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RESEARCH ARTICLE

Effect of Silver Nanoparticle Incorporation into an Epoxy Resin-Based Endodontic Sealer on Dentinal Tubule Penetration: An In Vitro Scanning Electron Microscopy Study

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Abstract

The long-term success of root canal treatment relies on effective chemomechanical disinfection and the ability of endodontic sealers to penetrate dentinal tubules, thereby improving the seal and limiting residual bacterial colonization. Silver nanoparticles (AgNPs) have attracted attention for their broad-spectrum antimicrobial activity; however, their influence on the dentinal tubule penetration of epoxy resin-based sealers remains incompletely understood. This study aimed to evaluate the effect of incorporating silver nanoparticles into an epoxy resin-based endodontic sealer on dentinal tubule penetration using scanning electron microscopy (SEM). Extracted human single-rooted teeth were instrumented, irrigated, and randomly allocated into a control group obturated with a conventional epoxy resin-based sealer and an experimental group obturated with the same sealer modified with silver nanoparticles. After setting, specimens were longitudinally sectioned and examined by SEM. The experimental sealer showed satisfactory adaptation and effective penetration comparable to the control group. Incorporating silver nanoparticles appears to preserve dentinal tubule penetration while potentially providing additional antimicrobial advantages, suggesting that modified sealers represent a promising strategy for enhancing endodontic disinfection without compromising sealing performance.

Keywords: silver nanoparticles, endodontic sealer, dentinal tubule penetration, epoxy resin-based sealer, root canal obturation, scanning electron microscopy, endodontics, nanotechnology.

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1 Introduction

Successful endodontic treatment depends on the effective elimination of microorganisms from the root canal system and the establishment of a durable three-dimensional seal that prevents reinfection [1]. Despite considerable advances in instrumentation techniques and irrigation protocols, complete disinfection of the root canal system remains a significant clinical challenge due to the complex anatomy of root canals, including fins, isthmuses, lateral canals, and dentinal tubules, which may harbor persistent microorganisms beyond the reach of mechanical preparation and chemical irrigants [2].

Microbial biofilms are recognized as the principal etiological factor in pulpal and periapical diseases [3]. Among the microorganisms associated with persistent endodontic infections, *Enterococcus faecalis* has received particular attention because of its ability to survive in nutrient-deprived environments, penetrate dentinal tubules, form resistant biofilms, and withstand conventional intracanal disinfection procedures [4]. Consequently, residual bacteria remaining within dentinal tubules continue to represent one of the major causes of post-treatment disease and endodontic failure [5].

Root canal obturation plays a fundamental role in preventing microbial recolonization after chemomechanical preparation [5]. While gutta-percha is the core filling material, it does not bond to dentin; therefore, endodontic sealers are essential to seal anatomical irregularities, accessory canals, and dentinal tubules, thereby improving the obturation system's sealing ability [6]. Among currently available materials, epoxy resin-based sealers are considered the gold standard because of their excellent sealing ability, dimensional stability, low solubility, favorable handling characteristics, and adequate adhesion to dentinal walls [7]. Their ability to penetrate dentinal tubules contributes not only to mechanical retention but also to the entombment of residual microorganisms and to reducing the potential for bacterial leakage [6, 7].

The incorporation of nanotechnology into endodontic biomaterials has emerged as a promising strategy to enhance antimicrobial performance while maintaining desirable physicochemical properties [8, 9]. Silver nanoparticles (AgNPs), typically ranging from 1 to 100 nm in size, exhibit broad-spectrum antimicrobial activity through multiple mechanisms, including disruption of bacterial cell membranes, generation of reactive oxygen species, inhibition of enzymatic activity, interference with DNA replication, and sustained release of silver ions [10]. Owing to their nanoscale dimensions and high surface-area-to-volume ratio, AgNPs can penetrate dentinal tubules efficiently and may improve bacterial eradication in areas inaccessible to conventional treatment methods [9, 10].

Although numerous studies have demonstrated the antimicrobial efficacy of silver nanoparticles, less is known about their influence on the physical behavior of endodontic sealers, particularly their ability to penetrate dentinal tubules [11, 12]. Since dentinal tubule penetration is considered an important indicator of sealer adaptation and sealing quality, any modification of the sealer composition should be carefully evaluated to ensure that its clinical performance is not compromised [11, 12]. Understanding whether incorporating AgNPs enhances antimicrobial properties without adversely affecting tubule penetration is essential before their routine clinical application. Therefore, investigating the interaction between silver nanoparticle-modified epoxy resin-based sealers and dentinal tissues may provide valuable insights into the development of next-generation endodontic biomaterials that combine superior antimicrobial activity with optimal sealing performance. To the best of our knowledge, no previous study has specifically characterized, through scanning electron microscopy, the pattern of dentinal tubule penetration of an epoxy resin-based sealer modified with 40 nm silver nanoparticles. Furthermore, rather than merely assessing the overall penetration of the sealer as a bulk material, this study specifically investigated, by means of SEM imaging, whether the silver nanoparticles incorporated into the sealer were able to reach and penetrate the dentinal tubules, providing direct visual evidence of nanoparticle-level penetration that has not been previously reported.

The aim of this *in vitro* study was to qualitatively evaluate the effect of incorporating AgNPs into an epoxy resin-based endodontic sealer on dentinal tubule penetration using SEM.

2 Materials and Methods

2.1 Study Design

This *in vitro* experimental study evaluated the effect of incorporating AgNPs into an epoxy resin-based endodontic sealer on dentinal tubule penetration. The investigation was performed using extracted human single-rooted teeth and SEM analysis. The study protocol included sample standardization, chemomechanical preparation, root canal obturation, specimen sectioning, SEM examination, and qualitative assessment of sealer penetration. The study was designed and reported following established standards for *in vitro* laboratory research in dentistry.

2.2 Sample Selection

Twenty freshly extracted human single-rooted permanent teeth with fully developed apices were selected for the study. Teeth presenting root fractures, external or internal resorption, previous endodontic treatment, severe canal curvature, calcifications, or anatomical abnormalities were excluded. Soft tissue remnants and calculus were removed mechanically, and the specimens were stored in physiological saline solution to prevent dehydration and structural alteration prior to use. The root surfaces were cleaned ultrasonically to remove superficial deposits and calculus, followed by decontamination through immersion in 5.25% NaOCl (Niclor, Olab Farmaceutica S.r.l., Muggiò, MB, Italy) for 5 minutes. Samples were standardized by sectioning at the level of the cemento-enamel junction using a diamond disc (Diatech, Swiss Dental Instruments, Heerbrugg, Switzerland) under water cooling.

2.3 Sample Preparation

Canal patency and working length were established manually with a stainless-steel size 10 K-file that was advanced until the tip was just visible at the anatomical apex and then withdrawn approximately 1 mm. Canals were subsequently instrumented using Mtwo rotary files (Sweden & Martina S.p.A., Due Carrare, Italy) in sequence (10/.04, 15/.05, 20/.06, 25/.06) to achieve a final taper of .06. Between each rotary instrument, canals were irrigated with a standardized volume of 3 mL of 5.25% NaOCl delivered through a 27-gauge needle (KerrHawe SA, Bioggio, Switzerland). At the completion of shaping, canals were irrigated with 10 mL of 17% EDTA (Olab Farmaceutica S.r.l., Muggiò, MB, Italy) followed by 10 mL of 5.25% NaOCl to remove the smear layer and expose the dentinal tubules, then rinsed with a final 10 mL of distilled water and dried with paper points (Inline, Torino, Italy).

2.4 Experimental Groups

The specimens were randomly divided into two experimental groups ($n = 10$) using a computer-generated randomization sequence:

- **Control Group (CG):** Root canals were obturated using gutta-percha associated with a conventional epoxy resin-based endodontic sealer (AH Plus, Dentsply Sirona, Konstanz, Germany).
- **Experimental Group (EG):** Root canals were obturated using gutta-percha associated with the same epoxy resin-based sealer modified by the incorporation of 2.5% (w/w) of 40 nm silver nanoparticles (AgNPs, cat. no. 730807, Merck KGaA, Darmstadt, Germany).

2.5 Root Canal Obturation

Root canal obturation was performed by cold lateral condensation. Sealer powder and liquid were hand-mixed on a glass slab with a spatula according to the manufacturer's instructions. In all groups, a gutta-percha master cone matching the taper and size of the last instrument used was fitted to working length with tug-back, lightly coated with sealer, and inserted into the canal; accessory cones were then added and laterally condensed with a manual spreader until no further space remained. Filled roots were stored for 7 days at 37 °C and 100% humidity to allow complete setting of the sealer.

2.6 SEM Analysis

To evaluate sealer penetration into the dentinal tubules, specimens were longitudinally grooved in a bucco-lingual direction along the vestibular and lingual root surfaces using a low-speed diamond disc (Diatech, Swiss Dental Instruments, Heerbrugg, Switzerland) and then split with a chisel and mallet, in order to minimize dentinal debris that might otherwise contaminate the specimen surface and affect the results. The specimens were dehydrated, mounted on metallic stubs, sputter-coated with gold, and examined using scanning electron microscopy.

SEM images were obtained at different magnifications to evaluate:

- adaptation of the sealer to canal walls,
- penetration of the sealer into dentinal tubules,
- distribution of the sealer along the dentinal surface,
- morphological characteristics of the sealer–dentin interface.

Representative micrographs were obtained from the coronal, middle, and apical thirds of each specimen for comparative evaluation. To evaluate the penetration of the AH Plus sealer into the dentinal tubules, specimens were first examined in secondary electron (SE) mode to assess tubule occlusion and sealer adaptation to the canal wall. To specifically determine whether the silver nanoparticles incorporated into the modified sealer had penetrated the dentinal tubules, the same fields were subsequently imaged in backscattered electron (BSE) mode, which provides compositional (Z) contrast and allows silver-containing structures — owing to the high atomic number of silver relative to the surrounding dentin and resin matrix — to be visualized as areas of increased brightness within the tubule lumina. Tubule penetration by the sealer and, where applicable, by silver nanoparticles, was assessed qualitatively by two independent, calibrated examiners blinded to the experimental group, based on the presence or absence of material within the tubule lumina at each observed level. Any disagreement between the two observers was resolved through discussion.

2.7 Outcome Assessment

The primary outcome was qualitative dentinal tubule penetration of the endodontic sealer and silver nanoparticles as observed by SEM. Penetration depth and distribution pattern were comparatively evaluated between the control and experimental groups.

3 Results

All specimens completed the experimental protocol without procedural complications. The qualitative analysis of SEM images demonstrated satisfactory adaptation of both sealers to the root canal walls and confirmed penetration into the dentinal tubules in both experimental groups.

3.1 Control group (AH Plus)

SEM analysis of the control group revealed continuous adaptation of the epoxy resin-based sealer (AH Plus) to the dentinal surface throughout the evaluated root regions. The sealer exhibited homogeneous adaptation along the canal walls with intimate contact at the sealer–dentin interface. Penetration into the dentinal tubules was consistently observed, with no evidence of major interfacial gaps or structural discontinuities (Figures 1–5). The images reported here are representative of all examined specimens.

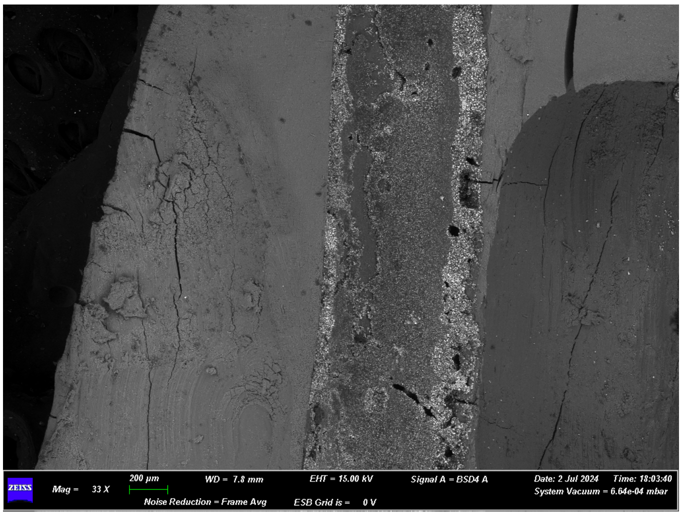

Figure 1. Apical region under backscattered electron (BSE) modality at 33× magnification. A layer of AH Plus cement is visible in the canal lumen, and its adaptation to the canal walls can be appreciated.

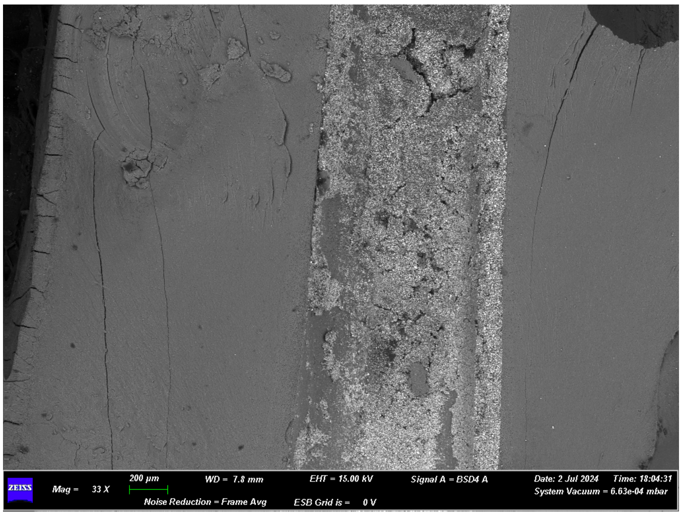

Figure 2. Middle-third region under backscattered electron (BSE) modality at 33× magnification. A layer of AH Plus cement is visible in the canal lumen, and its adaptation to the canal walls can be appreciated.

3.2 Experimental group (AH Plus + AgNPs)

SEM examination of the experimental group demonstrated that incorporation of AgNPs into the epoxy resin-based sealer did not adversely affect its adaptation to root canal dentin. The modified sealer exhibited a satisfactory pattern of adaptation, maintaining close contact with the dentinal walls and preserving penetration into the dentinal tubules. No structural disruption or deterioration of the sealer–dentin interface was detected following AgNPs incorporation (Figures 6–10). The images reported here are representative of all examined specimens. Silver nanoparticles could be observed within the lumen of certain dentinal tubules (Figure 10); a

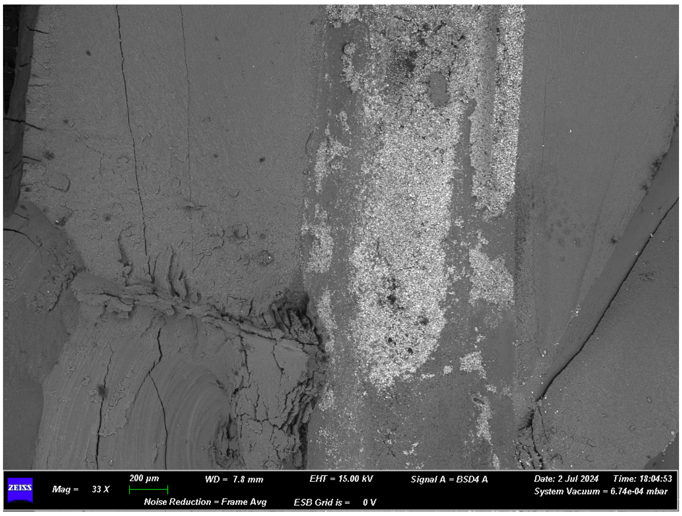

Figure 3. Coronal region under backscattered electron (BSE) modality at 33× magnification. A layer of AH Plus cement is visible in the canal lumen, and its adaptation to the canal walls can be appreciated.

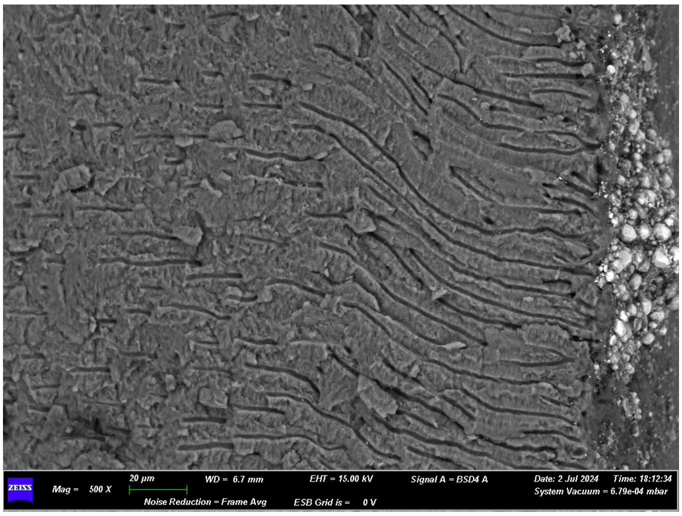

Figure 4. Dentinal tubule imaged under backscattered electron (BSE) modality at 500× magnification. A layer of AH Plus cement is visible on the internal wall of the canal lumen (right-hand side of the picture). Partial penetration of sealer particles inside some tubules can be observed.

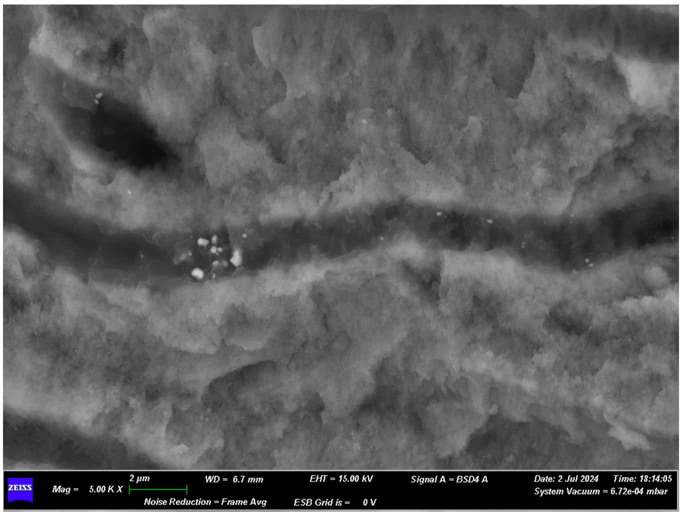

Figure 5. Dentinal tubule imaged under backscattered electron (BSE) modality at 5,000× magnification. Some particles of AH Plus cement are visible within a tubule.

Figure 6. Apical region under backscattered electron (BSE) modality at 33× magnification. A layer of AH Plus cement with nano-silver particles is visible in the canal lumen, and its adaptation to the canal walls can be appreciated.

higher magnification of 10,000× was employed for this image to allow direct visualization of individual nanoparticles within the tubule lumen.

3.3 Qualitative comparison between groups

Within the limits of the present investigation, qualitative comparison of the SEM images suggested comparable dentinal tubule penetration between the conventional and AgNP-modified sealers (Figures 1–10). In both groups, sealer penetration was generally greater in the coronal and middle thirds than in the apical third, consistent with the anatomical reduction in dentinal tubule density and diameter toward the root apex.

Representative SEM micrographs further demonstrated homogeneous sealer distribution and satisfactory adaptation to the canal walls in both experimental groups (Figures 1–10). No morphological alterations attributable to the incorporation of silver nanoparticles were observed.

Overall, the SEM findings indicate that the addition of silver nanoparticles preserved the penetration capacity and adaptation of the epoxy resin-based sealer while maintaining an intimate sealer–dentin interface; the nanoparticles themselves were observed within the lumina of some dentinal tubules. These observations support the potential application of

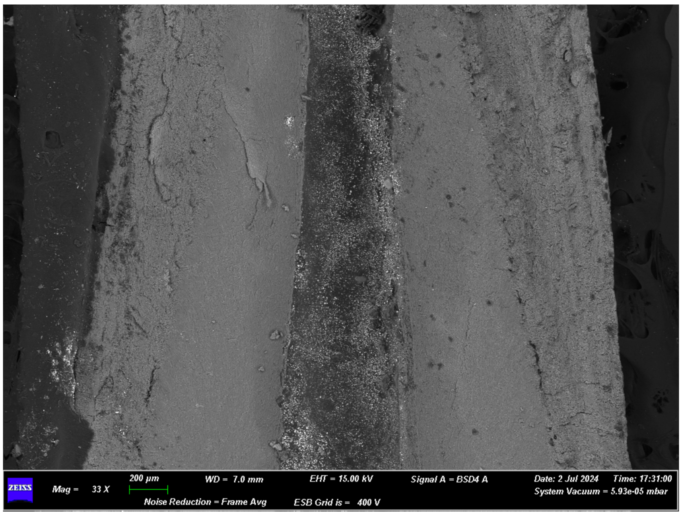

Figure 7. Middle-third region under backscattered electron (BSE) modality at 33× magnification. A layer of AH Plus cement with nano-silver particles is visible in the canal lumen, and its adaptation to the canal walls can be appreciated.

Figure 8. Coronal region under backscattered electron (BSE) modality at 33× magnification. A layer of AH Plus cement with nano-silver particles is visible in the canal lumen, and its adaptation to the canal walls can be appreciated.

AgNP-modified epoxy resin sealers as an adjunctive strategy for enhancing the biological performance of endodontic biomaterials without compromising their sealing characteristics.

4 Discussion

The present *in vitro* study evaluated the effect of incorporating AgNPs into an epoxy resin-based endodontic sealer on dentinal tubule penetration using SEM. The findings demonstrated that the addition of AgNPs did not adversely affect the penetration ability of the sealer. Indeed, the modified sealer

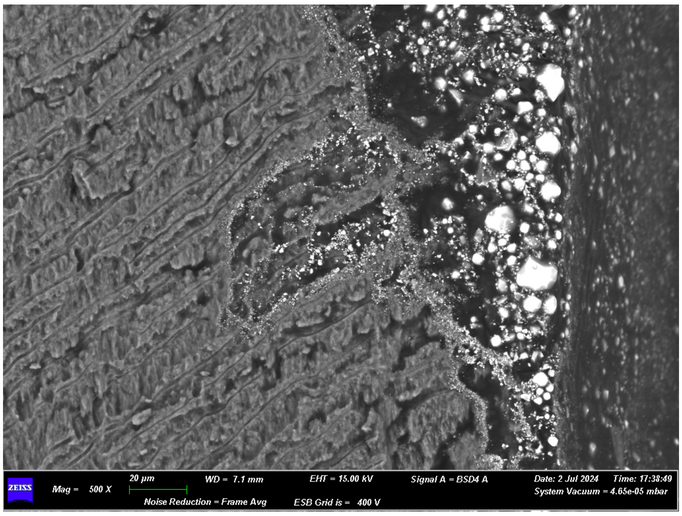

Figure 9. Dentinal tubule imaged under backscattered electron (BSE) modality at 500× magnification. A layer of AH Plus cement with nano-silver particles is visible on the internal wall of the canal lumen (right-hand side of the picture), as well as inside some of the tubules. Silver nanoparticles, which can be recognized from their brighter appearance under BSE modality, can be seen within the lumen of the tubules.

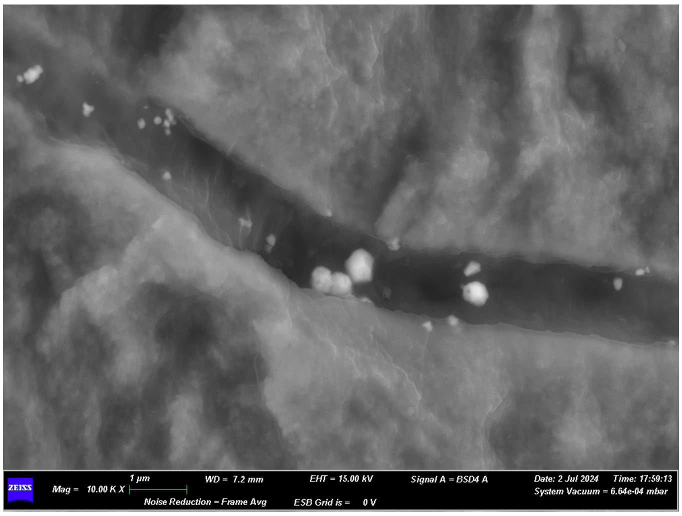

Figure 10. Dentinal tubules imaged under backscattered electron (BSE) modality at 10,000× magnification. Some particles of AH Plus cement with nano-silver particles are visible within a tubule.

exhibited satisfactory adaptation to root canal dentin and maintained an effective penetration pattern within dentinal tubules, suggesting that nanoparticle incorporation preserves the physicochemical behavior required for successful root canal obturation.

However, these findings should be interpreted with caution because they are derived from a qualitative assessment of two-dimensional SEM images. Nevertheless, SEM analysis was preferred over dye-penetration techniques — e.g., confocal laser scanning microscopy with rhodamine B — because it has been demonstrated that rhodamine B staining techniques present a substantial risk of error and false positives, since the dye can readily leak into tubules where no sealer is present [13]. These limitations suggest that such quantitative techniques may not necessarily provide more reliable information than a carefully conducted qualitative evaluation [14].

The long-term success of endodontic treatment depends on adequate chemomechanical preparation, followed by three-dimensional obturation that prevents bacterial recolonization [15]. Even with advanced rotary instrumentation systems, complete elimination of bacteria is hampered by complex canal morphologies such as C-shaped configurations, in which microorganisms persist in fins, isthmuses, and apical ramifications despite thorough preparation [16]. Although NaOCl, EDTA, and modern instrumentation systems significantly reduce the microbial burden, complete elimination of bacteria remains difficult because microorganisms persist within dentinal tubules, accessory canals, apical ramifications, and biofilms [17]. Consequently, endodontic sealers play an essential role in filling microscopic irregularities and entombing residual microorganisms that survive canal preparation [18].

Epoxy resin-based sealers, particularly AH Plus, remain the reference material in endodontics because of their excellent sealing ability, dimensional stability, low solubility, and favorable adhesion to dentin [19]. Numerous investigations have shown that deeper penetration of the dentinal tubules improves mechanical interlocking, enhances adaptation to canal walls, and reduces microleakage [19, 20]. The present findings support these observations by demonstrating intimate contact between the sealer and dentinal walls throughout the evaluated specimens, although through a qualitative evaluation.

Nanotechnology has recently emerged as a promising strategy for improving dental biomaterials. AgNPs are among the most extensively investigated nanomaterials because they possess broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi, and antibiotic-resistant microorganisms [20, 21]. Their antimicrobial effect is attributed to the multiple, simultaneously acting mechanisms already described in the Introduction [18, 20, 21]; unlike conventional antimicrobials, this multiplicity of mechanisms reduces the likelihood of bacterial resistance.

An important concern when modifying endodontic sealers with nanoparticles is the potential alteration of their physicochemical properties, including viscosity, flow, setting behavior, and dentinal tubule penetration. If nanoparticle incorporation compromises sealer flow, the quality of obturation may be negatively affected [22, 23]. In the present study, SEM observations indicated that the addition of AgNPs did not impair sealer adaptation or penetration into dentinal tubules, suggesting that the modified material maintained adequate flow characteristics after incorporation of nanoparticles.

These findings are consistent with previous laboratory investigations reporting that low concentrations of silver nanoparticles can improve the antimicrobial performance of epoxy resin-based sealers without negatively affecting their sealing ability or handling characteristics [22, 23]. The maintenance of dentinal tubule penetration observed in the present study suggests that AgNP incorporation may provide an additional antimicrobial benefit while preserving one of the principal determinants of successful root canal obturation.

From a clinical perspective, combining effective dentinal tubule penetration with prolonged antimicrobial activity may contribute to reducing residual bacterial contamination after endodontic treatment. Such an approach could be particularly beneficial in complex anatomical situations, retreatment cases, persistent apical periodontitis, and infections associated with resistant microorganisms such as *E. faecalis* [24]. Nevertheless, before routine clinical application, additional investigations should evaluate the long-term physicochemical stability, ion release, cytocompatibility, discoloration potential, bond strength, sealing ability, and resistance to bacterial leakage under clinically relevant conditions [22, 23].

The present study has some limitations. First, it was performed under *in vitro* conditions, which cannot fully reproduce the biological environment of the oral cavity. Second, SEM analysis primarily provides morphological evaluation and does not directly measure antimicrobial activity or long-term clinical performance. Third, the assessment was qualitative and conducted on two-dimensional images, without quantitative measurement of penetration depth or a reported measure of inter-examiner agreement. Finally, only one epoxy resin-based sealer formulation and one nanoparticle concentration were investigated. Future studies should evaluate different nanoparticle concentrations, alternative endodontic sealers, advanced imaging techniques such as confocal laser scanning microscopy (CLSM) or micro-computed tomography (micro-CT), and randomized clinical trials to validate the present findings. In addition, physicochemical characterization of the silver nanoparticles within the sealer matrix—including particle size distribution, zeta potential, and homogeneity of dispersion—should be reported in future investigations to improve reproducibility.

Overall, the results indicate that incorporating AgNPs into an epoxy resin-based endodontic sealer represents a promising strategy for developing bioactive endodontic materials capable of combining effective dentinal tubule penetration with enhanced antimicrobial properties. Further laboratory investigations and well-designed clinical studies are required to evaluate the long-term antimicrobial efficacy, biocompatibility, mechanical properties, and clinical effectiveness of silver nanoparticle-modified endodontic sealers.

5 Conclusions

Within the limitations of this *in vitro* study, the incorporation of AgNPs into an epoxy resin-based endodontic sealer did not negatively affect its penetration into dentinal tubules, as judged by qualitative assessment. The nanoparticle-modified sealer demonstrated satisfactory adaptation to root canal dentin and maintained intimate contact with dentinal walls, and the silver nanoparticles themselves were observed within the lumina of some dentinal tubules. Dentinal tubule penetration was preserved throughout the evaluated root regions, supporting the maintenance of adequate sealing performance.

Data Availability Statement

Data will be made available on request.

Funding

This work was supported without any funding.

Conflicts of Interest

Domenico Ciavarella served as an Editorial Board Member, and Michele Tepedino served as an Associate Editor of the *Journal of Oral and Dental Approaches* at the time of manuscript submission. To ensure the integrity of the peer-review process, neither Domenico Ciavarella nor Michele Tepedino was involved in the editorial handling, peer review, or decision-making process for this manuscript, which was handled independently by another editor. The remaining authors declare no conflicts of interest.

AI Use Statement

The authors declare that no generative AI was used in the preparation of this manuscript.

Ethical Approval and Consent to Participate

This study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was reviewed and approved by the Institutional Ethics Committee of University of L'Aquila (approval no. 22/2018). All teeth were extracted for reasons unrelated to the present study and were anonymized prior to analysis. Written informed consent for the use of the extracted teeth for research purposes was obtained from all donors prior to tooth extraction.

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