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# Effect of Chlorhexidine, Silver Nanoparticles, and Their Combination on the Fracture Resistance of Teeth: An In Vitro Comparative Study

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**KEYWORDS**

Fracture resistance,

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**ABSTRACT:**

**Introduction**: Silver nanoparticles (AgNPs) have demonstrated promising antimicrobial efficacy as intracanal medicaments; however, concerns remain regarding their interaction with radicular dentin and the potential compromise of mechanical integrity. The effect of combining AgNPs with chlorhexidine (CHX), a collagen-preserving agent, on fracture resistance has not been adequately explored.

**Objectives**: To comparatively evaluate the effect of Chlorhexidine, Silver nanoparticles and their combination as intracanal medicaments on fracture resistance of teeth.

**Methods**: Sixty extracted human single rooted mandibular premolars were decoronated and prepared using standard rotary instrumentation and randomly grouped as (n=20) Group 1 – 2% chlorhexidine gel, Group 2 – silver nanoparticles, and Group 3 –chlorhexidine-loaded-silver nanoparticles gel. After applying the intracanal medicaments and incubating for 7 days, the medicaments were removed, and universal testing machine was employed to measure the fracture resistance, and using one-way ANOVA the resulting data were subjected to statistical analysis followed by Tukey's post hoc test applied at a significance level of $p \leq 0.05$.

**Results**: Group 3 demonstrated the highest average fracture resistance, followed by Group 1 and Group 2 in decreasing order. Significant difference was observed between Group 2 and both Group 1 and Group 3 ($p \leq 0.05$), whereas no statistically significant differences were identified between Group 2 and Group 3.

**Conclusions**: The combination enhanced fracture resistance compared to silver nanoparticles alone and was comparable to that of chlorhexidine. These findings suggest that chlorhexidine mitigates the adverse effects of silver nanoparticles on dentin, making the combination a promising intracanal medicament with minimal compromise in mechanical integrity.

## 1. Introduction

Microbial persistence within the root canal system remains a principal cause of endodontic treatment failure. Complete elimination of microorganisms is challenging due to the complex anatomy of the canal system and the ability of certain pathogens to survive conventional chemo-mechanical preparation. Consequently, intracanal medicaments play a critical role in inter-appointment disinfection, particularly in cases of persistent infection, retreatment, and necrotic pulps. [1]

While intracanal medicaments are primarily selected for their antimicrobial efficacy, increasing attention has been directed toward their interaction with radicular dentin. [2] Prolonged exposure to certain medicaments has been shown to adversely affect the physicochemical and mechanical properties of dentin, including microhardness, elasticity, and fracture resistance. [3,4] Compromised dentin integrity is clinically significant, as endodontically treated teeth are inherently more susceptible to vertical root fractures, which remain a major cause of post-treatment failure.

Chlorhexidine (CHX) has been extensively investigated as an intracanal medicament owing to its broad-spectrum antimicrobial activity, substantivity, and relative biocompatibility. Importantly, CHX has been shown to inhibit matrix metalloproteinases (MMPs), thereby preserving the collagen matrix of dentin. Several studies have reported that CHX does not adversely affect the mechanical properties of root dentin, distinguishing it

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from calcium hydroxide, which has been associated with dentin weakening following prolonged application.

Silver nanoparticles (AgNPs) have emerged as a promising alternative intracanal medicament due to their potent antimicrobial action, high surface-area-to-volume ratio, and ability to penetrate dentinal tubules. [5] Their mechanism of action involves disruption of bacterial cell membranes, enzyme inhibition, and generation of reactive oxygen species. Although the antimicrobial efficacy of AgNPs has been well documented, concerns have been raised regarding their interaction with dentin substrates. Evidence suggests that nanoparticle size, surface charge, concentration, and duration of exposure may influence their effect on dentin structure, potentially altering its mechanical behaviour.

Recent studies have explored the synergistic antimicrobial potential of combining AgNPs with conventional medicaments such as chlorhexidine. Chlorhexidine-loaded silver nanoparticles have demonstrated enhanced antibacterial efficacy against resistant endodontic pathogens, including Enterococcus faecalis. However, while antimicrobial synergy has been established, there remains a paucity of evidence regarding the biomechanical consequences of such combination therapy on radicular dentin.

Specifically, the effect of silver nanoparticles—used alone or in combination with chlorhexidine—on the fracture resistance of endodontically treated teeth has not been adequately investigated. Understanding this interaction is essential, as a potentially suitable intracanal medicament should achieve effective disinfection without compromising dentin strength or increasing fracture susceptibility.

## 2. Objectives

The objective of this study was to comparatively evaluate the effect of chlorhexidine gel, silver nanoparticles, and chlorhexidine-loaded silver nanoparticles used as intracanal medicaments on the fracture resistance of human teeth. The null hypothesis tested was that there would be no significant difference in fracture resistance among the three groups.

## 3. Methods

The manuscript of this laboratory study has been written according to the Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) 2021 guidelines.[6]

Based on 95% power of the study and 0.05 permissible error, G*Power software (version 3.1.9.6) was employed to determine the sample count for this investigation, set at sixty. Upon securing ethical clearance from the university's ethical review board [IPDC/SS/2024/1860C (3)], sixty single rooted human permanent mandibular premolar teeth (n=20) were collected. Calculus and stains were removed using an ultrasonic scaler tip (Woodpecker, Guilin Woodpecker Medical Instrument Co., China) and the teeth were disinfected by immersion in sodium hypochlorite (NaOCl, 0.5%, Vishal Dentocare Pvt. Ltd., Ahmedabad, India) for 10 minutes. Each tooth was inspected under 3.5x optical magnification under a dental operating microscope (Labomed Inc., California, USA) to exclude the presence of cracks, caries, or any structural anomalies and subsequently stored in sterile saline at ambient temperature until experimentation.

Crowns were sectioned to a root length of 13 mm, then stored in distilled water.[7] Access opening was prepared and apical patency was obtained by means of a size 10 K-file (Mani Inc., Tochigi, Japan), carefully advanced 1 mm beyond the apex as seen under a dental operating microscope (Labomed Inc., California, USA). Following this, a deduction of 1 mm was made to determine the final working length, after which biomechanical preparation was performed with the ProTaper system (Dentsply Sirona, Ballaigues, Switzerland) using a torque and speed-controlled electric motor. The speed and torque values were set as recommended by the manufacturer. Between each change of instrument, irrigation of the canals was performed using 5 mL of 2.5% sodium hypochlorite (NaOCl). A final flush of 5 mL of normal saline (0.9% NaCl, Aculife Healthcare Pvt. Ltd., Ahmedabad, India) was done to neutralize any residual irrigants and then the canals were dried using sterile paper points (Dentsply Sirona, Dentsply India Pvt. Ltd., Gurugram, India)

The specimens were categorized into three separate groups randomly:

Group 1: 2% CHX gel

Group 2: 100 ppm AgNPs in propylene glycol

Group 3: CHX-loaded AgNPs gel (1:1)

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Synthesis of Silver-nanoparticles

Deionized water was used to dissolve silver nitrate to create a solution (typically, 0.01 M concentration). Sodium borohydride (or the chosen reducing agent) was dissolved in deionized water. The reducing agent solution was gradually introduced into the silver nitrate solution under continuous stirring. A visible colour change—typically yellow or brown—marked the successful synthesis of silver nanoparticles (AgNPs). A stabilizing agent like polyvinylpyrrolidone (PVP) or polyvinyl alcohol (PVA) was added to prevent the silver nanoparticles from aggregating. To ensure uniform nanoparticle size and prevent aggregation, the solution was subjected to ultrasonication for 10–15 minutes. The preparation was done according to a study by Fahmy (2024).[8]

Synthesis of Chlorhexidine-loaded Silver-nanoparticles (1:1)

100 ppm AgNPs were dispersed in 100 ml of CHX to yield a final concentration of 100mg/L, then the electrostatic binding between CHX and AgNPs was allowed by overnight mixing. The formulation utilized propylene glycol as its delivery vehicle providing a gel-like consistency.

The morphology, size, and dispersion of the synthesized silver nanoparticles were evaluated using transmission electron microscopy (TEM)[Figure 1]. A drop of the nanoparticle suspension was placed on a carbon-coated copper grid and allowed to air-dry prior to imaging. TEM analysis was performed to assess particle shape and nanoscale size distribution.

Raman spectroscopy was used to chemically identity the synthesized silver nanoparticles. Raman spectra were recorded over an appropriate spectral range under standardized conditions, and characteristic silver-associated vibrational peaks were analyzed to indicate nanoparticle formation [Figure 2].

Intercanal medicaments were placed according to the respective groups:

*Group 1: 2% Chlorhexidine gel*

One mL of commercially available CHX gel (0.2% chlorhexidine gel, Septodont, Mumbai, India) was delivered into the root canals using a 20-G needle and subsequently condensed up to the cemento-enamel junction (CEJ) using pluggers (Sybron Endo, Orange, California, USA).

*Group 2: 100 ppm AgNPs in propylene glycol*

A 100ppm AgNPs solution was introduced into the root canal in a manner discussed previously.

*Group 3: CHX-loaded AgNPs (1:1)*

The medicament was applied to the intra-radicular space similarly.

After the application of intracanal medicaments, all prepared specimens were coronally sealed with Cavit (3M ESPE, St. Paul, Minnesota, USA). For apical sealing, a flowable composite (Tetri Flow, Ivoclar Vivadent, Schaan, Liechtenstein) was subsequently applied. The samples were stored at 37°C in 100% humid conditions for a duration of one week. The seven-day medicament retention period was chosen to simulate the inter-appointment duration commonly employed in multi-visit endodontic therapy, particularly in cases of persistent infection and retreatment.

Following removal of intracanal medicaments using ultrasonic activation of 2.5% NaOCl for 60 seconds and a final rinse with 5 mL of saline, all specimens were vertically embedded in acrylic resin blocks using silicone Instrument Identification Rings (Hu-Friedy, Chicago, Illinois, USA), leaving 3 mm of the coronal root structure exposed to simulate alveolar bone support. For standardized load application, the long axis of each tooth was aligned perpendicular to the base of the acrylic block.

Fracture resistance testing was performed using a Universal Testing Machine (Instron, USA), A compressive load was applied along the long axis of the tooth using a stainless-steel spherical loading tip with a diameter of 4 mm. The load was applied at a constant crosshead speed of 1 mm/min until catastrophic fracture occurred. The maximum load at fracture was recorded in Newtons (N) for each specimen.

To reduce the potential for bias, the study employed meticulous sample selection, standardized protocols, probable randomization, uniform environmental conditions, and comprehensive statistical evaluation. Microsoft Excel 2010 was used for data entry, while Data analysis was conducted in SPSS software (version 27.0; SPSS Inc., Chicago, USA). One-way ANOVA and

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Tukey's post hoc test were employed, with statistical significance established at ($P < 0.05$) Effect size was calculated using eta squared ($\eta^2$) for one-way ANOVA to quantify the magnitude of intergroup differences.

## 4. Results

Transmission electron microscopy revealed predominantly spherical silver nanoparticles with nanoscale dimensions and relatively uniform distribution, indicating successful synthesis. Minimal particle aggregation was observed. Raman spectroscopic analysis demonstrated characteristic vibrational peaks corresponding to silver nanoparticles, indicating their chemical identity.

The mean fracture resistance values for all experimental groups are presented in Table 1. Teeth treated with chlorhexidine-loaded silver nanoparticles (CHX–AgNPs) demonstrated the highest mean fracture resistance, followed by the chlorhexidine (CHX) group, while the silver nanoparticles (AgNPs) group exhibited the lowest values.

One-way ANOVA revealed a statistically significant difference in mean fracture resistance among the three groups ($F = 7.11$; $df = 2,57$; $P = 0.026$). The effect size was large ($\eta^2 = 0.20$), indicating that a substantial proportion of the variance in fracture resistance was attributable to the type of intracanal medicament used.

Intergroup comparison using Tukey's post hoc test demonstrated statistically significant differences between the AgNPs group and both the CHX group ($P = 0.019$) and the CHX–AgNPs group ($P = 0.032$). No statistically significant difference was observed between the CHX and CHX–AgNPs groups ($P = 0.261$) (Table 2).

## 5. Discussion

The present in vitro study evaluated the effect of chlorhexidine, silver nanoparticles, and their combination on the fracture resistance of endodontically treated teeth. The results demonstrated that teeth treated with silver nanoparticles alone exhibited significantly lower fracture resistance compared to those treated with chlorhexidine or chlorhexidine-loaded silver nanoparticles. Accordingly, the null hypothesis was rejected.

Intracanal medicaments are indispensable for achieving microbial control between appointments; however, their prolonged contact with radicular dentin necessitates careful consideration of their biomechanical consequences. [9] Since endodontically treated teeth are inherently more susceptible to fracture, any medicament-induced alteration in dentin integrity may influence long-term prognosis. [10] Fracture resistance testing, therefore, provides a clinically relevant indicator of dentin structural preservation. [11]

In the present study, the chlorhexidine group demonstrated relatively high fracture resistance values, consistent with previous literature reporting minimal adverse effects of chlorhexidine on dentin mechanical properties. [12] This behaviour has been attributed to chlorhexidine's ability to inhibit matrix metalloproteinases, thereby preserving the collagen matrix and maintaining dentin's structural framework. [13]

In contrast, silver nanoparticles used alone resulted in significantly reduced fracture resistance. Although AgNPs are highly effective antimicrobial agents, their interaction with dentin may adversely influence mechanical behaviour. [14] The ability of nanoparticles to penetrate dentinal tubules and interact with organic components of dentin may alter the collagen–hydroxyapatite interface, potentially leading to structural weakening. [15] Such effects are known to be influenced by nanoparticle concentration, surface characteristics, and duration of exposure. [16]

A key novel finding of this study was that the combination of chlorhexidine with silver nanoparticles yielded fracture resistance values comparable to chlorhexidine alone and significantly higher than silver nanoparticles used independently. This suggests that chlorhexidine may mitigate the potential adverse mechanical effects associated with silver nanoparticles. The collagen-stabilizing and MMP-inhibitory properties of chlorhexidine may counterbalance the interaction of AgNPs with dentin organic components, thereby preserving dentin strength while retaining antimicrobial advantages. [5]

Most existing studies on silver nanoparticle–based intracanal medicaments have focused primarily on antimicrobial efficacy or dentin microhardness. The present study adds to the current body of evidence by demonstrating that combination therapy not only enhances antimicrobial potential, as previously reported,

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but also maintains the biomechanical integrity of radicular dentin. [6] This dual consideration of disinfection and fracture resistance represents the principal contribution of the present investigation. [17]

The large effect size observed in the present study further underscores the clinical relevance of intracanal medicament selection with respect to dentin fracture resistance. The limitations of this in vitro study should be acknowledged. Although TEM and Raman analyses indicated nanoparticle formation, advanced physicochemical characterization such as dynamic light scattering and zeta potential analysis were not performed. Additionally, the absence of thermocycling or cyclic loading protocols limits direct extrapolation to clinical conditions. The single exposure duration and controlled laboratory environment may not fully replicate the complex biomechanical stresses encountered intraorally. [18]

Further in vivo studies and long-term investigations incorporating functional loading and aging protocols are necessary to validate these findings and to determine the optimal concentration, exposure duration, and delivery systems for safe and effective clinical application.

Within these limitations, the findings suggest that while silver nanoparticles alone may compromise fracture resistance, their combination with chlorhexidine provides a more balanced intracanal medicament by preserving dentin strength. Further in vivo and long-term studies are warranted to validate these observations and to optimize concentration, exposure duration, and delivery systems for clinical application.

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**Legends for Tables**

Table 1. Comparison of mean fracture resistance (N) among different intracanal medicaments

| **Group** | **N** | **Mean Fracture Resistance (N)** | **SD** | **F-value (df)** | ***P*-value** |
|---|---|---|---|---|---|
| CHX gel | 20 | 227.00 | 10.93 | | |
| AgNPs | 20 | 183.00 | 26.88 | **7.11 (2,57)** | **0.026*** |
| CHX–AgNPs | 20 | 232.00 | 8.38 | | |

*One-way ANOVA; statistically significant at $P < 0.05$

Table 2. Intergroup comparison of fracture resistance values (N) using Tukey's post hoc test

| **Comparison** | **Mean Difference (N)** | ***P*-value** |
|---|---|---|
| AgNPs vs CHX | −43.48 | 0.019* |
| AgNPs vs CHX–AgNPs | −49.00 | 0.032* |
| CHX vs CHX–AgNPs | −5.56 | 0.261 |

*Statistically significant at $P < 0.05$

**Legends for Figures**

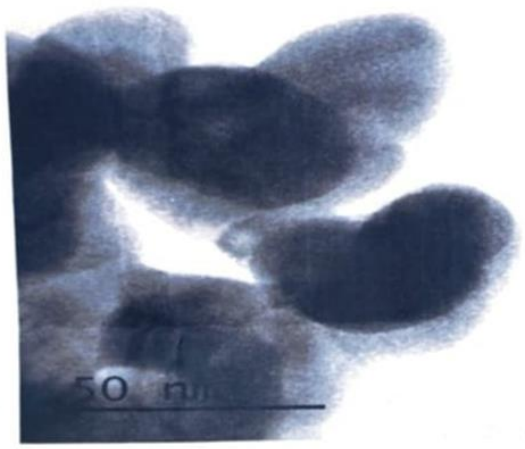


Fig 1: TEM analysis of Ag Nanoparticles.

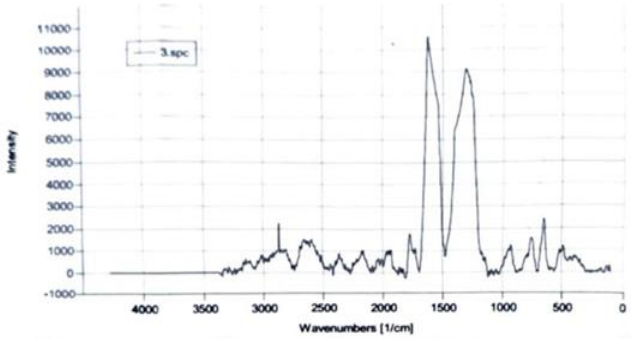


Fig 2: Raman Analysis of Silver nanoparticles