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Comparative antimicrobial efficacy of silver nanoparticle- nano-curcumin- and silver nano-curcumin-coated gutta-percha against *E. faecalis*: An *in vitro* study

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Abstract:
Persistent *Enterococcus faecalis* infection contributes to endodontic treatment failure, prompting interest in nanoparticle-modified gutta-percha for improved antimicrobial activity. Therefore, it is of interest to compare the silver nanoparticle, nanocurcumin and silver–nanocurcumin-coated gutta-percha against *E. faecalis* (ATCC 29212), with and without zinc oxide–eugenol sealer. Silver–nanocurcumin-coated gutta-percha showed the largest inhibition zone (16.25±0.28 mm), followed by silver nanoparticles (12.80±0.37 mm), nanocurcumin (10.35±0.28 mm) and control (0.33±0.15 mm). Zinc oxide–eugenol enhanced antimicrobial activity, with silver–nanocurcumin plus sealer producing the greatest inhibition (16.68±0.19 mm; $p<0.001$). These findings advance knowledge by supporting silver–nanocurcumin-coated gutta-percha as a promising infection-resistant obturation material against *E. faecalis*.

Keywords: Antimicrobial efficacy, endodontic materials, *Enterococcus faecalis*, Gutta-percha (GP), nanocurcumin, silver nanoparticles

Background:
Root canal therapy aims to eliminate infection within the root canal system while maintaining structural and functional integrity of tooth. Even after meticulous endodontic treatment, failure is still observed in approximately 3–15% of cases **[1].** These failures are primarily associated with incomplete debridement and complex canal anatomy leading to persistent microbial infection. Of all the microbial species that are known for causing post-treatment failure, *Enterococcus faecalis* is the most common organism isolated from the infected root canals **[2].** The reasons behind the persistence of this microorganism include its capacity for survival in harsh environment, deep penetration in dentinal tubules and formation of biofilm **[3]**. In addition, because of the survival of such resistant microorganisms, the attention has been diverted on the significance of obturation materials in avoiding reinfection **[4]**. Although Gutta-percha (GP) is considered the best root canal obturation material because of its biocompatibility and dimensional stability **[5]**. However, traditional GP does not have any antimicrobial property; it is used only as space filler and not as an antimicrobial material. Bacteria that survive become able to reinfection root canals **[6].** The advent of nanotechnology provides a novel approach towards enhancing the antimicrobial effect in endodontics **[7]**. The antimicrobial effect of metallic nanoparticles, including silver nanoparticles (AgNPs), is exhibited by the sustained release of ions that cause oxidation of the bacterial membranes **[8]**. In addition, the natural polyphenol curcumin, known for its antimicrobial and anti-inflammatory effects, gains enhanced bioavailability as well as efficacy when transformed to the nanoparticulate form (nanocurcumin) **[9, 10]**. Therefore, it is of interest to assess the antimicrobial activity of gutta-percha coated with AgNPs, nanocurcumin and AgNPs-nanocurcumin composite against *E. faecalis.*

Methodology:
Synthesis of nanoparticle solutions:
The silver nanoparticles were made using the method by Alejandra *et al.* **[11].** The nanocurcumin nanoparticles were made by the ionic gelation method proposed by Nair *et al.* **[12].** In case of silver-nanocurcumin composites, 100 µl of 0.1 M silver nitrate was added to 20 ml of nanocurcumin solution with 0.05 g of PVP as a stabilizer. All nanoparticle solutions were made under standardized conditions at Nano Research Lab, Jamshedpur.

Preparation of coated gutta-percha cones:
Eighty ISO sizes 25, 0.06 taper gutta-percha (GP) cones were sterilized by soaking in 5.25% sodium hypochlorite for one minute and rinsed properly with sterile distilled water. The GP cones were soaked separately in sterile Eppendorf tubes containing 200 µL of the corresponding nanoparticle solution for 24 hours to get an even coating. The GP cones were air dried for another 24 hours. In case of sealer coated subgroups, a ZOE sealer was made using two scoops of powder and one drop of liquid as recommended by the manufacturer. Half of the GP cones of each subgroup were coated with ZOE sealer and allowed to set for 24 hours before testing.

Microorganism and antimicrobial assay:
Enterococcus faecalis (ATCC 29212) was selected for its known resistance in endodontic infections and strong biofilm-forming capacity. Cultures were grown in nutrient agar at 37°C.

The study groups were divided as follows:
[1] Group A1: Uncoated GP (control)
[2] Group A2: AgNP-coated GP
[3] Group A3: Nanocurcumin-coated GP
[4] Group A4: Silver–nanocurcumin-coated GP
[5] Group B1: Uncoated GP with ZOE sealer
[6] Group B2: AgNP-coated GP with ZOE sealer
[7] Group B3: Nanocurcumin-coated GP with ZOE sealer
[8] Group B4: Silver–nanocurcumin-coated GP with ZOE sealer
Each specimen was placed on an agar plate inoculated with 0.1 mL of *E. faecalis* suspension (10^5–10^6 CFU/mL). The plates were incubated at 37°C for 24 hours. Antibacterial efficacy was determined by measuring the diameter of the inhibition zones (in mm) using a digital vernier caliper. All the data were

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evaluated using SPSS version 22. Comparison of antimicrobial efficacy among the groups was done using one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$).

Results:

The antimicrobial efficacy of the experimental groups was assessed by measuring the zone of inhibition against *Enterococcus faecalis*. Among the non-sealer groups, silver–nanocurcumin–coated gutta-percha demonstrated the highest mean inhibition zone (16.25 ± 0.28 mm), followed by silver nanoparticle–coated (12.80 ± 0.37 mm) and nanocurcumin-coated gutta-percha (10.35 ± 0.28 mm), whereas conventional gutta-percha showed negligible activity (0.33 ± 0.15 mm). The differences between all groups were statistically significant ($p < 0.001$). The zone of inhibition of four groups of without sealer coated gutta percha is summarised in **Table 1** and also depicted in **Figure 1.** In the sealer-coated groups, an overall increase in antimicrobial activity was observed. Silver–nanocurcumin-coated gutta-percha with ZOE sealer exhibited the maximum inhibition zone (16.68 ± 0.19 mm), followed by silver-coated (13.00 ± 0.24 mm) and nanocurcumin-coated samples (10.63 ± 0.24 mm), while the control group showed minimal activity (4.48 ± 0.15 mm). The difference between the groups was statistically significant. The zone of inhibition of four groups of with sealer coated gutta percha is summarised in **Table 2** and also shown in **Figure 2.** The comparison of mean zone of inhibition of four subgroups between two groups (without and with sealer coated gutta percha) is summarised in **Table 3** and also shown in **Figure 3.**

Table 1: Zone of inhibition (mm) of four groups of without sealer coated gutta percha

Group	Zone of inhibition (mm) (Mean ± SE) (n=10)	F value	*P* value
Uncoated gutta percha	0.33 ± 0.15	583.20	< 0.001
Nanocurcumin coated gutta percha	10.35 ± 0.28		
Silver nanoparticle coated gutta percha	12.80 ± 0.37		
Silver nanocurcumin coated gutta percha	16.25 ± 0.28		

Table 2: Zone of inhibition (mm) of four groups of with sealer coated gutta percha

Group	Zone of inhibition (mm) (Mean ± SE) (n=10)	F value	*P* value
Uncoated gutta percha	4.48 ± 0.15	599.70	< 0.001
Nanocurcumin coated gutta percha	10.63 ± 0.24		
Silver nanoparticle coated gutta percha	13.00 ± 0.24		
Silver nanocurcumin coated gutta percha	16.68 ± 0.19		

Table 3: Comparison of difference in mean zone of inhibition (mm) of each of four groups between without and with sealer coated gutta percha by Student's t test

Subgroup	Group		Mean diff.	t value	*P* value
	Without sealer coated gutta percha (n=10)	With sealer coated gutta percha (n=10)			
Gutta percha	0.33 ± 0.15	4.48 ± 0.15	4.15	19.46	< 0.001
Nanocurcumin	10.35 ± 0.28	10.63 ± 0.24	0.27	0.74	0.470
Silver nanoparticle	12.80 ± 0.37	13.00 ± 0.24	0.20	0.45	0.657
Silver nanocurcumin	16.25 ± 0.28	16.68 ± 0.19	0.43	1.25	0.228

difference, t value: Student's t test value.

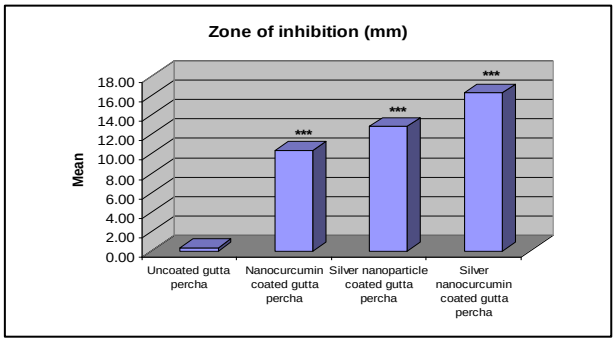


Figure 1: Mean zone of inhibition of four groups of without sealer coated gutta percha (***$P < 0.001$- as compared to Uncoated gutta percha)

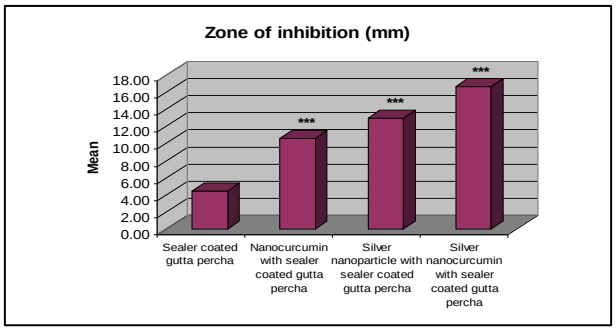


Figure 2: Mean zone of inhibition of four groups of with sealer coated gutta percha (***$P < 0.001$- as compared to Sealer coated gutta percha)

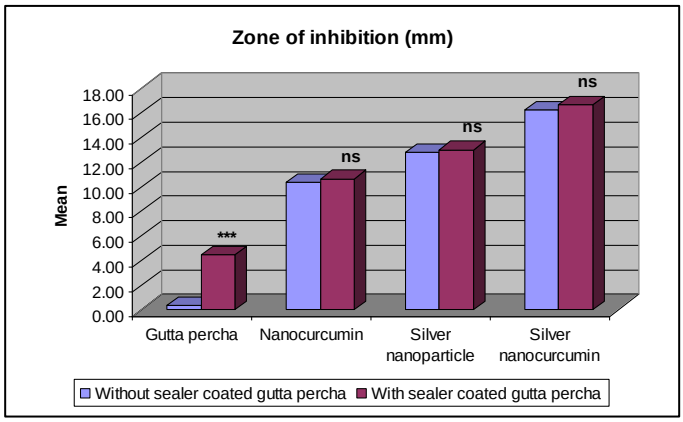


Figure 3: Comparison of difference in mean zone of inhibition of four groups between without and with sealer coated gutta percha (ns $P > 0.05$ or ***$P < 0.001$- as compared to without sealer coated gutta percha)

Discussion:

Root canal therapy aims to eradicate microorganisms and prevent reinfection within the complex canal system; however,

despite the introduction of newer and innovative disinfection approaches, infections caused by *Enterococcus faecalis* remain a significant contributor to endodontic failure **[11].** The ability of *E. faecalis* to survive under extreme conditions, penetrate dentinal tubules and form resilient biofilms underscores the desire for obturating materials with intrinsic and sustained antimicrobial properties **[12].** Conventional Gutta Percha (GP) is well-known but has no inherent anti-bacterial properties. Research indicates that GP can provide an ideal medium for microbial growth if improperly stored or handled. The combination of Zinc Oxide in addition to Gutta-Percha Matrix limits the release of ions into solution and therefore does not allow for direct anti-microbial activity. Therefore, traditional GP acts as a passive material providing a barrier from microorganisms that were present at time of obturation thus provides no active mechanism for elimination of residual bacteria; this establishes the rationale for incorporating anti-microbial agents into GP **[13]**. Previous attempts to introduce medicated GP, such as those containing iodoform or antibiotics, demonstrated inconsistent or transient efficacy **[14]**. In contrast, coating gutta-percha with antimicrobial nanoparticles can provide a continuous release of active ions or molecules, maintaining a bacteriostatic or bactericidal environment within the canal **[7]**. Nanoparticle-based coatings offer long-term, broad-spectrum antimicrobial activity due to their small size and high surface reactivity **[7]**. Coating nanoparticles onto GP rather than incorporating them within the matrix preserves the material's handling and thermoplastic properties while ensuring direct microbial contact at the surface **[14]**. Studies by Adnan *et al.* have suggested nanoparticle coating on gutta-percha which can impart antimicrobial activity to gutta percha without compromising physical properties **[4]**. In this study, the antimicrobial efficacy of GP was significantly enhanced following nanoparticle coating. Silver nanoparticle–coated GP (Group A2-12.80 ± 0.37 mm) and nanocurcumin-coated GP (Group A3-10.35 ± 0.28 mm) both showed measurable inhibition zones, with the combined silver–nanocurcumin formulation (Group A4-16.25 ± 0.28 mm) demonstrating the highest antibacterial activity. The enhanced efficacy of the combination can be attributed to the complementary mechanisms of silver and nanocurcumin. On the other hand, nanocurcumin acts primarily by integrating into bacterial cell membranes and generating reactive oxygen species (ROS), leading to lipid and protein oxidation **[15, 16].** It also interferes with bacterial division by binding to the filamenting temperature-sensitive mutant Z (FtsZ) protein, a prokaryotic homolog of tubulin that orchestrates cytokinesis, disrupting septum formation and replication **[17]**. Although nanocurcumin has exhibited strong antimicrobial properties, these are restricted due to its organic structure and that its effectiveness is based on diffusion so it will be weaker compared with silver nanoparticles **[9]**. In addition to creating oxidative stress, as described above, silver nanoparticles produce cytotoxicity within bacteria at the level of enzymes, metabolism and ultimately, by causing an influx of ions to penetrate the cytosol. Once inside the bacterial cell, Ag+ ions denature enzymes and disrupt the electron transport chain for ATP production. These disruptions cause a cascade of lethal events. A significant advantage of using silver nanoparticles is their "Trojan Horse" ability to carry Ag+ ions directly into the bacterial cell where they are released rapidly and with great potency **[18].** The greatest synergistic effect was observed when combining silver nanoparticles and nanocurcumin. Both exhibit unique mechanisms of bactericidal activity. Silver produces its bactericidal effects primarily via the release of Ag+ ions which then react with intracellular SH containing molecules (enzymes) and participate in generating ROS **[19]**, while nanocurcumin prevents aggregation of silver nanoparticles thereby providing increased availability of active silver species. Additionally, both compounds can generate oxidative damage on all structural components (proteins, DNA/RNA, membranes) of the bacterial cell **[20].** Furthermore, the use of zinc oxide-eugenol (ZOE) sealer enhanced the antibacterial efficacy in all groups. eugenol adds further antibacterial property by inhibiting bacterial membrane permeability and denaturation of proteins; while zinc oxide acts as Zn^{2+} ions source for generating ROS **[21]**. The synergy observed between ZOE and nanoparticle coated GP could be due to increased diffusion of the antimicrobial agents in the sealer. Although this research was an investigation of the antimicrobial impact of AgNP modified materials as they relate to their initial use, previous studies have shown that there is a prolonged release of silver ions from AgNP modified materials **[21].** Thus it can be inferred that these AgNP modified materials could potentially provide extended antibacterial protection. This evidence supports the higher rate of bacterial killing (short term) of AgNPs observed in this study and suggests the possibility for a long lasting method of disinfecting the root canal system. This nanoparticle modification constitutes an important step towards the development of active obturation materials which would ensure constant antimicrobial protection.

Conclusion:

Nanoparticle coatings significantly enhanced the antimicrobial action of gutta-percha. The combination of silver and nanocurcumin coated cones exhibited the largest zone of inhibition. The inclusion of a ZOE sealer added synergistic antimicrobial action, supporting infection-resistant obturation systems.

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