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Comparative Evaluation of the Antibacterial Efficacy of Nanocurcumin-, Neem-, Sodium Hypochlorite- and Chlorhexidine-Coated Gutta-Percha Against Enterococcus faecalis: An In Vitro Study

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ABSTRACT

Background: Residual microorganisms may persist after chemomechanical preparation and contribute to post-treatment endodontic disease. Incorporating antimicrobial agents onto gutta-percha could provide supplementary antibacterial activity within the obturated canal. Aim: To compare the antibacterial efficacy of gutta-percha coated with nanocurcumin, neem extract, 5.25% sodium hypochlorite and 2% chlorhexidine against Enterococcus faecalis. Materials and Methods: Sterilised ISO F2 gutta-percha cones were divided into five groups: conventional gutta-percha, 5.25% sodium hypochlorite-coated gutta-percha, 2% chlorhexidine-coated gutta-percha, neem extract-coated gutta-percha and nanocurcumin-coated gutta-percha. Each group comprised five observations. Nutrient agar plates were uniformly inoculated with an E. faecalis suspension containing approximately 10^5–10^6 cells/mL. Prepared gutta-percha specimens were placed on the inoculated plates and incubated at 37°C for 24 hours. The largest diameter of the inhibition zone was measured in millimetres using a digital vernier calliper. Results: Sodium hypochlorite-coated gutta-percha produced the largest mean inhibition zone (4.386±0.394 mm), followed by chlorhexidine (3.700±0.126 mm), nanocurcumin (2.704±0.071 mm), neem extract (2.026±0.038 mm) and conventional gutta-percha (0.464±0.021 mm). The overall difference was statistically significant ($F=338.50$; $p<0.001$), and every pairwise comparison was significant. Conclusion: All four coatings increased the antibacterial activity of gutta-percha. Sodium hypochlorite and chlorhexidine demonstrated the strongest effects, while nanocurcumin performed better than neem extract.

INTRODUCTION

Microorganisms are central to the development and persistence of pulpal and periapical diseases. The biological relationship between microorganisms and apical inflammation was established when pulpal exposure produced periapical pathology in conventionally raised animals but not in germ-free animals.[1] Endodontic treatment therefore aims to eliminate microorganisms, remove infected tissue and establish a three-dimensional seal that prevents reinfection. Complete microbial elimination is difficult because bacteria can survive in dentinal tubules, fins, isthmuses and other areas inaccessible to mechanical instruments. *Enterococcus faecalis* is particularly relevant because it is frequently recovered from root-filled teeth with persistent apical periodontitis.[2] This facultative anaerobic Gram-positive coccus can tolerate nutritional deprivation, alkaline conditions and environmental stress. It also adheres to dentine, penetrates dentinal tubules and forms biofilms, which can make it resistant to conventional endodontic procedures.[3,4]

Gutta-percha is the most widely used solid core material for root canal obturation because of its biocompatibility, dimensional stability, radiopacity and ease of removal during retreatment. Nevertheless, conventional gutta-percha has limited inherent antibacterial activity. Although zinc oxide and other constituents may produce a weak antimicrobial effect, this action may not be sufficient to inhibit microorganisms remaining after canal preparation.[5] Coating gutta-percha with antibacterial agents could create a biologically active surface capable of suppressing residual microorganisms near the root-filling interface.

Sodium hypochlorite and chlorhexidine are established endodontic antimicrobial agents. Sodium hypochlorite disrupts essential bacterial components and dissolves organic material, whereas chlorhexidine damages the bacterial cell membrane and possesses substantivity. Both agents have demonstrated activity against *E. faecalis*, although complete eradication is not consistently achieved.[6] Interest in plant-derived and nanoformulated antibacterial materials has increased because of concerns regarding cytotoxicity, chemical interactions and antimicrobial resistance. Neem contains several biologically active constituents with antibacterial and anti-inflammatory properties. Curcumin has antimicrobial potential, but its clinical application is limited by poor aqueous solubility and stability. Nanoformulation increases the available surface area and may enhance the dispersion, interaction and penetration of curcumin into bacterial cells. Nanocurcumin-coated gutta-percha has previously demonstrated greater inhibition of *E. faecalis* than uncoated cones.[7]

Despite growing evidence supporting active gutta-percha materials, differences in coating agents, test organisms and laboratory methods prevent direct extrapolation between studies.[8] The present study compared conventional gutta-percha with gutta-percha coated using 5.25% sodium hypochlorite, 2% chlorhexidine, neem extract and nanocurcumin. The null hypothesis was that the mean zones of inhibition would not differ among the five materials.

MATERIALS AND METHODS

An in vitro experimental study was conducted to compare the antibacterial activity of conventional and antibacterial agent-coated gutta-percha against *E. faecalis*. Antibacterial activity was evaluated using an agar diffusion assay.

A total of 25 observations were included. The prepared gutta-percha specimens were distributed equally into five groups, with five observations in each group:

Group A: Conventional gutta-percha

Group B: 5.25% sodium hypochlorite-coated gutta-percha

Group C: 2% chlorhexidine-coated gutta-percha

Group D: Neem extract-coated gutta-percha

Group E: Nanocurcumin-coated gutta-percha

ISO F2 gutta-percha cones, 5.25% sodium hypochlorite, 2% chlorhexidine, neem extract, nanocurcumin, nutrient agar, nutrient broth, sterile distilled water, sterile saline, micropipettes, inoculating loops, Petri plates and a digital vernier calliper were used. ISO F2 gutta-percha cones were initially immersed in 5% sodium hypochlorite for one minute for surface disinfection. The cones were subsequently rinsed with sterile distilled water to remove the sterilising solution. They were handled with sterile instruments throughout the preparation procedure.

Preparation of coated gutta-percha

Sterilised cones assigned to the experimental groups were manually coated with their respective agents: 5.25% sodium hypochlorite, 2% chlorhexidine, neem extract or nanocurcumin. The coated cones were allowed to dry under aseptic conditions so that a film formed over their external surface. The conventional gutta-percha group did not receive an experimental antibacterial coating. After drying, the cones were sectioned horizontally to obtain standardised cross-sectional specimens for microbiological testing. The concentrations and preparation methods for neem extract and nanocurcumin were maintained according to the formulations specified in the study protocol. No additional chemical agents were applied to the conventional gutta-percha specimens.

Preparation of Enterococcus faecalis

A pure culture of *E. faecalis* was used as the test microorganism. The organism was inoculated into nutrient broth and incubated to obtain active bacterial growth. The bacterial suspension was adjusted to contain approximately 10^5–10^6 cells/mL. The identity and purity of the culture were confirmed through colony morphology and standard microbiological procedures before the agar diffusion test was performed.

Preparation and inoculation of agar plates

Sterile nutrient agar plates were prepared and labelled according to the five study groups. Each agar surface was heavily and uniformly seeded with 0.1 mL of the standardised *E. faecalis* suspension. The inoculum was evenly distributed over the plate to produce confluent bacterial growth. Prepared conventional and coated gutta-percha specimens were aseptically positioned on the corresponding inoculated agar plates. Care was taken to ensure consistent contact between the test surface and agar.

Following placement of the specimens, the agar plates were incubated aerobically at 37°C for 24 hours. The plates were maintained under identical incubation conditions for all five groups.

Measurement of antibacterial activity

Antibacterial activity was evaluated by observing the clear zone surrounding each gutta-percha specimen. This area represented inhibition of bacterial growth produced by the conventional material or the applied coating. The largest diameter of each inhibition zone was measured in millimetres using a digital vernier calliper. Measurements were recorded for all five observations in every group. A larger inhibition zone was interpreted as greater antibacterial activity against *E. faecalis*. The primary outcome was the diameter of the zone of inhibition, expressed in millimetres. The independent variable was the type of gutta-percha coating. The dependent variable was the measured inhibition-zone diameter after incubation.

Statistical analysis

Data were entered into Microsoft Excel and analysed using IBM SPSS Statistics version 25. Mean, standard deviation, minimum and maximum were calculated for each group. Mean inhibition-zone diameters were compared using one-way analysis of variance. After identifying a statistically significant overall difference, post-hoc pairwise comparisons were performed to determine which groups differed. $p<0.05$ was considered statistically significant.

RESULTS

Antibacterial activity was detected in all five groups, although its magnitude differed substantially. Conventional gutta-percha produced only a small inhibition zone. Every antibacterial coating increased the mean inhibition-zone diameter. Sodium hypochlorite-coated gutta-percha produced the greatest antibacterial effect, with a mean zone of 4.386±0.394 mm. Chlorhexidine-coated gutta-percha ranked second, followed by nanocurcumin-coated and neem-coated gutta-percha.

Table 1. Zone of inhibition produced by the five gutta-percha groups

Group	Material tested	n	Mean	Minimum–maximum
A	Conventional gutta-percha	5	0.464±0.021	0.44–0.49
B	5.25% sodium hypochlorite-coated gutta-percha	5	4.386±0.394	4.09–4.87
C	2% chlorhexidine-coated gutta-percha	5	3.700±0.126	3.52–3.84
D	Neem extract-coated gutta-percha	5	2.026±0.038	1.99–2.08
E	Nanocurcumin-coated gutta-percha	5	2.704±0.071	2.62–2.78

$F=338.50$; $p<0.001$

The material used for coating significantly affected antibacterial performance. Sodium hypochlorite produced the largest zone of inhibition, while conventional gutta-percha produced the smallest. Nanocurcumin demonstrated greater antibacterial activity than neem extract but remained less effective than sodium hypochlorite and chlorhexidine.

Every pairwise comparison was statistically significant. Sodium hypochlorite-coated gutta-percha produced a mean inhibition zone 0.686 mm larger than chlorhexidine-coated gutta-percha. Chlorhexidine produced a mean zone 0.996 mm larger than nanocurcumin, while nanocurcumin exceeded neem extract by 0.678 mm.

Table 2: Pairwise comparison of inhibition zones

Comparison	Mean difference (mm)*	P value
Conventional vs sodium hypochlorite-coated GP	−3.922	<0.001
Conventional vs chlorhexidine-coated GP	−3.236	<0.001
Conventional vs neem-coated GP	−1.562	<0.001
Conventional vs nanocurcumin-coated GP	−2.240	<0.001
Sodium hypochlorite-coated vs chlorhexidine-coated GP	0.686	0.014
Sodium hypochlorite-coated vs neem-coated GP	2.360	<0.001
Sodium hypochlorite-coated vs nanocurcumin-coated GP	1.682	<0.001
Chlorhexidine-coated vs neem-coated GP	1.674	<0.001
Chlorhexidine-coated vs nanocurcumin-coated GP	0.996	<0.001
Neem-coated vs nanocurcumin-coated GP	−0.678	<0.001

*Mean difference was calculated as the mean of the first group minus that of the second group. GP: gutta-percha.

Each antibacterial coating performed significantly better than conventional gutta-percha. Sodium hypochlorite was significantly superior to the other coatings, and chlorhexidine was significantly superior to both herbal formulations. Among the plant-derived agents, nanocurcumin produced significantly greater inhibition than neem extract.

DISCUSSION

The present study compared the antibacterial efficacy of four coated gutta-percha formulations against *E. faecalis*. The null hypothesis was rejected because significant differences were observed among all five groups. The descending order of antibacterial activity was 5.25% sodium hypochlorite-coated gutta-percha, 2% chlorhexidine-coated gutta-percha, nanocurcumin-coated gutta-percha, neem-coated gutta-percha and conventional gutta-percha.

The agar diffusion method provided a visible and measurable indication of bacterial inhibition around each specimen. However, the inhibition-zone diameter depends on both antimicrobial activity and the ability of an agent to diffuse through agar. It does not directly quantify bacterial killing on the gutta-percha surface or within dentinal tubules. Luddin and Ahmed highlighted that agar diffusion results are affected by concentration, molecular size, solubility, agar composition and diffusion characteristics.[9] The present findings should therefore be interpreted as comparative antibacterial activity under the specified experimental conditions.

Sodium hypochlorite-coated gutta-percha demonstrated the largest mean inhibition zone. Its antimicrobial action is associated with the release of active chlorine, oxidation of cellular components and disruption of essential bacterial enzymes. The sodium hypochlorite-coated group was significantly more effective than the chlorhexidine-coated group, although both substantially outperformed the remaining materials. This finding differed from Sinha et al., who reported strong activity of chlorhexidine and neem against *E. faecalis*, with no significant difference among neem, sodium hypochlorite and chlorhexidine.[10] Differences in agent concentration, extract preparation, diffusion behaviour and testing of free solutions rather than coated cones may explain the contrasting results. The current result demonstrated short-term activity after sodium hypochlorite coating and drying; it did not confirm sustained activity after obturation. Unlike irrigation, during which fresh sodium hypochlorite can be replenished, the active chlorine available from a dried coating may decline over time. Therefore, its stability, release profile, sealer compatibility and effects on gutta-percha properties require further assessment.

Chlorhexidine-coated gutta-percha produced the second-largest inhibition zone. Chlorhexidine is a cationic bisbiguanide that interacts with negatively charged bacterial surfaces, alters membrane permeability and, at higher concentrations, causes precipitation of intracellular contents. Its adsorption to biological surfaces contributes to substantivity. Mustafa also demonstrated antibacterial activity of 2% chlorhexidine against *E. faecalis* when compared with neem and sodium hypochlorite.[11] Chandrappa et al. similarly reported activity against *E. faecalis* with chlorhexidine and neem preparations.[12] These findings support the use of chlorhexidine as a reference agent when evaluating plant-derived endodontic antimicrobials. Nevertheless, chlorhexidine does not dissolve organic tissue and may form an undesirable precipitate when combined with sodium hypochlorite. Its compatibility with irrigants and sealers must therefore be established before clinical application as a gutta-percha coating.

Nanocurcumin-coated gutta-percha ranked third, with a mean inhibition zone of 2.704±0.071 mm. It performed significantly better than neem-coated and conventional gutta-percha. Panwar et al. likewise reported that nanocurcumin-coated gutta-percha produced a larger inhibition zone against *E. faecalis* than conventional gutta-percha.[7] Curcumin can interfere with bacterial membranes, cell division and intracellular processes. However, conventional curcumin has limited aqueous solubility and stability. Nanoformulation increases its surface-area-to-volume ratio and may improve dispersion and interaction with bacterial cells.[7]

Sharma et al. found that curcumin-loaded chitosan nanoparticle-coated gutta-percha demonstrated greater antibacterial activity than untreated cones.[13] Because chitosan possesses antimicrobial properties, the observed effect could not be attributed exclusively to nanocurcumin. Another study by Sharma et al. showed that nanocurcumin-coated gutta-percha exhibited greater activity than conventional curcumin-coated and uncoated cones.[14] Although that study used *Escherichia coli*, it supports the potential influence of nanoformulation on antibacterial performance. The narrow range observed in the present nanocurcumin group also indicated relatively consistent activity. Neem-coated gutta-percha demonstrated significantly greater inhibition than conventional gutta-percha but was less effective than the other coatings. Neem contains biologically active compounds, including nimbidin, nimbolide and azadirachtin, which may interfere with bacterial membranes and cellular metabolism.[10-12] However, neem preparations vary according to plant source, extraction solvent, concentration, storage and phytochemical composition. The findings therefore apply specifically to the formulation used in this study.

Rumhein et al. demonstrated that the activity of antimicrobial gutta-percha points against *E. faecalis* depended on the incorporated substance and experimental environment.[15] Their findings support the concept of biologically active filling points while emphasising that laboratory inhibition does not necessarily indicate clinical effectiveness. Conventional gutta-percha produced the smallest inhibition zone of 0.464±0.021 mm. Its weak activity may be related to zinc oxide and other constituents, but every antibacterial coating produced significantly greater inhibition. A systematic review of nanoparticle-coated gutta-percha similarly reported promising antimicrobial effects but identified substantial methodological heterogeneity and limited clinically relevant evidence.[16]

The study used standardised microbial, incubation and measurement conditions, with equal allocation across groups. Nevertheless, only five observations were included per group. Coating thickness and uniformity were not quantified, and minimum inhibitory concentration, minimum bactericidal concentration and direct-contact bacterial survival were not assessed. Agar diffusion may favour readily diffusible substances, while the single-species, 24-hour model did not reproduce mature biofilms, dentinal tubules, sealer interactions or intracanal conditions.[9,16] Future studies should standardise coating characteristics and evaluate release kinetics, surface stability, physical properties, cytotoxicity, sealer compatibility and activity against mature multispecies biofilms using complementary direct-contact, CFU and imaging methods.

CONCLUSION

Within the limitations of this in vitro agar diffusion study, coating gutta-percha with 5.25% sodium hypochlorite, 2% chlorhexidine, neem extract or nanocurcumin significantly increased its antibacterial activity against *Enterococcus faecalis*. Sodium hypochlorite-coated gutta-percha produced the largest inhibition zone and was significantly more effective than all other materials. Chlorhexidine-coated gutta-percha demonstrated the second-highest activity. Among the plant-derived formulations, nanocurcumin produced significantly greater inhibition than neem extract, while both performed better than conventional gutta-percha. Conventional cones exhibited only weak antibacterial activity. These results indicate that antimicrobial surface modification may improve the biological function of gutta-percha and could help suppress residual microorganisms after root canal preparation. However, agar diffusion findings do not

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