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Nanoparticle-Enhanced Bioceramic Sealers in Root Canal Therapy: Evaluating Their Antimicrobial Efficacy and Dentin Bond Integrity under Biofilm-Stressed Conditions

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Abstract

This study aimed to compare the antimicrobial and versatility of bioceramic sealer with nanoparticles in relation to root canal dentin. Materials and Methods: Totalfill BC sealer, 2.3% and 15% volumes of Silver and chitosan nanoparticles, were added, respectively. Flexibility of root dentin was assessed in terms of thirty single-rooted human teeth, which have been prepared and obturated by the use of the lateral condensation method. The longitudinal sectioning of samples was performed, and the gap area (expressed as a percentage) was indicated at the following magnifications: x45 and x1000, using a stereomicroscope and a scanning electron microscope, respectively. After positioning the sealer under the spectrophotometer, the antimicrobial activity of the sealer was determined against E. faecalis using a direct contact test to assess its bacteriostatic nature. The data indicated a parametric distribution; therefore, intergroup comparisons were performed using one-way ANOVA, followed by the Tukey post hoc test, and intragroup comparisons were performed using ANOVA, followed by the Bonferroni post hoc test. Findings: The adaptability test with SEM results indicated that the highest gap value was obtained in BC + Silver NP (0.59 ± 0.27), followed by BC (0.29 ± 0.15) and then BC + Chitosan NP (0.22 ± 0.16). The gap value between BC and + Silver NP was significantly greater than the gap values between other groups, and this was shown on post hoc pairwise comparisons. According to the direct contact test, the highest value, indicating the highest bacterial growth, was observed in BC + Chitosan NP (0.19 ± 0.01), followed by BC (0.18 ± 0.01), and the lowest value was obtained in BC Silver NP (0.17 ± 0.01). Post hoc pairwise tests revealed a significant difference in abundance between the BC + Chitosan NP group and the other groups ($p < 0.001$). Conclusion: When incorporated into the bioceramic sealer, nanoparticles can significantly enhance the properties of the latter.

Keywords: Antimicrobial efficacy, Adaptability, Bioceramic sealer, Nanoparticles.

Introduction

The removal of infecting microorganisms is crucial to the success of endodontic treatment. This is typically achieved through the chemo-mechanical preparation of root canals; however, this process may not be sufficient, and microorganisms may persist. This challenge is mainly attributed to anatomical and microbial factors. Waltimo et al.1 observed that even with proper cleaning and shaping, 20-30% of cases might remain unresolved. Grossman2 highlighted the essential requirements for an optimal sealer, including its ability to form a hermetic seal, provide sufficient adhesion to canal walls, be antimicrobial, biocompatible with periradicular tissues, and remain insoluble in tissue fluid. A sealer that demonstrates strong adhesion and adaptation offers two significant benefits: first, it ensures proper sealing of the canal due to the enhanced interface between the sealer and dentin, and second, it helps entomb residual bacteria within the dentinal tubules, thus preventing reinfection of the periradicular tissues.

Various sealers are employed in endodontics, with bioceramic (BC) sealers gaining popularity due to their bioactivity and biomineralisation properties. The Totalfill BC sealer (FKG Dentaire, La Chaux-de-Fonds, Switzerland) is one of the earliest commercial BC sealers introduced. Its

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structure is similar to that of the Endosequence BC sealer, both of which are known for their excellent physical bio-compatibility.5 The small particle size, hydrophilicity, and low contact angle of Totalfill BC sealer allow for easy spreadability on the canal wall, ensuring better adaptation to dentin and effective filling of lateral micro-channels.6 BC sealers also exhibit antimicrobial properties due to their alkalinising effect. A study by Patri et al.7 found that the EndoSequence BC sealer demonstrated the best marginal adaptation, followed by ProRoot MTA and then EndoREZ. Poggio et al.8 compared the antimicrobial activity of various root canal sealers, including Totalfill BC, against Enterococcus faecalis (E. faecalis) using the ADT and DCT methods. Results from DCT tests revealed that Totalfill BC sealer was bactericidal against E. faecalis, eradicating all bacteria with prolonged exposure. Bronzel et al. also found that Totalfill BC was significantly more effective in reducing CFU of E. faecalis compared to AH Plus and the control.

Nanoparticles (NPs) have gained considerable attention in the medical field, with applications in targeted therapy, including the treatment of cancer. NPs have also been utilised in endodontics to reduce treatment failure by enhancing the disinfection process when used alone or in combination with irrigants.10 These nanoparticles can be either metallic or organic. Among the metallic NPs, silver nanoparticles (AgNPs) are particularly effective. The interaction between the positively charged silver NPs and the negatively charged bacterial cell walls leads to disruption of the bacterial cell membrane and permeability.11 On the other hand, chitosan, a non-toxic, biocompatible biopolymer, exhibits biodegradability and offers biological benefits such as bactericidal, anti-inflammatory, antioxidant, and antitumor properties.12

Anaerobic bacterial species, such as E. faecalis, have been identified in both failed root canal treatments and persistent periradicular lesions, with E. faecalis showing a 38% prevalence, as reported by Sundqvist et al. in 1998.14 The present study aims to evaluate the flexibility and antimicrobial activity of bioceramic sealer combined with silver or chitosan nanoparticles. The null hypothesis posits that there are no significant differences between the tested materials in terms of canal dentin wall adaptation and antimicrobial efficacy.

Materials and Methods

A. Evaluation of Root Canal Dentin Adaptability Using Scanning Electron Microscope and Stereomicroscope

Thirty single-rooted anterior teeth were sourced from the MIU teeth bank for this study. The teeth were decontaminated by submerging them in 5.25% sodium hypochlorite (NaOCl) for 5 minutes, followed by ultrasonic scaling to remove any deposits or calculus. The teeth were then decapitated at the cement-enamel junction using an Isomet saw under water coolant.

Sample Size Calculation:

Power analysis was conducted using a one-way fixed-effects analysis of variance with three experimental groups. Based on the findings of Wang et al. (15 the expected enhancement in antibacterial effect due to nanoparticles was estimated to be 10%. With an alpha level of 5% and a beta level of 20%, the power was set at 80%. The minimum sample size was calculated to be 30 teeth (10 per group) using IBM SPSS SamplePower, Release 3.0.1.

Randomisation and Grouping of Samples:

The teeth were randomly assigned to three experimental groups (10 teeth per group) based on the materials to be tested:

- Group I (n=10): Totalfill BC + Silver NP
- Group II (n=10): Totalfill BC + Chitosan NP
- Group III (n=10): Totalfill BC (Control group)

Preparation of Silver NP:

Silver nanoparticles were synthesised using a chemical reduction process described by Wang et al.16,17. A 3.4g quantity of silver nitrate ($AgNO_3$) was dissolved in 20 ml of cold distilled water. Polyvinylpyrrolidone (PVP) was used as a stabilising agent, and the two solutions were combined dropwise while stirring at 500 rpm at 60°C. The solution gradually turned a greyish-yellow colour, indicating the formation of AgNPs.

Preparation of Chitosan NP:

Chitosan nanoparticles were prepared using an ionotropic gelation process 18 Briefly, 1 g of chitosan powder was dissolved in 200 mL of 1% acetic acid (pH=4), and the solution was stirred at 400 rpm to obtain a homogeneous solution. Tripolyphosphate (TPP) was added dropwise, and the solution became opaque, indicating the formation of nanoparticles. The suspension was then centrifuged three times (30 minutes at 9000 rpm) with distilled water to isolate the nanoparticles.

Cleaning and Shaping:

The working length was determined using a #15 K file, and the final working length was set 1 mm short of the recorded length. The canals were irrigated with 5.25% NaOCl between each instrument size. Cleaning and shaping were performed with M Pro rotary NiTi files, followed by manual preparation to a #40 master apical file. The canals were rinsed with 5 mL of 17% EDTA, 5 mL of 2.25% NaOCl, and then with saline to remove the smear layer.

Preparation of Tested Materials:

The Totalfill BC sealer was used in accordance with the manufacturer's instructions. Silver and chitosan NPs were added to the sealer using calibrated syringes and micropipettes at the following concentrations:

- Silver NPs: 2.3% volume
- Chitosan NPs: 15% volume

Root Canal Obturation:

After drying the canals with paper points, a master cone (size 40, taper 0.04) was adjusted to the working length. Cold lateral condensation was used for obturation, with accessory cones added to complete the filling. Radiographic evaluation was performed in two angulations to ensure adequate obturation of the root canal.

Stereomicroscope Evaluation:

Specimens were longitudinally sectioned and analysed under a stereomicroscope at x45 magnification, divided into cervical, middle, and apical thirds. Images were processed using the Image Analysis Unit software for detailed measurements.

Scanning Electron Microscope (SEM):

Root samples were analysed in an environmental scanning electron microscope (ESEM) at x1000 magnification, with images processed using Image J software to calculate gap surface area percentages between the endodontic filling and root dentin.

Image Analysis:

Image J was used for image correction (contrast and brightness) and to calculate the gap areas between the endodontic filling and root dentin. The gaps were quantified and expressed as a percentage of the canal surface area.

B. Evaluation of Antimicrobial Efficacy Using the Direct Contact Test

Bacterial Inoculation:

Enterococcus faecalis (ATCC 4083) was cultured overnight at 37°C in tryptic soy broth (TSB) supplemented with 1% glucose. The bacterial suspension was adjusted to a turbidity of 0.5 McFarland scale.

Direct Contact Test:

A 96-well microtiter plate was used, with wells coated with 100 µL of freshly mixed material. The samples were incubated at 37°C and 100% humidity for 7 days. Bacterial suspension (1.5 x 10^8 bacteria/mL) was applied to the material surface, and bacterial growth was measured by optical density at 620 nm using a spectrophotometer after incubation.

Results

Root Canal Dentin Adaptability: Stereomicroscope Analysis

Effect of Root Third on Sealer Adaptability: The

Table (1): Statistical analysis of gap area percentage for different sealer types within each specimen third.

Specimen third	Gap area of the canal (%) (Stereomicroscope) (mean±SD)			p-value
	BC + Silver NP	BC + Chitosan NP	BC	
Coronal	5.20±0.06^{A}	0.90±0.01^{C}	1.77±0.02^{B}	<0.001*
Middle	3.09±0.05^{A}	0.74±0.02^{C}	1.42±0.02^{B}	<0.001*
Apical	2.16±0.15^{A}	0.64±0.03^{B}	0.71±0.02^{B}	<0.001*

Different superscript letters indicate a statistically significant difference within the same row.

**= significant ($p \leq 0.05$)*

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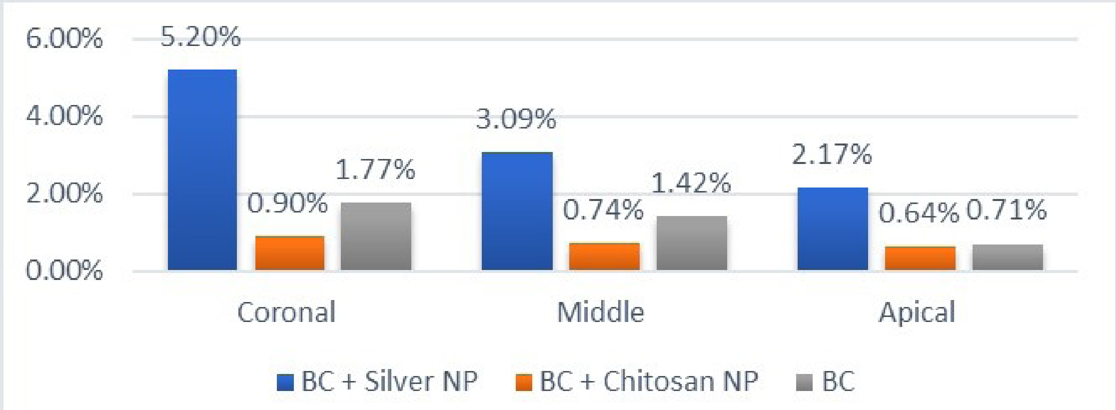

Figure (1): Bar chart showing comparison of average gap area (%) for different types of sealers at different thirds.

mean and standard deviation (SD) values of the percentage of gap area in the root canal (as observed under the stereomicroscope) for various sealer types across different root thirds are presented in Table 1 and Figure 1.

Table (2): Statistical analysis of gap area percentage for different sealer types.

Gap area of the canal (%) (Stereomicroscope) (mean±SD)			p-value
BC + Silver NP	BC + Chitosan NP	BC	
3.48±1.30ᴬ	0.76±0.11ᶜ	1.30±0.45ᴮ	<0.001*

Different superscript letters indicate a statistically significant difference within the same row.
**= significant ($p \leq 0.05$)*

****Total gap area percentage within each root: Table (4) figure (4)** contains the mean, Standard deviation (SD) values of gap area of the canal (%)(SEM) of different types of sealers.

Percentage of Total Gap Area in Each Root: The mean and SD values of the percentage of gap area in the canal for various sealer types are outlined in Table 2 and Figure 2.

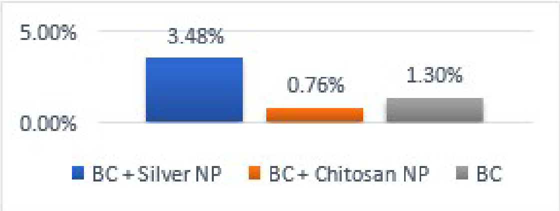

Figure (2): Bar chart showing comparison of average gap area (%) for different types of sealers at different thirds.

****Antimicrobial efficacy (Spectrophotometer evaluation): Table (5) figure (5)** shows mean, Standard deviation (SD) values of optical density of various types of sealer.

Scanning Electron Microscope (SEM): Effect of Root Third on Sealer Adaptability: The mean and SD values of the percentage of gap area in the canal (SEM) for each type of sealer across all root thirds are reflected in Table 3 and Figure 3.

Discussion

Endodontic bioceramic (BC) sealers have garnered significant popularity in recent years. One such sealer is the Totalfill BC sealer (FKG Dentaire, La Chaux-de-Fonds, Switzerland), which contains inorganic constituents such as tricalcium silicate, dicalcium silicate, calcium phosphates, colloidal silica, and calcium hydroxide.26 This biocompatible and non-toxic sealer is not eliminated in human biology and exhibits antimicrobial properties due to its alkalinising effect. The elevated pH

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Table (3): Statistical analysis of gap area percentage for different sealer types within each specimen third.

Root third	Gap area of the canal (%) (SEM) (mean±SD)			p-value
	BC + Silver NP	BC + Chitosan NP	BC	
Coronal	0.96 ± 0.02^{A}	0.43 ± 0.01^{C}	0.50 ± 0.01^{B}	**<0.001***
Middle	0.34 ± 0.01^{A}	0.18 ± 0.01^{C}	0.20 ± 0.01^{B}	**<0.001***
Apical	0.48 ± 0.01^{A}	0.06 ± 0.01^{C}	0.17 ± 0.01^{B}	**<0.001***

Different superscript letters indicate a statistically significant difference within the same row.
**= significant ($p \leq 0.05$)*

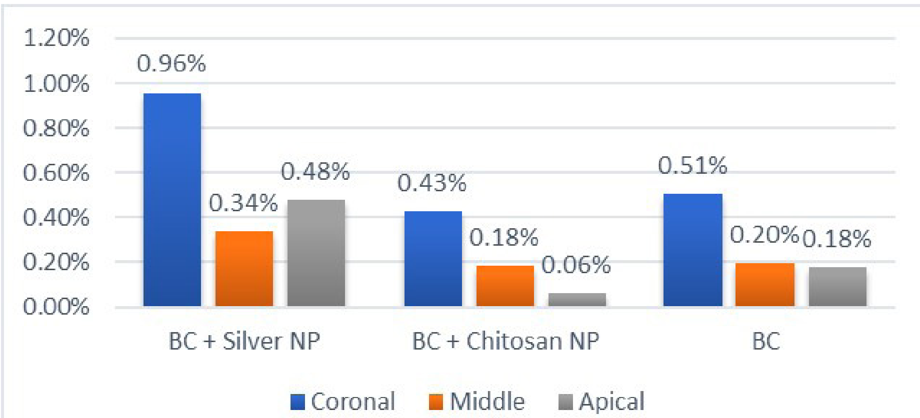

Figure (3): Bar chart showing comparison of average gap area (%) for different types of sealers at different thirds.

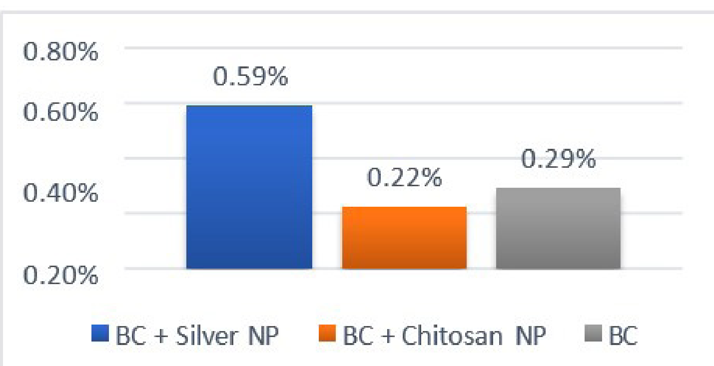

Figure (4): Bar chart showing average gap area of the canal (%) (SEM) for different types of sealers.

Table (4): Statistical analysis of gap area percentage for different sealer types.

Gap area of the canal (%) (SEM) (mean±SD)			p-value
BC + Silver NP	BC + Chitosan NP	BC	
0.59 ± 0.27^{A}	0.22 ± 0.16^{B}	0.29 ± 0.15^{B}	**<0.001***

*Different superscript letters indicate a statistically significant difference within the same horizontal row *= significant ($p \leq 0.05$)*

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Table (5): Statistical analysis values of optical density for different sealer types.

Measurement	Optical density (mean±SD)			p-value
	BC + Silver NP	BC + Chitosan NP	BC	
Contact of bacteria with sealer after 60 min.	0.17±0.01 B	0.19±0.01 A	0.18±0.01 B	**0.007***

Different superscript letters indicate a statistically significant difference within the same horizontal row.
**= significant ($p \leq 0.05$)*

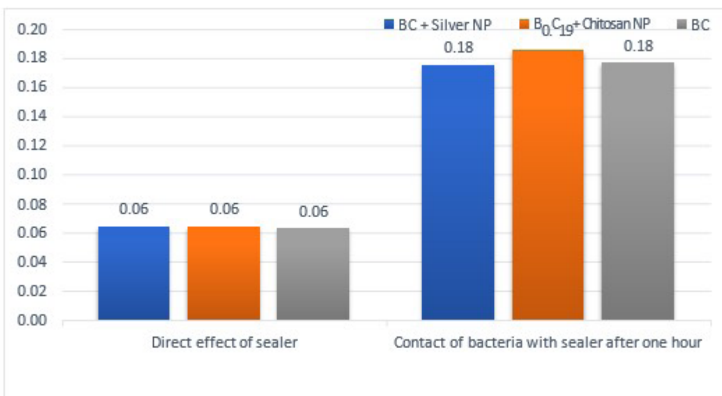

Figure (5): Bar chart showing average optical density at 620 nm for different types of sealers within each measurement.

of these materials results from the formation of calcium hydroxide and calcium silicate hydrogel.27 This alkalinity is responsible for the high pH observed, which is linked to its antimicrobial capabilities.7,9

The Totalfill BC sealer relies on the moisture present within the dentinal tubules to set. Once the material has fully set, calcium silicate aids in forming a calcium silicate hydrate gel and calcium hydroxide, which then react with dentinal phosphate ions to form hydroxyapatite and water. This process also results in a 0.20% growth, but the lack of chemical bonding between the dentinal walls and the sealer may limit its long-term performance. Nanomaterials, including metallic and organic nanoparticles, have garnered attention in dentistry due to their enhanced antimicrobial properties.29 Silver nanoparticles (AgNPs), in particular, are widely used for their ability to interact with the negatively charged bacterial cell walls. Silver NP's concentration-dependent toxicity works by interacting with sulfhydryl groups of proteins and DNA, altering hydrogen bonding, and disrupting cell wall synthesis.32 Although silver NPs have proven to be effective, their potential adverse effects on human health and the environment at lower concentrations have been a concern.33,19 In this study, we used a 23 ppm concentration of silver NP to mitigate such concerns.

Chitosan, a natural biopolymer, exhibits strong antimicrobial properties due to its high affinity for bacterial cells. Chitosan nanoparticles, due to their quantum-size effect and high surface area, can closely adhere to bacterial cell membranes, leading to the leakage of intracellular contents and cell destruction.20 The addition of chitosan nanoparticles to Totalfill BC sealer at a 15% concentration was aimed at enhancing its adaptability and flexibility while maintaining its antimicrobial properties, as outlined in Bonde et al.'s study.20 Adaptation of sealants to dentin has been extensively studied through various methods, including the use of stereomicroscopes and SEM. Studies by Akman et al.,34 Al Hadad et al.,35 and Palanivelu et al.36 have adopted stereomicroscopes for measuring gap areas, while SEM has been utilised in studies by Punithia et al.,37 Mohammadian et al.,26 Asawaworant et al.,38 and Patri et al.7. Both methods offer advantages, with SEM providing better magnification control and more precise visualisation due to its use of electron beams rather than lenses.

In the present study, the adaptability of the root canal filling material to the dentin was assessed by sectioning the roots into longitudinal sections. The results indicated that the adaptability of BC sealer to the root dentin improved towards the apical direction.39–41 This finding is consistent with the results of several studies,42,43 which suggest that the morphology of the root canal varies depending on the root third. The apical third generally showed better sealer adaptation than the coronal third, potentially due to the use of a manual dynamic activation technique, which enhances sealer penetration by effectively removing the smear layer. The addition of chitosan NP to Totalfill BC sealer improved its adaptability to root dentin compared to Totalfill BC alone and Totalfill BC mixed with silver NP, as observed under both the stereomicroscope and SEM. This outcome aligns with previous studies that demonstrated that chitosan, being hydrophilic, establishes better contact with dentin, thus improving sealer penetration. Chitosan's amino and hydroxyl functional groups enable it to interact with calcium ions in dentin, enhancing its adaptability and providing ionic bonding.47

On the other hand, silver NP combined with Totalfill BC sealer exhibited the largest gap size when compared to the different groups, both under the stereomicroscope and SEM. This finding is consistent with earlier studies (48, 49) that noted a decrease in the flow capacity of the sealer when silver NP was added. This reduction in flow may hinder the sealer's ability to adhere effectively to dentin. Totalfill BC sealer alone showed lower gap mean values compared to the Totalfill BC mixed with silver NP, supporting the notion that the alkaline byproducts of calcium silicate hydration may damage the collagenous aspect of the interfacial dentin, allowing better penetration into dentinal tubules 51

The elimination of bacteria from the root canal system remains a significant challenge, despite advancements in chemomechanical disinfection protocols. Effective obturation, which involves sealing the root canal and preventing reinfection, requires the use of sealers with antimicrobial properties.52 The addition of antimicrobial agents like silver NP enhances the sealer's bacteriostatic properties, thus providing additional protection against bacterial persistence in the root canal space. The direct contact test (DCT) has been widely employed in studies, such as those by Barros et al.,23 Poggio et al.,8 Colombo et al.,53 and Jerez-Olate et al.54, to measure the antimicrobial efficacy of root canal sealers. In this study, the bacteriostatic effects of the sealers were measured using turbidimetric values, which estimate bacterial proliferation by analysing optical density.25

Our findings indicated that both Totalfill BC sealer and Totalfill BC with 2.3% silver NP exhibited similar antimicrobial effects against E. faecalis and were significantly more effective than Totalfill BC with 15% chitosan NP. The antimicrobial activity of Totalfill BC sealer is primarily attributed to the release of hydroxyl ions, which increase the pH and create an environment hostile to bacterial growth.56 Moreover, the study found that the antimicrobial effect of Totalfill BC sealer persisted for up to 30 days after setting, providing extended protection against bacterial reinfection.57 In contrast, chitosan NP demonstrated lower antimicrobial activity against E. faecalis compared to silver NP, particularly due to its more substantial impact on gram-negative bacteria.58 This suggests that while chitosan enhances the flexibility of the sealer, it compromises its antimicrobial efficacy.

Conclusion:

The addition of silver NP to Totalfill BC sealer significantly improved its antimicrobial activity against E. faecalis, but adversely affected its adaptability to root dentin. Chitosan NP enhanced the flexibility of Totalfill BC sealer, improving its adaptability to root dentin, but reduced its antimicrobial action against E. faecalis. The best adaptation to root dentin was observed in the apical third, where higher magnifications (x45 to x1000) provided more detailed insights into sealer-dentin interactions.

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