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Silver Nanoparticles; Endoflas; AH Plus; Endodontic Sealer; *Enterococcus Faecalis*; *Staphylococcus Aureus*; Bacterial Viability.

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Evaluation of the Antimicrobial Efficacy of Silver Nanoparticle-Incorporated Endodontic Sealers Against Enterococcus faecalis and Staphylococcus aureus

Abstract
This in-vitro experiment compared the antimicrobial activity of silver nanoparticle-impregnated endodontic sealers to Enterococcus faecalis and Staphylococcus aureus at the sealer-dentin interface. Endoflas and AH Plus are two endodontic sealers that were impregnated with silver nanoparticles in concentration of 0.05, 0.1, and 0.5%. Removed single-rooted human teeth were cut into blocks of dentin, incubated individually with *E. faecalis* and *S. aureus*, and subjected to the experimental and control sealer formulations. Confocal laser scanning microscopy in conjunction with LIVE/DEAD bacterial viability staining was used to determine bacterial viability. Live bacterial cells were green fluorescence, and non-viable cells were red fluorescence. These findings revealed that there was a reduction in the percentage of live bacteria with respect to concentration post incorporation of the silver nanoparticles. Endoflas + 0.5 percent silver nanoparticles proved to be the most effective formulation tested, with a significant difference in bacterial viability, in particular against *E. faecalis.* AH Plus, too, displayed enhanced antimicrobial activity following incorporation with silver nanoparticle although the effect was more pronounced at elevated concentrations. *E. faecalis* tended to be more persistent than *S. aureus*, particularly in the AH Plus and the silver nanoparticle-alone group. In general, the results indicate that silver nanoparticles can be used to augment the antimicrobial efficacy of endodontic sealers and minimize the remaining bacteria survival at the sealer-dentin interface. Additional research is needed to determine the long-term antimicrobial stability, cytotoxicity, biocompatibility, and physical characteristics before clinical use.

1. Introduction

The microbial nature of endodontic infections is mostly persistent as microorganisms can enter the anatomical complexities within dentinal tubules, lateral canals, isthmuses, and sealer-dentin interface. Despite chemomechanical preparation and irrigation, biofilms can still harbor residual bacteria, which are less susceptible to traditional antimicrobial agents. The ongoing survival of microorganisms is a significant issue since it may undermine periapical healing and be a predisposing factor to post-treatment disease. Thus, antimicrobial activity can be taken as a significant feature of endodontic materials, in particular, sealers, which continue to be in direct contact with dentinal walls following obturation. Sealers with improved antibacterial potential may help reduce residual bacterial viability at the sealer–dentin interface and improve the biological success of root canal treatment [1,2].

Among endodontic pathogens, *Enterococcus faecalis* is one of the most frequently studied microorganisms because of its ability to survive harsh environmental conditions, invade dentinal tubules, resist starvation, and form persistent biofilms. Its association with failed endodontic treatment makes it a suitable organism for evaluating antimicrobial materials used in root canal therapy. Previous investigations have shown that *E. faecalis* biofilms can survive despite the use of intracanal medicaments and antimicrobial irrigants, which emphasizes the need for modified endodontic materials with enhanced antibacterial efficacy [3,4]. *Staphylococcus aureus* is also clinically relevant as an opportunistic pathogen with biofilm-forming ability and has been used in antimicrobial testing of endodontic sealers. Its inclusion allows broader evaluation of antibacterial effectiveness against Gram-positive organisms that may contribute to persistent oral and endodontic infections [1].

Endodontic sealers are essential components of obturation because they fill gaps between gutta-percha and root canal dentin, seal irregularities, and reduce microleakage. However, conventional sealers may not provide sufficient long-term antimicrobial activity once they are set. Therefore, researchers have focused on modifying sealers with nanoparticles to enhance their antibacterial performance without compromising their physicochemical properties. Endoflas, a zinc oxide–based sealer, and AH Plus, a resin-based epoxy sealer, represent two different material categories used in endodontics. Their different compositions may influence nanoparticle interaction, antimicrobial release, and antibacterial behavior. Comparative evaluation of these sealers after silver nanoparticle incorporation is therefore important to determine whether sealer type affects antimicrobial performance [5].

Silver nanoparticles have attracted considerable attention in dentistry because of their broad-spectrum antimicrobial activity, small particle size, large surface area, and ability to interact with bacterial cell walls and membranes. Their antibacterial action has been associated with silver ion release, disruption of membrane permeability, oxidative stress, protein dysfunction, and interference with DNA replication. In endodontics, silver nanoparticles have been studied in combination with irrigants, medicaments, calcium hydroxide, and sealers to improve antibiofilm efficacy against resistant organisms such as *E. faecalis* [4,6]. Recently, it was also shown that incorporation of silver nanoparticles in endodontic sealers can result in improvements in antimicrobial effects, though the effects can be variable, depending on nanoparticle concentration, composition of the sealer and bacterial species tested [7,8].

It is vital to test various nanoparticle concentrations since the antimicrobial efficacy might increase with the concentration of nanoparticles, but higher concentrations can also affect the sealer properties, cytotoxicity, setting behavior, solubility and dentin adaptation. Thus, concentration-based assessment can be used to identify formulations that offer an antimicrobial advantage, and that could be used in clinical practice. Recent reports on nanop/article-modified sealers have stated that antimicrobial efficacy needs to be regarded along with physical and biological characteristics, such as bond strength, marginal adaptation, and cytocompatibility [5,9].

Though some studies have evaluated antimicrobial activity of endodontic sealers or silver nanoparticles individually, there is a lack of evidence that compares antimicrobial activity of silver nanoparticle-modified Endoflas and AH Plus against *E. faecalis* and *S. aureus* in different concentrations. Therefore, the study at hand will assess the antimicrobial effectiveness of silver nanoparticles in endodontic sealers against Enterococcus faecalis and Staphylococcus aureus. The aims are to test 0.05 per cent, 0.1 per cent and 0.5 per cent silver nanoparticles incorporated in Endoflas and AH Plus in the presence of the two organisms; compare the antimicrobial activity of Endoflas and AH Plus following the incorporation of silver nanoparticles, and assess bacterial viability at the sealer endodontium interface with the silver nanoparticle modification.

2. Materials and Methods

2.1 Study Design

The present research study gained the form of an in-vitro experimental study to determine the antimicrobial activity of silver nanoparticle-impregnated Endoflas and AH Plus sealers against Enterococcus faecalis and Staphylococcus aureus. Confocal laser scanning microscopy (CLSM) with LIVE/DEAD bacterial viability staining was used to determine the viability of bacteria at the sealer-dentin interface.

2.2 Materials

The study used two endodontic sealers; Endoflas, an endodontic sealer, which consists of zinc oxide, and AH Plus which is an endodontic sealer made of resin. Silver nanoparticles had an antimicrobial additive value, and were added to both sealers in varying concentrations. Microbiological and microscopic analyses were performed using Brain Heart Infusion (BHI) medium, nutrient agar plates, sterile paper disk, extracted single-rooted human teeth, 5.25% sodium hypochlorite, 6% citric acid, sterile distilled water, LIVE/DEAD BacLight bacterial viability stain and a confocal laser scanning microscope.

2.3 Preparation and Incorporation of Silver Nanoparticles

Nanoparticles of silver were acquired in the form of a liquid at a concentration of 0.6 mg/mL of silver. The average size of particles was between about 3 nm and 22 nm. Silver nanoparticles were added to the chosen endodontic sealers in the concentration of 0.05%, 0.1% and 0.5%. In the manipulation of the material, each sealer was mixed with the nanoparticles in a 1:1 ratio by weight. The manipulation of all materials was carried out based on the instructions of the manufacturers in order to make the experimental procedure standardized.

2.4 Microorganisms and Bacterial Suspension Preparation

Enterococcus faecalis and Staphylococcus aureus were used as antimicrobial activity assays of the experimental materials. The bacterial strains were grown in Brain Heart Infusion medium individually and 24 hours were incubated at 37 C. After incubation, a dilution of the microbial cells was carried out in sterile distilled water to give a standardized bacterial suspension that was equal to an approximate of 0.5 McFarland standard which is equal to an approximate of 1.5-1.6 x 10 o CFU/mL. *E. faecalis* and S. aureus were separately prepared in separate standardized suspensions and agar plates and dentin specimens inoculated.

2.5 Experimental Grouping

The experimental materials were divided according to sealer type and silver nanoparticle concentration. The first group consisted of the zinc oxide–based sealer Endoflas incorporated with silver nanoparticles at concentrations of 0.05%, 0.1%, and 0.5%, along with an Endoflas control group without silver nanoparticles. The second group consisted of the resin-based sealer AH Plus incorporated with silver nanoparticles at the same concentrations of 0.05%, 0.1%, and 0.5%, along with an AH Plus control group without silver nanoparticles. A third comparative group included silver nanoparticles alone at concentrations of 0.05%, 0.1%, and 0.5%. These groups were used to assess the antibacterial efficacy of the sealers and the concentration-dependent effect of silver nanoparticles.

2.6 Dentin Block Preparation for CLSM Analysis

For microscopic evaluation of bacterial viability at the sealer–dentin interface, 32 extracted single-rooted human teeth were used. From each tooth, a root dentin block of approximately 4 mm in length was horizontally sectioned at about 1 mm below the cementoenamel junction. The root canal space within each dentin block was enlarged using Gates Glidden drill #6 to obtain an internal canal diameter of approximately 1.5 mm.

Each cylindrical dentin block was fractured into two semicylindrical halves, resulting in 64 semicylindrical dentin specimens. The external surfaces of the specimens were shaped using a carbide bur to obtain a standardized thickness of approximately 2 mm. The smear layer was removed by immersing the specimens in 5.25% sodium hypochlorite and 6% citric acid for 4 minutes each in an ultrasonic bath. After smear layer removal, the specimens were rinsed with sterile water and sterilized by autoclaving at 121°C for 20 minutes.

2.7 Bacterial Inoculation of Dentin Specimens

Following sterilization, the dentin specimens were inoculated with the test microorganisms. Thirty-two specimens were immersed in a suspension of *E. faecalis*, while the remaining thirty-two specimens were immersed in a suspension of *S. aureus*, both prepared in BHI medium. The specimens were incubated for 48 hours to allow bacterial infiltration into the dentinal tubules and to simulate microbial colonization of root canal dentin.

2.8 Application of Sealers at the Sealer–Dentin Interface

After bacterial inoculation, the dentin specimens were randomly allocated according to sealer type and silver nanoparticle concentration. Freshly prepared Endoflas or AH Plus, with or without silver nanoparticles, was applied onto the dentin surface corresponding to the root canal wall. The sealer layer was maintained at an approximate thickness of 0.5 mm. Following sealer application, the specimens were fractured through the root canal to expose a fresh longitudinal dentin surface and the sealer–dentin interface for CLSM evaluation.

2.9 Confocal Laser Scanning Microscopy and Bacterial Viability Analysis

The fractured dentin specimens were stained using LIVE/DEAD BacLight bacterial viability stain according to the manufacturer's instructions. Two additional uninfected semicylindrical dentin specimens were stained using the same protocol and served as negative controls. The stained specimens were examined using confocal laser scanning microscopy. The sealer–dentin interface and freshly fractured dentin surface were first located under the microscope, and randomly selected areas were scanned for bacterial viability assessment.

Dual-channel fluorescence imaging was used to differentiate viable and non-viable bacterial cells. Green fluorescence indicated live bacterial cells, whereas red fluorescence indicated dead bacterial cells. CLSM images were analyzed using imaging software, and the volume ratio of green fluorescence to total fluorescence was calculated to determine the proportion of live bacteria within the biofilm at the sealer–dentin interface.

2.10 Statistical Analysis

The collected data were analyzed using SPSS software. One-way analysis of variance was performed to compare antimicrobial efficacy among the experimental and control groups. Post-hoc multiple comparison testing was applied where required to identify significant intergroup differences. The level of statistical significance was set at $P < 0.05$

3. Results

3.1 CLSM Analysis of Bacterial Viability

Confocal laser scanning microscopy was used to evaluate bacterial viability at the sealer–dentin interface after exposure to silver nanoparticle-incorporated endodontic sealers. The proportion of live bacteria was expressed as green fluorescence percentage, where higher green fluorescence indicated a higher proportion of viable bacterial cells and lower green fluorescence indicated greater antimicrobial activity. The CLSM values for *Enterococcus faecalis* and *Staphylococcus aureus* in the Endoflas, AH Plus, and silver nanoparticle-only groups are presented in **Table 1**. The proportion of live bacteria varied according to sealer type and silver nanoparticle concentration. Overall, lower green fluorescence values were observed in the higher-concentration silver nanoparticle groups, particularly at 0.5% SN, indicating improved antibacterial efficacy.

Table 1. CLSM analysis showing the proportion of live bacteria in silver nanoparticle-incorporated Endoflas, AH Plus, and silver nanoparticle-only groups against *Enterococcus faecalis* and *Staphylococcus aureus*

	SILVER NANOPARTICLES (SN)	*E. faecalis* Green (%)	*S. aureus* Green (%)
Group 1: Zinc oxide based sealer (ENDOFLAS)	ENDOFLAS + 0.05% SN	68.47 68.03 65.13 64.68	32.31 33.00 32.56 31.59
	ENDOFLAS +0.1% SN	40.64 41.01 42.03 39.89	21.65 22.03 21.54 20.19
	ENDOFLAS +0.5%SN	20.31 21.09 20.86 22.35	20.11 21.99 23.00 19.89
CONTROL	ENDOFLAS	70.34 71.02 70.98 69.99	52.14 53.91 51.93 52.75
Group 2: Resin based sealer (AH PLUS)	AH PLUS + 0.05% SN	98.67 97.89 97.55 98.60	62.31 63.12 61.56 62.48
	AH PLUS + 0.1% SN	69.08 62.99 68.34 69.87	59.65 59.24 58.19 58.12
	AH PLUS + 0.5% SN	51.34 52.03 50.99 52.06	31.62 32.56 31.48 30.89
CONTROL	AH PLUS	98.86 97.58 99.01 98.65	64.16 65.81 64.22 63.99
Group 3: SILVER NANOPARTICLES (SN)	0.05% SN	98.09 99.01 97.59 98.23	69.41 69.89 68.12 70.10
	0.1% SN	71.09 72.03 71.68 71.92	60.33 61.00 62.14 60.85
	0.5% SN	51.36 52.00 50.64 51.94	32.11 33.15 31.55 32.47

3.2 Bacterial Viability in the Endoflas Group

In the zinc oxide–based Endoflas group, the control specimens showed a high proportion of viable *E. faecalis*, with green fluorescence values ranging from **69.99% to 71.02%**. After incorporation of silver nanoparticles, a concentration-dependent reduction in live *E. faecalis* was observed. Endoflas + 0.05% SN showed green fluorescence values ranging from **64.68% to 68.47%**, whereas Endoflas + 0.1% SN showed a further reduction, with values ranging from **39.89% to 42.03%**. The lowest live bacterial percentage was observed in the Endoflas + 0.5% SN group, with values ranging from **20.31% to 22.35%** .

For *S. aureus*, Endoflas control showed green fluorescence values ranging from **51.93% to 53.91%**. Incorporation of silver nanoparticles reduced live bacterial percentage, with Endoflas + 0.05% SN showing values between **31.59% and 33.00%**, Endoflas + 0.1% SN showing values between **20.19% and 22.03%**, and Endoflas + 0.5% SN showing values between **19.89% and 23.00%**. These findings indicate that silver nanoparticle incorporation enhanced the antimicrobial activity of Endoflas against both tested microorganisms, with the strongest reduction observed at higher concentrations. The CLSM images of the Endoflas + 0.05% SN group showed live/dead bacterial distribution at the sealer–dentin interface, with green fluorescence indicating viable cells and red fluorescence indicating non-viable cells (**Figure 1**).

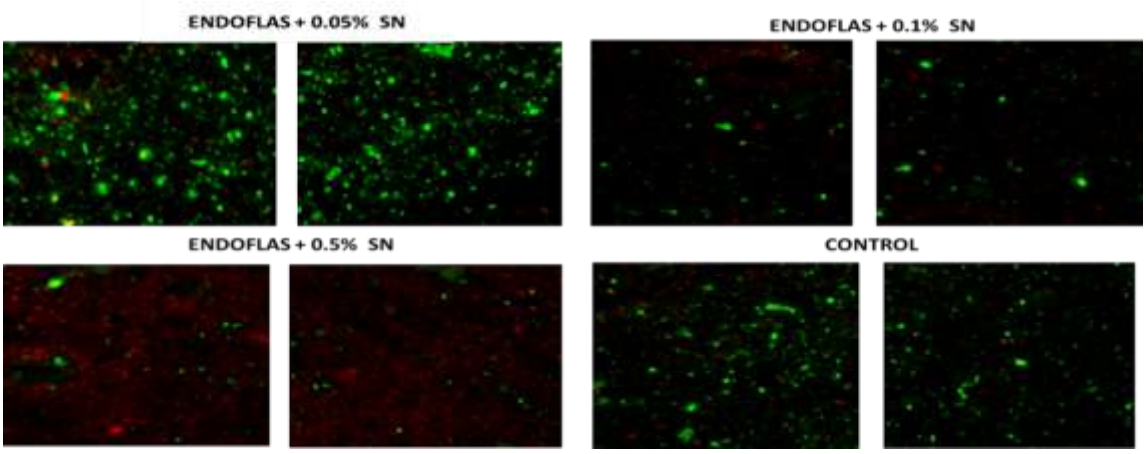

Figure 1. Representative CLSM images showing bacterial viability after treatment with Endoflas + 0.05% silver nanoparticles

3.3 Bacterial Viability in the AH Plus Group

In the resin-based AH Plus group, the control specimens showed very high viability of *E. faecalis*, with green fluorescence values ranging from **97.58% to 99.01%**. AH Plus + 0.05% SN showed values ranging from **97.55% to 98.67%**, indicating minimal reduction compared with the AH Plus control. However, greater reduction was observed at higher silver nanoparticle concentrations. AH Plus + 0.1% SN showed green fluorescence values ranging from **62.99% to 69.87%**, while AH Plus + 0.5% SN further reduced live bacterial percentage to values ranging from **50.99% to 52.06%** (**Table 1**).

For *S. aureus*, AH Plus control showed green fluorescence values ranging from **63.99% to 65.81%**. AH Plus + 0.05% SN showed values between **61.56% and 63.12%**, while AH Plus + 0.1% SN showed values between **58.12% and 59.65%**. The greatest reduction within the AH Plus group was observed with AH Plus + 0.5% SN, where green fluorescence values ranged from **30.89% to 32.56%**. These results suggest that AH Plus demonstrated improved antibacterial activity mainly at the higher concentration of 0.5% SN. Representative CLSM images demonstrated differences in live/dead bacterial distribution between the control and silver nanoparticle-incorporated sealer groups, confirming the concentration-dependent reduction in bacterial viability (**Figure 2**).

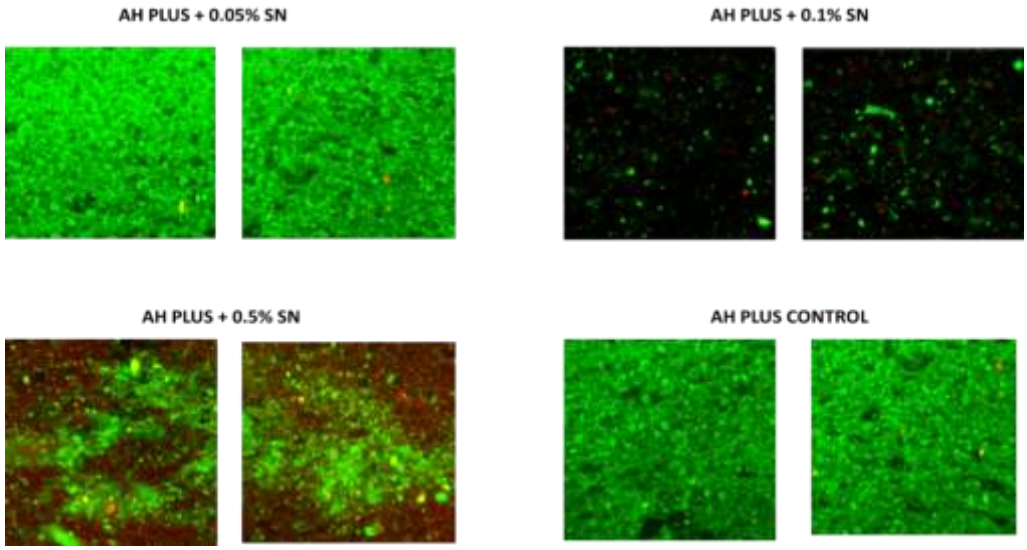

Figure 2. Representative CLSM images showing live/dead bacterial viability in control and silver nanoparticle-incorporated endodontic sealer groups

3.4 Bacterial Viability in the Silver Nanoparticle-Only Group

Silver nanoparticles alone also showed a concentration-dependent reduction in bacterial viability. For *E. faecalis*, 0.05% SN showed high green fluorescence values ranging from **97.59% to 99.01%**. This decreased with 0.1% SN, where values ranged from **71.09% to 72.03%**, and further decreased with 0.5% SN, where values ranged from **50.64% to 52.00%**.

For *S. aureus*, 0.05% SN showed green fluorescence values ranging from **68.12% to 70.10%**, while 0.1% SN showed values ranging from **60.33% to 62.14%**. The lowest values were observed with 0.5% SN, ranging from **31.55% to 33.15%**. These findings show that the antimicrobial effect of silver nanoparticles alone increased with concentration, with 0.5% SN showing the greatest reduction in live bacterial percentage for both microorganisms.

3.5 Comparative Findings Between *E. faecalis* and *S. aureus*

In the groups studied, *E. faecalis* tended to exhibit a higher value of green fluorescence as compared to S. aureus, especially in the AH Plus and the silver nanoparticle-alone groups. This implies that *E. faecalis* was more persistent in the test conditions. The viability of *E. faecalis* decreased more in the Endoflas group with increasing concentration of silver nanoparticle up to 0.5% SN. In contrast, *S. aureus* showed comparatively lower live bacterial percentages even at lower silver nanoparticle concentrations, especially in the Endoflas groups. The CLSM images showed visible differences in live/dead bacterial distribution among the control and silver nanoparticle-incorporated sealer groups, indicating reduced bacterial viability in the experimental groups (**Figure 3**).

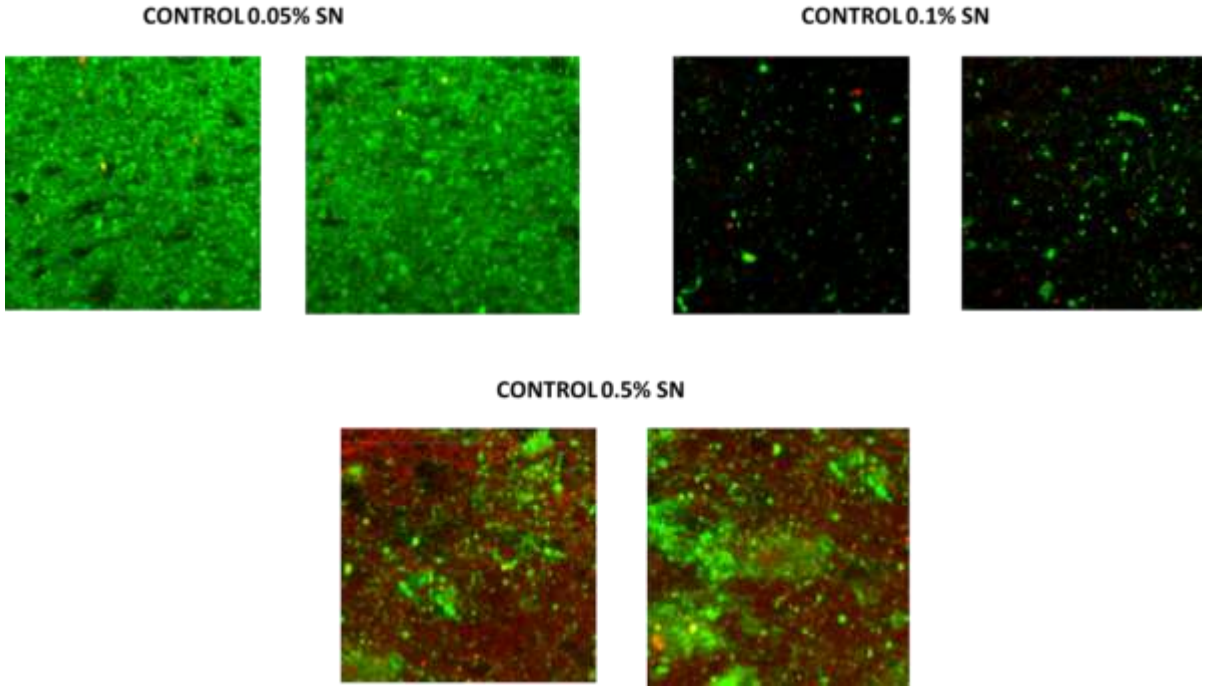

Figure 3. Representative CLSM images showing bacterial viability after exposure to control and silver nanoparticle-incorporated endodontic sealer groups

3.6 Comparative Findings Between Endoflas and AH Plus

Comparison between the two sealer groups showed that Endoflas incorporated with silver nanoparticles produced a stronger reduction in live bacterial percentage than AH Plus. For *E. faecalis*, Endoflas + 0.5% SN showed green fluorescence values ranging from **20.31% to 22.35%**, whereas AH Plus + 0.5% SN showed higher values ranging from **50.99% to 52.06%**. Similarly, for *S. aureus*, Endoflas + 0.5% SN showed values ranging from **19.89% to 23.00%**, while AH Plus + 0.5% SN showed values ranging from **30.89% to 32.56%**. Thus, Endoflas + 0.5% SN demonstrated the greatest antimicrobial efficacy among the sealer-based experimental groups.

4. Discussion

The current study compared antimicrobial effect of endodontic sealers containing silver nanoparticles with Enterococcus faecalis and Staphylococcus aureus through the CLSM-based bacterial viability measurement. The main observation was that addition of silver nanoparticles enhanced the antimicrobial activity of both sealers tested but the extent of reduction depended on the concentration of the nanoparticles, the type of sealer and bacterial species. There was a concentration-related trend, with the highest decrease in viable bacterial cells observed with 0.5% silver nanoparticles. The result is similar to earlier research that indicated that nanoparticle-modified endodontic materials have greater antimicrobial activity than unmodified sealers [10–12].

The enhanced antibacterial activity with increased concentrations of silver nanoparticles can be explained

by the antimicrobial effects of silver nanoparticles, such as sustained silver ion release, cell membrane destabilization, production of oxidative stress, binding of silver nanoparticles to thiol groups of bacterial enzymes, and disruption of DNA replication. These processes can synergistically lead to a decrease in bacterial viability in biofilms and sealer-dentin interface. Silver nanoparticles can be used in dentistry with wide antibacterial activity due to nanoscale size, high surface area, and ability to come into direct contact with bacteria cells [13]. Silver nanoparticles can also reduce bacterial adhesion and biofilm formation in endodontic models, supporting their potential role in antimicrobial endodontic applications [14].

Endoflas reinforced with silver nanoparticles was found to perform well in terms of antibacterial activity as compared to AH Plus reinforced with silver nanoparticles. The difference can be associated with the chemical composition, setting reaction, the matrix structure and the nanoparticle interaction in the respective sealers. Sealers containing zinc oxide might allow more efficient contact or release of antimicrobial agents, but resin sealers like AH Plus can entrap nanoparticles in a polymeric network and thus become less accessible to the materialbacteria interface. Previous researchers have demonstrated that antimicrobial effects of sealers can be different based on the formulation, solubility, diffusion properties, and release of active antibacterial agents [15,16]. Incorporation of nanoparticles can improve biological and antimicrobial properties, although the effect depends strongly on the type of sealer and nanoparticle used [17,18].

Comparison between the bacterial species revealed that E. faecalis appeared to be more resistant than *S. aureus*, particularly in the AH Plus groups. Its clinical implications are that *E. faecalis* is usually associated with the lack of full eradication of endodontic infections and endodontic root canal failure due to its survival during nutritional deprivation, invasion of dentinal tubes, tolerance to alkalinity and development of resistant biofilms. E. faecalis biofilms are very versatile and hard to eliminate in endodontic infections [19]. The evaluation of endodontic materials against *E. faecalis* is important because of its resistance and persistence in root canal systems [20]. Conversely, *S. aureus* exhibited a relatively higher percentage of live bacterial reduction implying that the bacterium might be more vulnerable to silver nanoparticle-incorporated formulations at the current experimental conditions.

The clinical implication of the study is that the antimicrobial potential of root canal sealers may be enhanced. Antimicrobial sealers could offer an added protective effect at the sealer-dentin interface since residual microorganisms could still exist post-instrumentation and post-irrigation. This is significant since the disinfection of root canals is not only related to the use of irrigants like sodium hypochlorite and chlorhexidine but also the antimicrobial effect of materials remaining in the canal following obturation [21]. Better antimicrobial sealers can help achieve better control of microbes and possibly lead to success of endodontics in the long term, but clinical outcomes also rely on obturation quality, coronal restoration, and biological factors related to the case [22].

One of the key strengths of the current study is the comparison of two clinically relevant sealers Endoflas and AH Plus at three levels of silver nanoparticles. Use of two types of bacteria gave a better insight into antimicrobial activity on resistant and opportunistic Gram-positive microorganisms. The other strength was the application of CLSM with live/dead stain that made it possible to directly visualize and quantify viable bacterial cells at the sealer/dentin interface. These laboratory techniques are suggested to enhance the quality of endodontic material studies and their reproducibility [23].

Nevertheless, there are limitations of the study. It was a small in-vitro study that had a small sample size and only two bacteria species. The antibacterial activity was tested in the short term, and the multispecies biofilm behavior was not measured. Moreover, there was no investigation of physical, chemical, sealing, flow, setting time, bond strength and biocompatibility characteristics of the modified sealers. These considerations are critical since clinical performance must not be affected by antimicrobial improvement [24,25]. To determine the long-term antimicrobial, cytotoxicity, biocompatibility, dentin adaptation, and sealing capacity of sealers containing silver nanoparticles, future research is necessary with mature multispecies biofilm and preclinical models. These modified sealers will require clinical validation before they are recommended to be used as routine endodontic.

5. Conclusion

The current in-vitro experiment showed that silver nanoparticles increased the antimicrobial activity of endodontic sealers when using it against Enterococcus faecalis and Staphylococcus aureus. The CLSM results revealed that percentage of live bacteria decreased as a function of concentration of silver nanoparticles, so that, the bigger concentrations of silver nanoparticles the greater were the antibacterial effects at the sealer, dentin interface. The most effective concentrations of silver nanoparticles were 0.5% in which the bacterial viability of both microorganisms were reduced. Endoflas with silver nanoparticles had superior antimicrobial activity to AH Plus with silver nanoparticles especially with E. faecalis. This implies that the antimicrobial properties of silver nanoparticles can not just be determined by the concentration, but also by the composition and interaction of the sealer material. A relatively higher resistance of E. faecalis than S. aureus also demonstrated its clinical importance as a chronic endodontic pathogen. Generally, the results indicate that incorporation of silver nanoparticles could be a viable approach in enhancing the antibacterial capabilities of endodontic sealers and lowering the bacteria survival rates in the sealer dentin interface. Nevertheless, this was an in-vitro study; therefore, the long-term antimicrobial stability, cytotoxicity, biocompatibility, sealing ability, flow, setting time, and bond strength of the silver nanoparticle-modified sealers have to be investigated before they can be used in a clinical setting.

References

1. Al-Quraine NT, Al-Ibraheem JFA, Zyara YHE, Abdulridha WM. In vitro assessment of antibacterial activity in four endodontic sealers against Staphylococcus aureus and Kocuria rhizophila using agar diffusion test. Journal of Medicine and Life. 2023;16(4):610.
2. Pallavi P, Bansal A, Kukreja N, Kaur N, Chhabra S, Ahuja J. Comparative evaluation of antimicrobial efficacy of different endodontic sealers against Enterococcus Faecalis: An in-vitro study. International Journal of Health Sciences. 2022;(I):9838–45.
3. Balto H, Bukhary S, Al-Omran O, BaHammam A, Al-Mutairi B. Combined effect of a mixture of silver nanoparticles and calcium hydroxide against Enterococcus faecalis biofilm. Journal of Endodontics. 2020;46(11):1689–94.
4. Ghahramani Y, Yaghoobi F, Motamedi R, Jamshidzadeh A, Abbaszadegan A. Effect of endodontic irrigants and medicaments mixed with silver nanoparticles against biofilm formation of enterococcus faecalis. Iranian Endodontic Journal. 2018;13(4):559.
5. Almutairi B, Alkhudhairy F. Nanoparticles modified bioceramic sealers on solubility, antimicrobial efficacy, pushout bond strength and marginal adaptation at apical-third of canal dentin. PeerJ. 2025;13:e18840.
6. Didar AA, Ghadimi S, Yousefbeigi A, Farahani P, Bahman Z, Khajeh Salehani H, et al. Antibacterial properties of bioengineered silver nanoparticles from Pastinaca sativa against Enterococcus faecalis biofilms in endodontic therapy. Scientific Reports. 2025;15(1):39600.
7. Ahmed K, Nik Abdul Ghani NR, Mahmoud O. Physicochemical and Antimicrobial Properties of Bioceramic Sealer Enhanced with Silver Nanoparticles: An in vitro Evaluation. CCIDE. 2025 Aug;Volume 17:423–34. doi:10.2147/CCIDE.S534254
8. Marashdeh M, Stewart C, Kishen A, Levesque C, Finer Y. Drug-silica coassembled particles improve antimicrobial properties of endodontic sealers. Journal of Endodontics. 2021;47(5):793–9.
9. Hassan N, Riad M, Ibrahim SH, Mahmoud K, Abulnoor BA, Hassan R. Antibacterial and Cytotoxicity characteristics of experimental epoxy-based endodontic sealer loaded with silver gold nanoparticles: in vitro study. BDJ open. 2024;10(1):81.
10. Loyola-Rodríguez JP, Torres-Méndez F, Espinosa-Cristobal LF, García-Cortes JO, Loyola-Leyva A, González FJ, et al. Antimicrobial activity of endodontic sealers and medications containing chitosan and silver nanoparticles against *Enterococcus faecalis*. Journal of Applied Biomaterials & Functional Materials. 2019 Apr;17(3):2280800019851771. doi:10.1177/2280800019851771
11. Seung J, Weir MD, Melo MAS, Romberg E, Nosrat A, Xu HH, et al. A modified resin sealer: physical and antibacterial properties. Journal of endodontics. 2018;44(10):1553–7.
12. Vilela Teixeira AB, De Carvalho Honorato Silva C, Alves OL, Cândido Dos Reis A. Endodontic Sealers Modified with Silver Vanadate: Antibacterial, Compositional, and Setting Time Evaluation. BioMed Research International. 2019 May 9;2019:1–9. doi:10.1155/2019/4676354
13. Yin IX, Zhang J, Zhao IS, Mei ML, Li Q, Chu CH. The Antibacterial Mechanism of Silver Nanoparticles and Its Application in Dentistry. IJN. 2020 Apr;Volume 15:2555–62. doi:10.2147/IJN.S246764
14. Pérez-Sáenz MG, Martínez-Martínez RE, Zaragoza-Contreras EA, Domínguez-Pérez RA, Reyes-López SY, Donohue-Cornejo A, et al. Antibacterial and Anti-Adherence Efficacy of Silver Nanoparticles Against Endodontic Biofilms: An In Vitro and Ex Vivo Study. Pharmaceutics. 2025;17(7):831.
15. Dalmia S, Gaikwad A, Samuel R, Aher G, Gulve M, Kolhe S. Antimicrobial Efficacy of Different Endodontic Sealers against Enterococcus faecalis: An: In vitro: Study. Journal of International Society of Preventive and Community Dentistry. 2018;8(2):104–9.
16. Kapralos V, Koutroulis A, Ørstavik D, Sunde PT, Rukke HV. Antibacterial activity of endodontic sealers against planktonic bacteria and bacteria in biofilms. Journal of endodontics. 2018;44(1):149–54.
17. Jung MK, Park SC, Kim YJ, Park JT, Knowles JC, Park JH, et al. Premixed calcium silicate-based root canal sealer reinforced with bioactive glass nanoparticles to improve biological properties. Pharmaceutics. 2022;14(9):1903.
18. Navarrete-Olvera K, Niño-Martínez N, De Alba-Montero I, Patiño-Marín N, Ruiz F, Bach H, et al. The push-out bond strength, surface roughness, and antimicrobial properties of endodontic bioceramic sealers supplemented with silver nanoparticles. Molecules. 2024;29(18):4422.
19. Yang S, Meng X, Zhen Y, Baima Q, Wang Y, Jiang X, et al. Strategies and mechanisms targeting Enterococcus faecalis biofilms associated with endodontic infections: a comprehensive review. Frontiers in cellular and infection microbiology. 2024;14:1433313.
20. Moon SH, Shin SJ, Oh S, Bae JM. Antibacterial Activity and Sustained Effectiveness of Calcium Silicate-Based Cement as a Root-End Filling Material against Enterococcus faecalis. Materials. 2023;16(18):6124.
21. Ruksakiet K, Hanák L, Farkas N, Hegyi P, Sadaeng W, Czumbel LM, et al. Antimicrobial efficacy of chlorhexidine and sodium hypochlorite in root canal disinfection: a systematic review and meta-analysis of randomized controlled trials. Journal of endodontics. 2020;46(8):1032–41.
22. Sadaf D. Survival Rates of Endodontically Treated Teeth After Placement of Definitive Coronal Restoration: 8-Year Retrospective Study. TCRM. 2020 Feb;Volume 16:125–31. doi:10.2147/TCRM.S223233

23. Nagendrababu V, Murray PE, Ordinola-Zapata R, Peters OA, Rôças IN, Siqueira JF, et al. PRILE 2021 guidelines for reporting laboratory studies in Endodontology: A consensus-based development. Int Endodontic J. 2021 Sep;54(9):1482–90. doi:10.1111/iej.13542
24. Kapralos V, Rukke HV, Ørstavik D, Koutroulis A, Camilleri J, Sunde PT. Antimicrobial and physicochemical characterization of endodontic sealers after exposure to chlorhexidine digluconate. Dental Materials. 2021;37(2):249–63.
25. Wang Y, Fang L, Wang P, Qin L, Jia Y, Cai Y, et al. Antibacterial effects of silica nanoparticles loading nano-silver and chlorhexidine in root canals infected by Enterococcus faecalis. Journal of Endodontics. 2025;51(1):54–63.