method. Data were entered into the computer, and the diameter of the growth inhibition zone at three points perpendicular to each other was measured using a software program. The mean of the three values was calculated and reported as the diameter of the growth inhibition zone.

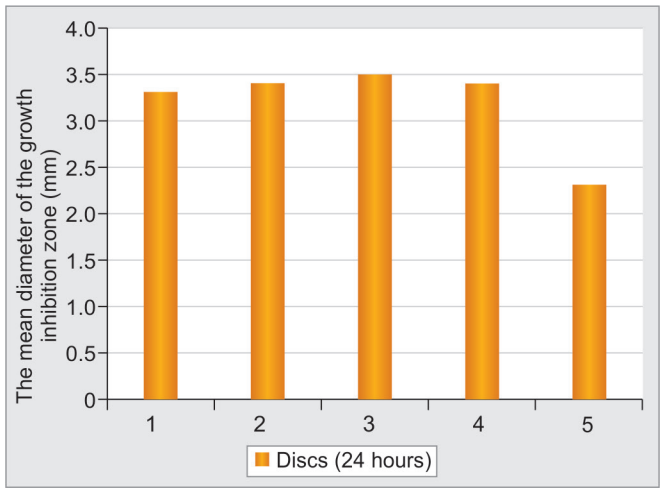
Method of Sampling

A 0.5 McFarland standard of the microbial suspension was prepared, and a specific volume of the solution was randomly used for testing. After sampling and data collection, the results were analyzed using analysis of variance (ANOVA), and the mean diameter of the growth inhibition zone at different time points was analyzed using Duncan's new multiple range test. The diameter of the growth inhibition zone was measured in three different points using a software program, and the mean of the three values was calculated and reported.

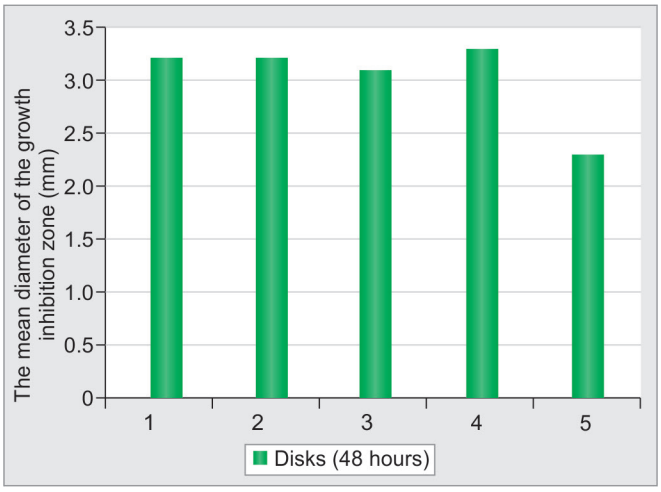
Data were analyzed using ANOVA and Duncan's new multiple range test (considering the distribution of data). After descriptive analysis of data, repeated measures ANOVA with the effect of treatment groups as the independent variable was used for further analysis of the data.

RESULTS

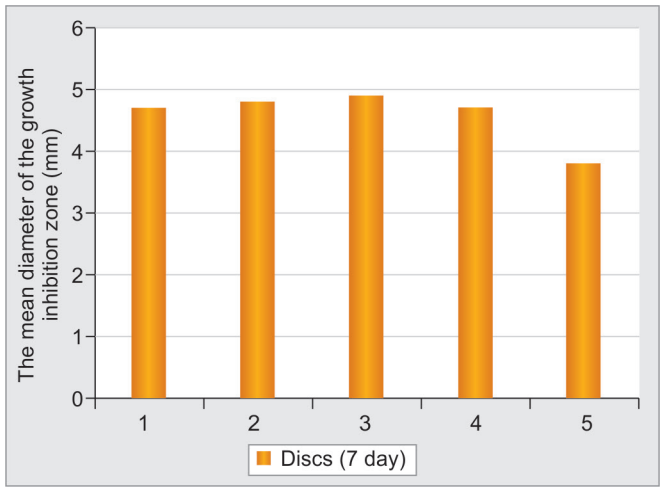
This study evaluated the antimicrobial effect of 0, 0.5, 2, and 5 wt% concentrations of nanosilver added to ZOE on *E. faecalis* using the disk diffusion method in agar medium. The following results were obtained (Graphs 1 to 4 and Table 1).



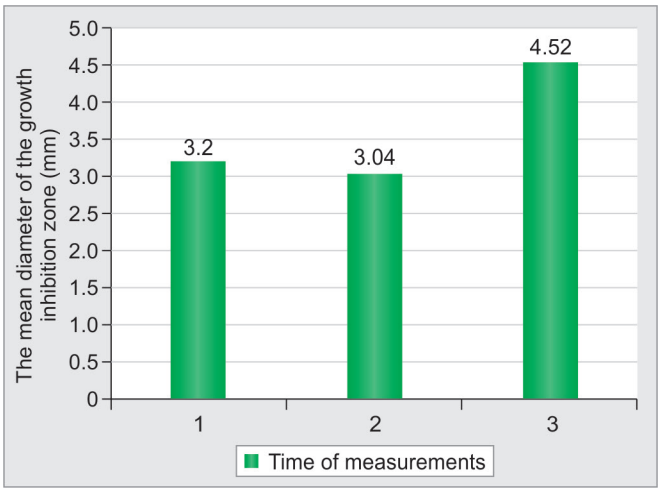
Graph 1: The mean diameter of the growth inhibition zone at 24 hours (columns 1 to 4 indicate 0, 0.5, 2, and 5 wt% concentrations of nanosilver and column 5 indicates azithromycin disk)



Graph 2: The mean diameter of the growth inhibition zone at 48 hours (columns 1 to 4 indicate 0, 0.5, 2 and 5 wt% concentrations of nanosilver respectively, and column 5 indicates azithromycin disk)



Graph 3: The mean diameter of the growth inhibition zone at 1 week (columns 1 to 4 indicate 0, 0.5, 2, and 5 wt% concentrations of nanosilver respectively, and column 5 indicates azithromycin disk)



Graph 4: The mean diameter of the growth inhibition zone around disks at 24 hours (column 1), at 48 hours (column 2), and at 1 week (column 3)

Table 1: The mean diameter of the growth inhibition zone at 24 and 48 hours and 1 week

S.O.V	*df*	*24 hours*	*48 hours*	*7 days*
Media	4	0.361	0.181	0.319
Disk	4	1.209**	0.765**	1.029**
Error	16	0.037	0.033	0.099

The mean diameters of the growth inhibition zones were not significantly different around the four concentrations of nanosilver at 24 and 48 hours; however, the diameters significantly increased at 1 week.

Disk Diffusion Test

In the disk diffusion test, the diameters of the growth inhibition zones around 0, 0.5, 2, and 5 wt% concentrations of nanosilver at 24 and 48 hours and 1 week were not significantly different; however, the difference in this regard with the azithromycin disk was highly significant.

DISCUSSION

Enterococcus faecalis can cause pulpal and periapical infections. Root canal treatment is performed with an aim to eliminate the microorganisms and to improve the periapical status of teeth. The ZOE sealer has a long history as root canal filling material. High antimicrobial activity of ZOE makes it a suitable material for pulpectomy. Many attempts have been made to improve the properties of ZOE sealer. Silver and its compounds have a long history of being used as antibacterial and disinfecting agent. This study evaluated the antibacterial efficacy of 0, 0.5, 2, and 5 wt% concentrations of nanosilver added to ZOE sealer against *E. faecalis* using the disk diffusion method.[1-3,6] The results of the disk diffusion method, in this study, showed that all disks containing different weight percentages of nanosilver formed growth inhibition zones. However, the diameters of the growth inhibition zones around 0, 0.5, 2, and 5 wt% concentrations of nanosilver at 24 and 48 hours and 1 week were not significantly different. The mean diameter of the growth inhibition zone around disks did not significantly increase at 48 compared with 24 hours. However, measurements at 1 week revealed a significant increase in the diameter of the growth inhibition zones. It appears that addition of different percentages of nanosilver to ZOE sealer did not cause a significant change in antibacterial efficacy.

Kaiwar et al[2] evaluated the effect of ZOE on *E. faecalis* using the disk diffusion method. They evaluated the diameter of the growth inhibition zones around four commonly used sealers for the root canal treatment of primary teeth in the culture media containing *E. faecalis* after 24 and 48 hours and noted a significant difference in the diameter of the growth inhibition zones around

ZOE sealer compared with other sealers. Formation of a growth inhibition zone around ZOE sealer at 24 and 48 hours in their study was similar to our findings regarding the formation of growth inhibition zone around the ZOE disk without (0% concentration) nanosilver.

Anumula et al evaluated the antimicrobial activity of ZOE sealer on *E. faecalis* using direct contact test. They assessed the kinetics of bacterial growth following exposure to ZOE and reported a significant difference between the antimicrobial activity of ZOE sealer and other sealers on *E. faecalis*. Apparently, they used direct contact test to overcome the shortcomings of agar diffusion test. The optical density values in their study were measured by a spectrophotometer every 30 minutes, for 16 hours. They reported that sealers lost their antimicrobial property over time. In contrast, we found a significant increase in the antimicrobial efficacy of ZOE sealer over time; such finding in our study may be due to the addition of nanosilver to ZOE.[18]

Poggio et al, in their study in 2011, used agar diffusion test and showed that Argoseal containing ZOE and silver powder along with liquid eugenol caused the largest growth inhibition zone. However, they did not mention anything regarding the effect of incorporation of silver in this sealer, and they solely attributed the antimicrobial activity to the presence of eugenol in its formulation. This result is in line with our findings since we did not find any significant difference in the diameter of the growth inhibition zone around the nanosilver containing sealer and the plain ZOE sealer, either.[19]

Mussolino et al compared the antibacterial effect of ZOE sealer on Gram-positive and Gram-negative bacteria isolated from primary teeth with endodontic infections and showed that the antimicrobial effect of ZOE sealer on Gram-negative bacteria was greater than that on Gram-positive bacteria, such as *E. faecalis*. They stated that liquid eugenol was mainly responsible for the antimicrobial efficacy of this sealer.[1]

Toosi et al compared the efficacy of calcium hydroxide-based and eugenol-based sealers and reported that calcium hydroxide-based sealers had significantly greater antimicrobial effects on *E. faecalis*. They added that calcium hydroxide-based root canal filling materials showed antibacterial activity due to the release of hydroxyl ions and resulted in denaturation of proteins and disintegration of bacterial DNA. Furthermore, the alkaline pH of these materials can deactivate the bacterial cell membrane enzymes.[10]

CONCLUSION

It can be concluded that different results obtained by direct contact test and disk diffusion method may be due to the difference between the solid and liquid media and also the difference in the release pattern of the antibacterial agents present in the cements in these two media. It appears that higher contact between the bacteria and antibacterial agents in the broth medium may have contributed to this finding. However, the results of direct contact test in the broth medium indicated the positive effect of the addition of silver nanoparticles to glass ionomer cement. Thus, it can be concluded that addition of up to 5 wt% of silver nanoparticles to ZOE sealer has no clinical efficacy for root canal treatment of primary teeth. The current study focused on the antimicrobial efficacy of nanosilver in 0, 0.5, 2, and 5 wt% concentrations in ZOE sealer. Future studies are required to assess the antibacterial susceptibility of other bacterial species involved in endodontic infections to the mixture of ZOE sealer and nanosilver. Moreover, the cytotoxicity of nanosilver and its biocompatibility for use in the clinical setting must be evaluated. The efficacy of addition of specific amounts of nanosilver to different sealers used in the endodontic treatment of primary teeth can also be the subject of future studies to further assess the change in their antimicrobial properties.

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