Original Article

Comparative evaluation of the antibacterial activity of curcumin-coated gutta-percha versus nanocurcumin-coated gutta-percha against *Escherichia coli*: An *in vitro* study

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Abstract

Introduction: Due to its biological and antibacterial qualities, many plants, including curcumin, are used as phytomedicines in dentistry. They are primarily used as intracanal medication in endodontics to prevent probable chemical side effects and also to address antimicrobial resistance. Curcumin nanoformulations have improved antibacterial activity and improved dispersion, making them the superior form of curcumin. The purpose of this study was to assess curcumin and nanocurcumin's antibacterial properties. As a gutta-percha coating, they are to be tested against *Escherichia coli*.

Materials and Methods: The study employs the standard strain of *E. coli*, ATCC 25922. The antibacterial activity of gutta-percha cones against *E. coli* is assessed after coating them with suspensions of curcumin and nanocurcumin. Scanning electron microscopy is utilized to evaluate the coatings' continuity.

Results: The gutta-percha cones that are untreated, coated with curcumin, and coated with nanocurcumin exhibit significantly different levels of antibacterial activity. There is statistically significant variation in their antibacterial activity.

Conclusion: (1) Compared to curcumin-coated and untreated gutta-percha cones, those coated with nanocurcumin exhibit a stronger antibacterial activity. (2) Compared to uncoated gutta-percha cones, gutta-percha cones coated with curcumin exhibit more antibacterial action.

Keywords: Curcumin; gutta-percha; nanocurcumin; scanning electron microscopy

INTRODUCTION

Endodontic treatment points to dispense with diseases from the internal root canal framework and avoid reinfection by obturation.[1] The complexity of the root canal can be attributed to the presence of isthmuses, accessory canals, and dental tubules. Root canals' conventional cleaning procedures do not prevent microbes from surviving due to this complex anatomy.[2] In addition to helping to prevent and treat apical periodontitis, the removal of bacteria from the root canal system is essential in lowering the number of re-treatment cases and, consequently, treatment failure.[3]

There are many different types of bacteria present at the primary endodontic level of infection, with Gram-negative

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bacteria, primarily anaerobes, predominate. In contrast, the diversity of bacterial flora at the secondary level of infection is minimal and distinct. Because of their virulence factors and pathogenicity markers, these bacteria are able to reenter the roots even after treatment. Secondary or persistent root canal infections usually contain facultative bacteria that were formerly hard to isolate from primary infections.[4-6]

The primary virulence characteristics of these Gram-negative bacteria that are common in root canals include their ability to build biofilms, their resistance to intracanal medications, their ability to produce endotoxin lipopolysaccharide (LPS), and their capacity to survive on their own in an environment deficient in nutrients.

Antibiotic resistance has resulted in a shift toward the use of phytochemicals and a search for other methods of treating these infections.[7-9] Direct broad-spectrum antibacterial activity of curcumin against both Gram-positive and Gram-negative bacteria has been demonstrated.[10-12] The anti-inflammatory, anti-arthritic, anti-asthmatic, anti-oxidant, anti-microbial, cardiac protein, and immune-modulatory properties of curcumin have been demonstrated.[13] Curcumin's broad-spectrum pharmacological characteristics notwithstanding, its low intestinal absorption, hydrophobic nature, and fast metabolism provide a significant obstacle to the drug's desired therapeutic applications. After oral administration, it has extremely little systemic bioavailability.[14]

A number of methods have been developed recently to boost curcumin's effectiveness.[15] Using curcumin nanoformulations is the most popular and efficient way to improve the stability and solubility of curcumin.[16] The purpose of this study is to evaluate the antibacterial activity of uncoated, curcumin-coated, and nanocurcumin-coated gutta-percha against *Escherichia coli*.

MATERIALS AND METHODS

Nanocurcumin synthesis

Ionic gelation method was used to prepare curcumin-loaded chitosan nanoparticles, the methodology procedure was described by Nair *et al*.[17] To prepare this, to 1% (v/v) acetic acid solution, 0.2% w/v chitosan were added and it was homogenized by overnight stirring at 500 rpm on magnetic stirrer. 4M NaOH was used to adjust the pH of the solution to 5. Then, the curcumin (6%) dissolved in tween 80 was gently mixed with the chitosan solution. To achieve mass ratios of chitosan and tripolyphosphate (TPP) of between 3:1 ratio, an anionic cross-linker TPP (0.1% w/v) was added dropwise to this solution. The suspension obtained was further stirred using magnetic stirrer for 10–45 min at room temperature.

Bacterial strain

Reference strain *E. coli* ATCC 25922 was the bacterial strain employed in this investigation. To obtain the isolated colonies, the freeze-dried strain preparation was reconstituted using brain heart infusion (BHI) broth, subcultured on BHI agar plates, and then incubated at 37°C for 18–24 h. Following that, the bacterial solution was diluted until the final concentration met the McFarland criterion of 0.5.

Preparation of coated gutta-percha cones

In this study, curcumin and nanocurcumin coatings were applied to gutta-percha. Size 35 taper 4% gutta-percha cones certified from the International Organization for Standardization were autoclaved to sterilize them. The sterilized gutta-percha cones were then placed in Eppendorf tubes containing the tested coatings of nanocurcumin and curcumin for a duration of 24 h. Cones were taken out and let to air dry for an additional day. Together with the untreated gutta-percha cone samples, these coated gutta-percha cones were also submitted for scanning electron microscopy (SEM) to check the uniformity of the coatings on the surface of the gutta-percha cones. Furthermore, tests were conducted on the antibacterial activity of the three different varieties of gutta-percha.

Antimicrobial activity of coated gutta-percha and uncoated gutta-percha cones

The agar diffusion method was utilized to ascertain the antibacterial activity.[18] The nanoparticle-coated gutta-percha cones and the curcumin-coated cones were compared for their antibacterial activity along with that of ordinary uncoated gutta-percha. For this, a reference strain of *E. coli* was used to seed the BHI plates. The plates were split into two sections. On one half of the plate, normal gutta-percha and coated gutta-percha were inserted on the other half. The plates were incubated for 18–24 h at 37°C. The results were compared and read as a zone of inhibition. Three assays were run, and the mean value was taken into account for statistical analysis.

Scanning electron microscopy study

SEM was used to assess the surface topography of the coated and uncoated gutta-percha cones. Cones coated with curcumin and nanocurcumin and uncoated gutta-percha were critical point dried and then gold coated using an ion sputter JFC 1100. After the cones were cut horizontally, pictures were made by scanning the cross sections of the cones.[19] A JSM-6100 scanning electron microscope with a slub was used to investigate the coated gutta-percha cones' outer surface at Punjab University Chandigarh's Central Instrument Laboratory.

Statistical analysis

The comparison between the results of the antibacterial activity of three groups (uncoated gutta-percha, curcumin-coated gutta-percha, and nanocurcumin-coated gutta-percha) was done using a one-way ANOVA test.

RESULTS

Antimicrobial activity of coated gutta-percha versus uncoated gutta-percha cones

The agar diffusion method was utilized to assess antimicrobial activity. The results clearly indicate that coated gutta-percha exhibits a well-defined distinct zone of inhibition against *E. coli*, while the assay findings demonstrate no antimicrobial activity of conventional gutta-percha against *E. coli.* When we compared the zones of inhibition of the three groups, we found out that nanocurcumin had wider zones as compared to the other two groups. The test outcomes are shown in Figures 1a and b.

Scanning electron microscopy study

The gutta-percha cones' surface coatings have dramatically altered the topography of the object. Comparing the uncoated gutta-percha surface to coated cones, unevenness and irregularities have been observed. While curcumin coatings and nanocurcumin coatings were closely adherent to the surface, but latter showed greater uniformity and evenness; for further details, see Figure 2a-c.

Statistical analysis result

As clear from Table 1 which shows test results of one-way ANOVA that there is significant difference between the antibacterial activity of nanocurcumin-coated gutta-percha as compared to curcumin-coated and uncoated gutta-percha. The F-ratio value is 6056. The $P < 0.00001$. The result is significant at $P < 0.05$, indicating that nanocurcumin-coated gutta-percha has the highest antibacterial activity and uncoated gutta-percha has the least antibacterial activity.

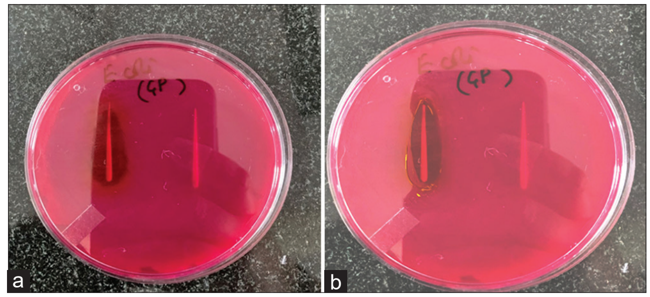

Figure 1: (a) Antibacterial activity of nanocurcumin-coated gutta-percha versus uncoated gutta-percha, (b) Antibacterial activity of curcumin-coated gutta-percha versus uncoated gutta-percha

Table 1: Statistical details

Summary of Data

	Treatments			Total
	1	2	3	
N	5	5	5	15
∑X	2.25	4.85	7.75	14.85
Mean	0.45	0.97	1.55	0.99
∑X2	1.0135	4.7055	12.0135	17.7325
Std.Dev.	0.0158	0.0158	0.0158	0.4653

Result Details

Source	SS	df	MS	*F* ratio
Between-treatments	3.028	2	1.514	F=6056
Within-treatments	0.003	12	0.0003	
Total	3.031	14		

SS: sum of squares; df: degree of freedom and MS: mean sum of squares

DISCUSSION

A number of microbial taxa, primarily facultative anaerobes, are important contributors to endodontic case failure.[20,21] These microorganisms either enter the canal through coronal or apical leaks following obturation, or they survive all endodontic cleaning methods and remain inside the canal and dentine tubule.[22,23] Microbes that cause endodontic failure and flare-ups include Gram-positive bacteria (*Staphylococcus*, *Enterococcus*), Gram-negative bacteria (*E. coli*), and yeasts (*Candida albicans*).[24,25] Bacterial prevalence differences in the literature may be due to differences in detection techniques, sample collection, and patients' clinical conditions. Some microorganisms have a similar prevalence in both primary infections and secondary infections, suggesting that they may not be completely eliminated during treatment with endodontics.[26,27]

The aim of the study was to synthesize and test the antibacterial activity of a curcumin-loaded chitosan nanoparticle against *E. coli*, as well as to compare it with that of curcumin alone and also with that of uncoated gutta-percha. Curcumin has a wide range of therapeutic potential due to its antimicrobial properties, anti-inflammatory properties, anticancer properties, and anti-oxidation properties. However, its potential remains limited due to low bioavailability, large size, and stability problems.[14] However, because nanocurcumin is much smaller than curcumin, it penetrates cells more easily and is taken up by them more fully. Our study's findings show that gutta-percha coated with nanocurcumin has higher antibacterial activity than gutta-percha coated with conventional curcumin, suggesting that nanocurcumin has more antimicrobial potential than curcumin. To match the zone of inhibition of curcumin to that of nanocurcumin, curcumin at higher concertation is to be used; since at the same concentration levels, they showed different zone diameters. In this investigation, a size 35 gutta-percha cone was selected since it is an intermediate size that works well with most canals and obturation methods.[28] When compared to the surface of uncoated and curcumin-coated gutta-percha cones, the nanocurcumin coating is more consistent and tightly adheres to the gutta-percha cones.

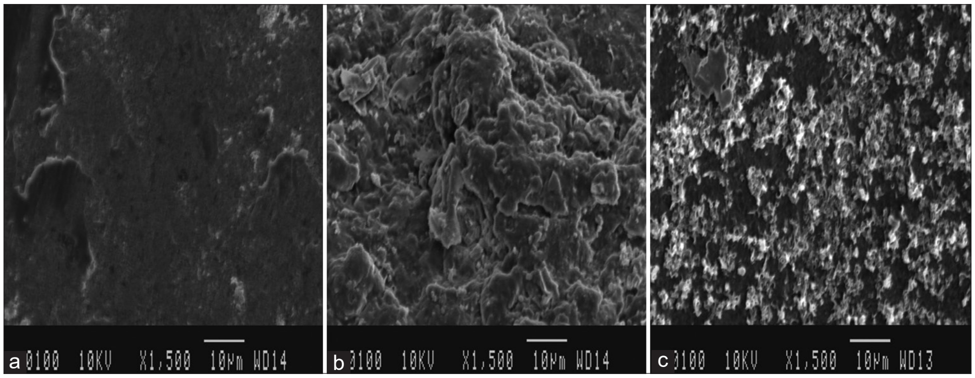

Figure 2: (a) Scanning electron microscopy (SEM) image of uncoated gutta percha at ×1500, (b) SEM image of curcumin-coated gutta-percha at ×1500, (c) SEM image of nanocurcumin-coated gutta-percha at ×1500

Our study's findings concur with those of Adahoun *et al*., who also showed that curcumin nanoformulations have improved antibacterial activity and increased water solubility.[29] A further study by Mun *et al*. showed that curcumin and other antibiotics had a synergistic impact on methicillin-resistant *Staphylococcus aureus* and that using curcumin in combination with each of these drugs significantly improved the inhibition of bacterial growth.[30] Researchers Panwar *et al*. showed that gutta-percha coated with nanocurcumin had an antibacterial impact on *Enterococcus faecalis*,[31] whereas Sharma *et al*. observed an antimicrobial effect on *S. aureus*.[32] Our findings also support the findings of Tyagi *et al*., who demonstrated that curcumin had potent antibacterial activity against *E. coli*, *S. aureus*, and *E. faecalis*.[33] Our investigation revealed that conventional gutta-percha lacked antibacterial activity; these findings are consistent with those of Melker *et al*.,[20] who also showed that normal gutta-percha was unable to eradicate crucial endodontic pathogens.

CONCLUSION

Nanocurcumin represents a significant advance as an antimicrobial agent against *E. coli* and other pathogens prevalent in endodontic infections. Curcumin nanoparticles have good stability and solubility, therefore, difficulties inherent in curcumin administration can be circumventers. Gutta-percha cones coated with nanocurcumin have antimicrobial activity against various microbes and may be of great help in combating residual microbes in the root canals after obturation, thus limiting flare-ups and decreasing the number of failure cases in endodontic treatment.

Limitations of the study

1. Minimum inhibitory concentration of curcumin as well as nanocurcumin would add to the precision of the study
2. A study designed on large population sample is required to generalize the results
3. Carrier used for nanoparticle preparation may also alter the properties of nanoparticles. Detailed characterization of nanoparticles is required for precise results.

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Conflicts of interest

There are no conflicts of interest.

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