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A Comparative evaluation of the effectiveness of nitrofurantoin and silver nanoparticles with chlorhexidine on the microhardness of root canal dentin – an *in vitro* study

Abstract

Context: Disinfecting the root canal system is incredibly challenging due to its complications, as well as the presence of multiple varieties of canal microflora. Irrigation followed by placement of intracanal medicaments helps combat the microbes present in the intricate areas of the endodontic space.

Aim: This study evaluated and compared the effects of nitrofurantoin and silver nanoparticles with chlorhexidine on radicular dentin microhardness when used as an intracanal medicament.

Methods and material: Decoronation of thirty single-rooted teeth was achieved, and canal enlargement was accomplished using F3 rotary Protaper files. Roots were sectioned transversely into three segments using self-cure acrylic resin. Depending on the intracanal medication utilised, samples were split into three groups at random. Group 1: Silver nanoparticles with chlorhexidine, Group 2: Nitrofurantoin and Group 3: Control group (Triple antibiotic paste). Baseline dentin microhardness values were recorded using Vickers hardness tester. After seven days post-treatment, dentin microhardness was recorded.

Statistical analysis: One-way ANOVA and *post hoc* Tukey were used to analyse the data.

Results: Radicular dentin microhardness reduced significantly after placement of intracanal medicaments. Silver nanoparticles with chlorhexidine showed a lesser reduction in dentin microhardness compared to nitrofurantoin and triple antibiotic paste.

Conclusion: Although the medicaments reduced the dentin microhardness, their potential clinical benefits, such as efficacy against the varied microflora seen in infected root canals, can occasionally outweigh the disadvantages.

Introduction

Root canal instrumentation involves the combined utilisation of endodontic instruments and irrigating solutions to remove existing organic and inorganic debris resulting from the operative procedures, as well as to reduce microbial content and its by-products.¹ Intracanal medicaments also have a significant part in disinfecting the root canal. Intracanal medicaments are mainly used in multi-appointment endodontic treatments to minimise microbial growth between visits.²

Calcium hydroxide stands out as the most frequently utilised intracanal medicament. However, its antibacterial efficacy has been questioned due to several factors like the buffering action of dentin, the resistance of *E. faecalis*, low solubility and diffusibility.³

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Silver nanoparticles are known to be biocompatible, non-cytotoxic and antibacterial. Silver nanoparticles derived from plant extracts have been used in this study. The safety of naturally derived silver nanoparticles from plant extracts has been established for therapeutic applications.⁴ According to a study by Nasim *et al.*, silver nanoparticles along with graphene oxide have shown to improve dentin microhardness.⁵

Nitrofurantoin was introduced as an alternative to TAP, a single drug rather than a combination of multiple drugs. Nitrofurantoin is a unique antibiotic that features a hydantoin ring and a nitro-substituted furanyl side chain, which bacteria metabolise into reactive compounds that exert bactericidal effects.⁶ It is a broad-spectrum antibiotic and works well against the majority of gram-positive and gram-negative bacteria.⁷ Notably, in a comprehensive study involving 300 *Enterococcus* isolates, none showed resistance to Nitrofurantoin, including *E. Faecalis*.⁸

Hoshino *et al.* introduced triple antibiotic paste (TAP), combining metronidazole, ciprofloxacin and minocycline in equal parts (1:1:1 ratio). They asserted that this specific antibiotic mix could effectively eliminate root canal bacteria and aid in the healing of periapical tissues.⁹ Also, TAP demonstrates effectiveness against a variety of Gram-positive and Gram-negative odontogenic bacteria, particularly against *E. faecalis*.¹⁰⁻¹² Its outstanding antimicrobial properties make it a valuable medicament for infected root canals.¹³ TAP has been used as a control in this study.

An intracanal medicament must not compromise the physical and mechanical characteristics of root dentin. However, some medicaments may negatively impact the physical properties of root dentin, such as microhardness, which indicates changes in mineral content in dental hard tissues measured at a microscopic level using an indenter.¹ Extended exposure of various medications to radicular dentin has demonstrated varying adverse effects on dentin microhardness and mineralisation, which are directly associated with both concentration and exposure duration.¹⁴ Due to the diminished microhardness of dentin following exposure to intracanal medicaments, a compromised structure could lead to an increased risk of tooth fracture.¹³

A study suggested combining Silver Nanoparticles with Chlorhexidine to target multispecies biofilms. The combination showed synergistic antimicrobial efficacy against root canal flora.^{15,16} Although its antibacterial effects are confirmed, limited research exists on its influence on dentin microhardness. Nonetheless, no studies have determined the effects of nitrofurantoin and silver nanoparticles in combination with chlorhexidine on the microhardness of the radicular dentin. Thus, the purpose of the study was to test the null hypothesis that the intracanal medicaments under study do not change the dentine microhardness.

Materials and Methods

This in vitro experimental study was conducted following approval from the Institutional Ethics Committee (Approval No. YEC2/2024/002). The study aimed to evaluate the effect of different intracanal

medicaments on the microhardness of radicular dentin using extracted human teeth under standardized laboratory conditions.

Sample Selection

Thirty single-rooted premolars of humans with single canals were extracted and used in the study. To maintain uniformity of samples, teeth with caries, fractures, developmental abnormalities, immature apices, calcifications, excessive root curvature, or multiple canals were excluded. Each specimen was carefully debrided to eliminate the remnants of the soft tissue and calculus, and then kept in 0.2% thymol solution at room temperature (4 °C) until the experimental procedures started to eliminate dehydration and microbial contamination.¹⁷

Specimen Preparation

All chosen teeth were removed at the cemento-enamel junction with a diamond disc under continuous cooling with water to achieve a standard root length of 14 mm. Pulp tissue was extirpated with a barbed broach and biomechanical preparation of the root canals was done using Protaper Gold rotary system up to file size F3. The length of work was set 1 mm below the apex. Irrigation was performed with 3% sodium hypochlorite in the instrumentation process to provide sufficient debridement with a final rinse in saline solution to remove any remaining irrigant.

After the preparation of the canals, the roots were impregnated in self-cure acrylic resin blocks to enable their manipulation and cutting. To reduce the variability in anatomy, each root was cut transversely and the middle third of the root was chosen to analyze it. Dentin discs of about 2 mm were cut out of this area. The samples were sequentially polished with silicon carbide abrasive papers of decreasing grit sizes (320-2000) under continuous water irrigation to produce a smooth and standardized surface. The last polishing was done using alumina suspension to remove the irregularities in the surface and for proper microhardness measurements.

Preparation of Intracanal Medicaments

Silver nanoparticles were prepared by use of aqueous extract of *Catharanthus roseus* as reducing agent. The plant extract was made by washing, cutting and boiling fresh leaves in distilled water, and then filtration to get a clear solution. The extract was added to 2% chlorhexidine and stirred with the silver nitrate solution until a color change to dark brown occurred which was a sign of the formation of nanoparticles. The particle size of the synthesized nanoparticles was 20-30 nm and was mixed with chlorhexidine in equal amounts.^{18,19}

The triple antibiotic paste which served as the control condition was prepared by dissolving the nitrofurantoin powder in sterile water to produce a concentration of 25 mg/ml.⁶ The triple antibiotic paste was prepared by mixing the metronidazole, ciprofloxacin, and minocycline powders in a 1:1:1 ratio and then adding sterile water to the mixture to achieve a concentration of 1 mg/ml.^{9, 20}

Grouping of Samples

The ready specimens were randomly divided into three experimental groups, each group consisting of ten specimens. Group 1 involved silver nanoparticles with chlorhexidine treatment, Group 2 involved nitrofurantoin paste treatment, and Group 3 was the control group that involved triple antibiotic paste treatment. To reduce selection bias and equal distribution across the groups randomization was carried out.

Microhardness Testing

Baseline dentin microhardness measurements were obtained using a Vickers microhardness tester.²⁰ All specimens were placed in the testing platform and a load of 200 g with a dwell time of 15 seconds was used to make indentations using a diamond indenter. The

positions of the indentations were set at the standard distance of 0.5 mm from the canal wall in the mid-root dentin so that all the samples are similar.^{16,21}

After the baseline measurements, 0.1 ml of the respective intracanal medicament was applied to each specimen, making sure that the entire surface of the dentin was covered. The specimens were then put in separate Petri dishes and allowed to incubate at 37 °C and 100 percent humidity over a period of seven days to mimic clinical conditions.²²⁻²⁶ The specimens were then rinsed with distilled water to remove any medicament and dried in air after incubation period. Microhardness was then measured at post-treatment with the same testing parameters as at the baseline. The Vickers microhardness testing apparatus used in the study is shown in Figure 1.



Figure 1: Vickers microhardness testing machine used for evaluating dentin microhardness of the specimens
Sample Size Determination

The sample size was determined using the information from past research considering a standard deviation of 7.93, expected mean difference of 7.²⁷ A significance level of 1% and a statistical power of 80. A sample size of nine specimens per group was decided to be the minimum required. In order to increase the reliability of the study and counter the possible loss of specimen in the course of the experiment, the sample size was raised to ten specimens per group.

Statistical Analysis

All the results of the study were tabulated and analyzed with the help of SPSS version. Descriptive statistics were in terms of mean and standard deviation. One-way analysis of variance (ANOVA) was used to perform intergroup comparisons to establish whether there are statistically significant differences between the groups. The test of post hoc Tukey was then adopted to make

pair-wise comparisons. The p-value below 0.05 was regarded as statistically significant.

Results

The current experiment examined the impact of three intracanal medicaments on microhardness of radicular dentin with baseline and post-treatment Vickers hardness number (VHN). It was possible to analyze all the specimens and no sample was lost in the course of the experiment.

Descriptive Statistics

Table 1 shows the descriptive statistics of the values of post-treatment VHN. Group 1 (silver nanoparticles with chlorhexidine) had the largest mean microhardness value (16.29 ± 4.22), Group 2 (nitrofurantoin) had the second largest mean value (12.82 ± 6.42), and Group 3 (triple antibiotic paste) had the lowest mean value of microhardness (10.88 ± 5.33).

Table 1: Mean and standard deviation of various groups using descriptive statistics

	n	Mean	SD	SE	95% CI for Mean	
					Lower bound	Upper bound
Group 1	10	16.29	4.22	1.34	13.27	19.31
Group 2	10	12.82	6.42	2.03	8.22	17.42
Group 3	10	10.88	5.33	1.69	7.07	14.69

SD: Standard deviation, SE: Standard error, CI: Confidence interval

Figure 2 depicts representative dentin specimens after sectioning and preparation, showing that all samples used in the experimental groups were uniform.



Figure 2: Transverse sections of prepared root dentin specimens obtained from the middle third of single-rooted teeth used for microhardness evaluation

Figure 3 illustrates the grouping and allocation of treatment to the acrylic-embedded specimens that were exposed to various intracanal medicaments.



Figure 3: Root specimen embedded in self-cure acrylic resin block prior to sectioning and microhardness testing

Intragroup Comparison

Comparative analysis of the baseline and post-treatment microhardness showed that all three groups had a decrease in dentin microhardness after the administration of intracanal medicaments. This regular decrease points to the fact that dentin structure changes under the influence of exposure to intracanal medicaments and these changes are measurable. Figure 4 demonstrates the visual representation of the treated specimens after the application of the medicaments where the differences in the appearance of the surface of the groups can be observed after the incubation period.



Figure 4: Distribution of acrylic-embedded root specimens into experimental groups: silver nanoparticles with chlorhexidine (AgNP+CHX), nitrofurantoin (Nif), and triple antibiotic paste (TAP)

Intergroup Comparison

Table 2 shows the results of intergroup comparison of VHN values after treatment through one-way analysis of variance (ANOVA). The statistical comparison showed that there was a statistically significant difference between the three groups (F = 3.4974, p = 0.0446) meaning that the type of intracanal medicament had a significant effect on dentin microhardness.

Table 2: One-way ANOVA to assess significant difference between the groups

ANOVA					
	Sum of squares	df	Mean square	F	P-Value
Between groups	169.5887	2	84.7943	3.4974	0.0446
Within groups	654.621	27	24.2452		
Total	824.2097	29	28.421		

P < 0.05

Post Hoc Analysis

Pairwise comparisons using Tukey’s post hoc test are shown in **Table 3**. A statistically significant difference was observed between Group 1 (AgNP + CHX) and Group 3 (TAP) (p = 0.0439), indicating superior preservation of dentin microhardness by the silver nanoparticle–chlorhexidine combination. No statistically significant differences were found between Group 1 and Group 2 (p = 0.1547), or between Group 2 and Group 3 (p = 0.8068).

Table 3: Post hoc Tukey for multiple comparisons between the groups

Pair	Difference	SE	Lower CI	Upper CI	P-value
Group1-Group2	4.21	1.5571	-1.2498	9.6698	0.1547
Group1-Group3	5.59	1.5571	0.1302	11.0498	0.04399
Group2-Group3	1.38	1.5571	-4.0798	6.8398	0.8068

SE: Standard error, CI: Confidence interval, P < 0.05

Overall Findings

In general, the application of all intracanal medicaments led to a decrease in dentin microhardness after 7 days of use. Silver nanoparticles with chlorhexidine had the least reduction followed by nitrofurantoin and triple antibiotic paste had

the highest reduction among the groups. Figure 5 shows the microhardness testing procedure and indentation process, which confirms the standardized measurement conditions of all the specimens.



Figure 5: Close-up view of specimen positioning under the Vickers indenter during microhardness testing

Discussion

Disinfecting the endodontic therapy is exceptionally challenging due to the complexity and variability of the root canal system, and the occurrence of different microbial communities in biofilm. The enduring presence of microbes appears to be the main factor contributing to failures in root canal treatments, which can subsequently affect both pain levels and the general quality of life.²⁴ Utilising potent antimicrobial medications within the root canal can address the limitations of instrumentation and effectively diminish the bacteria found in the intricate structure of root canals. Therefore, it is essential to have an effective antimicrobial agent to disinfect the canals and guarantee successful root canal therapy.²⁵

Certain intracanal medicaments may adversely affect the physical characteristics of root dentine, including its microhardness.¹ The aim of this study was to compare and contrast the effect of nitrofurantoin and silver nanoparticles with chlorhexidine on the radicular dentin microhardness. Each specimen was determined to have a Mean Vickers hardness number (VHN). In this research, the mid-root dentin area was selected for experimentation to reduce the impact of structural differences among various teeth and establish a suitable baseline for assessment.¹⁶

A study integrated silver nanoparticles with chlorhexidine to enhance antimicrobial effectiveness against multispecies biofilm.¹⁵ In this study, silver nanoparticles with chlorhexidine showed a lesser reduction in dentin microhardness compared to nitrofurantoin and TAP. In a study by Nasim *et al.*, a combination of silver nanoparticles and graphene oxide improved the microhardness values of dentin, which the authors attributed to the deposition of silver nanoparticles on the surface of dentin and within the dentinal tubules.⁵

Nitrofurantoin is an antibiotic widely used to treat urinary tract infections. It has been utilised in dentistry as an intracanal treatment, especially targeting *E. faecalis*.⁶ Dentine microhardness was reduced with the use of Nitrofurantoin, attributed to the demineralising effect of nitrofurantoin on radicular dentin.²⁶

TAP is an intracanal medicament that possesses outstanding antimicrobial characteristics. TAP reduced dentin microhardness in this study. Studies have shown that TAP decreased dentin microhardness over time.^{13,27} A study suggested this could be due to the breakdown of the organic component of the dentin, which is primarily made up of collagen.²⁷ The decrease in microhardness might have resulted from the demineralising effects of the acidic antibiotic combinations employed, as confirmed by several

studies.^{13,28} Due to the negative effects of the intracanal medicaments examined, the null hypothesis was rejected.

All studies have certain strengths and weaknesses. This study had too. Weighing the merits of the study, this study demonstrated the extent of reduction in dentin microhardness of samples that were treated with intracanal medicaments. Additionally, EDTA was not used as a final irrigant, a well-known chelating agent that decalcifies dentin to a depth of 20-30 µm in 5 min,²⁹ leading to a reduction in dentin microhardness,³⁰ thereby not altering the study results. Considering limitations, this is an *in vitro* study; the same result may not be observed clinically. Additionally, we are unable to manage the properties or characteristics, such as the composition of dentin in a non-vital tooth, in a clinical setting due to the variability among different patients. The mechanical properties and characteristics of non-vital teeth are also changed and tend to be less predictable when compared to vital teeth.¹³ While the medications demonstrated a decrease in the microhardness of dentin, the potential clinical benefits, such as effectiveness against the diverse microflora found in infected root canals, can sometimes surpass the drawbacks.

Conclusion

As the conclusions of this *in vitro* research, it is possible to say that the use of intracanal medicaments leads to a measurable decrease in microhardness of radicular dentin. All medicaments tested, namely, silver nanoparticles with chlorhexidine, nitrofurantoin and triple antibiotic paste, showed reduction in dentin microhardness after 7 days of application. The degree of reduction was however different among the groups. Silver nanoparticles combined with chlorhexidine had the lowest microhardness reduction, which means that it was more likely to preserve dentin structure, and triple antibiotic paste had the highest reduction. These results indicate the significance of a close choice of intracanal medicaments since they do not only affect antimicrobial effectiveness but also change the mechanical characteristics of dentin. The decrease in microhardness can undermine the structural integrity of the tooth and predispose it to fracture, which is a key factor in the planning of endodontic treatment. In spite of these discoveries, the clinical implications are to be approached with caution because of the limitations that are inherent in the *in vitro* studies. Differences in oral environment, individual factors in the patient and long-term effects cannot be completely reproduced in the laboratory. Thus, additional *in vitro* and carefully designed clinical research is needed to assess the long-term impact of these medicaments on the nature of dentin and to determine the best treatment regimens balancing antimicrobial efficacy with dentin integrity.

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