Original Article

An *in vitro* evaluation of antibacterial effect of curcumin-loaded chitosan nanoparticle-coated gutta-percha against *Staphylococcus aureus*

Jyoti Sharma, Deepika Panwar[1], Jagat Bhushan[1], Manjula Mehta, Kitty Sidhu[1], Swaty Jhamb[1], Vandita Kakkar[2]

Departments of Microbiology and [1]Conservative Dentistry and Endodontics, Dr. Harvansh Singh Judge Institute of Dental Sciences and Hospital, Panjab University, [2]University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India

Abstract

Background: The persistence of microorganisms in root canal system is pivotal factor pertinent to endodontic failure. Even if you meet the highest technical and asepsis standards and also minimize the procedural errors, failures result. The rapidly increasing antibiotic resistance among these bacteria and the adverse effects of these antibiotics along with their toxicity are the main situations indicating the utmost urgent requirement of a safe, effective, natural phytochemical like curcumin with tremendous medicinal potential. Nanoformulations of curcumin are their improved version with enhanced antibacterial activity.

Materials and Methods: A thin layer of nanocurcumin was coated on the surface of gutta-percha cones. To observe the uniformity and adherence of nanocurcumin coating on the exterior surface of gutta-percha, scanning electron microscopy (SEM) was done. Further agar gel diffusion technique was used to assess the antimicrobial activity of nanocurcumin-coated gutta-percha cones and conventional gutta-percha cones and their results were compared statistically.

Results: The results of SEM study showed a layer of nanocurcumin adhering uniformly to the surface of gutta-percha cones. Furthermore, the nanocurcumin-coated gutta-percha cones demonstrated higher antibacterial activity as compared to the conventional cones.

Conclusions: Our study results reveal that the coating of nanocurcumin on gutta-percha cones has augmented their antibacterial activity.

Keywords: Gutta-percha; nanocurcumin; root canal failure; *Staphylococcus aureus*

INTRODUCTION

The fundamental and very essential basis of endodontic treatment is comprehensive debridement and antisepsis of root canal system of the clinically involved pulp tissue to shape the canal space and to equip it to be replete with the safest inert material, thereby reducing the chances of re-infection to the bare minimum. However, this is not absolute because sometimes the endodontic procedures fail because of various factors such as the microorganisms that are capable to survive harsh chemical as well as strict mechanical debridement of the root canal.[1] These bacteria are usually resistant to antibiotics, have capacity to form tenacious biofilms, and are capable of surviving in the minimal nutritional environment; hence, they reign in the filling material.[2] The relation of such bacteria with the periradicular infection is deep rooted, is well established, and has been extensively documented in the literature. That is why, at the time of root canal obturation if such robust microorganisms persist, they become the major cause

Address for correspondence:
Dr. Jyoti Sharma,
Department of Microbiology, Dr. Harvansh Singh Judge Institute of Dental Sciences and Hospital, Panjab University, Chandigarh, India.
E-mail: contactjyotisharma@yahoo.co.in

Date of submission : 08.05.2023
Review completed : 15.07.2023
Date of acceptance : 17.07.2023
Published : 16.09.2023

Access this article online	
Quick Response Code:	Website: https://journals.lww.com/jcde
	DOI: 10.4103/jcd.jcd_302_23

How to cite this article: Sharma J, Panwar D, Bhushan J, Mehta M, Sidhu K, Jhamb S, *et al*. An *in vitro* evaluation of antibacterial effect of curcumin-loaded chitosan nanoparticle-coated gutta-percha against *Staphylococcus aureus*. J Conserv Dent Endod 2023;26:560-3.

of endodontic root canal treatment failure.[3] To address this problem, several studies have been undertaken. To reduce the density of such bacteria in root canal system, a biocompatible intracanal drug with antibacterial properties is introduced in the roots, between appointments, which is expected to increase the likelihood of root canal therapy success.[4] Out of several approaches which are undertaken to reduce the number of root canal treatment failure cases, enhancing the antibacterial potential of commercially available gutta-percha cones remains the most common and most relevant approach.

An untreated tooth differs remarkably from the root canal-failed tooth in terms of their microflora. The endodontic treatment failure can be attributed to these residual bacteria only if they have the pathogenicity determinants and are in significant proportion at the site and presented with the virulence factors which facilitate their spread to periradicular tissue to establish periradicular disease. Since these bacteria have no strict nutritional requirement, it is easy for them to remain viable in the canals for prolonged duration even in the nutritionally deprived environment. In addition, because of their capacity to form biofilm, they are more resistant to regular irrigants, medicaments, and antibiotics as compared to their planktonic counterparts. In the current scenario, the antibiotic resistance and the bacterial infections caused by this multidrug resistance are ranked among the first 10 health threats faced throughout the world as the antibiotic depository is running short[5] and soon will reach its exhaustion level. To deal with this forthcoming crunch of antimicrobial agents, several products are being tried by various researchers. Out of all the known products, a potential plant-based antimicrobial – curcumin – is extensively studied. Curcumin alone and its different formulations are being evaluated and substantiated against different multidrug-resistant bacterial strains. The number of phytochemicals has been tried till date in conservative dentistry and endodontics.[6]

Curcumin, which is a plant-derived biomolecule, has abundant therapeutic applications including antimicrobial activity. Its therapeutic potential cannot be exploited to its fullest because of its few properties such as its particle size is very large, its water solubility is very low, its bioavailability is less, and cellular uptake is diminished and has stability issues.[7] Various nanoformulations of curcumin can be synthesized. The nanocurcumin of curcumin is upgraded form of this promising candidate with tremendous improved properties as compared to its crude form. They now have their bioavailability limits enhanced; their particle size is reduced, so now, solubility is also augmented along with its improved antimicrobial activity.[8] Its antimicrobial action can be attributed to its more penetrating power and its ability to disrupt the outer membrane barrier.[9] Another important mode of action is that after they enter inside the cell, they trigger the synthesis of various reactive oxygen species interceding membrane and cytoplasmic leakage. It also curbs various crucial bacterial metabolic processes which are essential for the survival of bacteria, bacterial growth, and formation of biofilm, thereby leading to stunted bacterial growth and subsequently will cause bacterial death.[10] Curcumin-loaded liposomes boost its water dispersibility and chemical stability as well as its antioxidant and anti-inflammatory activities.[11] Curcumin nanoparticles that have higher antimicrobial action against various bacteria such as *Staphylococcus aureus, Escherichia coli*, *Enterococcus faecalis,* and *Pseudomonas aeruginosa* are in size of 2–40 nm.[12,13] According to a membrane permeability analysis, it is shown to cause membrane leakage in Gram-positive and Gram-negative bacteria.[14] These are the supporting reasons for the fact that its embodiment into gutta-percha could be clinically applicable in clearing residual microorganisms from root canals.

MATERIALS AND METHODS

Preparation of nanocurcumin

In this study, the method used for the synthesis of curcumin nanoparticles was done according to the procedure described by Nair *et al*.[15] Ionic gelation method was used for the preparation of curcumin-loaded chitosan nanoparticles. Concisely, 0.2% w/v of chitosan was added to a 1% (v/v) acetic acid solution. To dissolve this, the solution was stirred overnight using a magnetic stirrer maintained at a speed of 500 rpm. NaOH (4 mM) was added to this solution to adjust the final pH of the solution to 5.0. To this chitosan solution, 6% curcumin dissolved in tween 80 was mixed gently. Then, to this solution, 0.1% w/v tripolyphosphate (TPP) which acts as an anionic cross-linker, TPP was added dropwise so as to achieve mass ratios of chitosan and TPP of 3:1. The obtained suspension was further stirred at room temperature for 10–45 min.

Bacterial strain

A standard strain of *S. aureus*, ATCC 25923, was used in the present study. The strain was revived using brain–heart infusion (BHI) broth, and later, the bacteria were subcultured on BHI agar media plates. The plates were then incubated at 37°C for 18–24 h to get the isolated colonies.

Preparation of coated gutta-percha

The gutta-percha cones used for the study were of the International Organization for Standardization size 25, taper 4%. The gutta-percha cones were first sterilized by autoclaving. These autoclaved gutta-percha cones were then placed in the Eppendorf Tubes which were containing the nanocurcumin coating to be tested, for 24 h. The cones were removed and air-dried for another 24 h.

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

The antibacterial activity was determined using the agar diffusion method following the Clinical and Laboratory Standards Institute guidelines.[16] The activity of conventional gutta-percha was compared with that of curcumin-loaded chitosan nanoparticle-coated gutta-percha cones. For this, the BHI plates were swabbed with a suspension of the standard stain of *S. aureus*. The plates were divided into two halves; on one half, conventional gutta-percha was placed, and on the other, coated gutta-percha was placed. Then, these media plates were incubated at 37°C for 18–24 h, and the results were read in the form of zones of inhibition and compared. The assay was performed in triplicate, and the mean value was considered for statistical analysis.

Scanning electron microscopy study

Both nanocurcumin-coated gutta-percha cones and noncoated cones were critical point dried and were gold coated using ion Sputter JFC-1100. The gutta-percha cones were cut horizontally, and to acquire the images, the cross-section of the cones was scanned. The exterior plane of the overlaid gutta-percha cones was scanned using the scanning electron microscope JSM-6100 with slub at the Central Instrumentation Laboratory, Panjab University, Chandigarh.[17]

Statistical analysis

A comparison of the antibacterial activity of coated gutta-percha cones with that of noncoated gutta-percha cones was evaluated using an independent *t*-test.

RESULTS

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

The antibacterial activity of *S. aureus* was estimated using the agar gel diffusion method. The assay results show no antimicrobial activity of conventional gutta-percha against *S. aureus*, whereas a well-defined, clear zone of inhibition was shown by coated gutta-percha against *S. aureus*, as evident from Figure 1a and b.

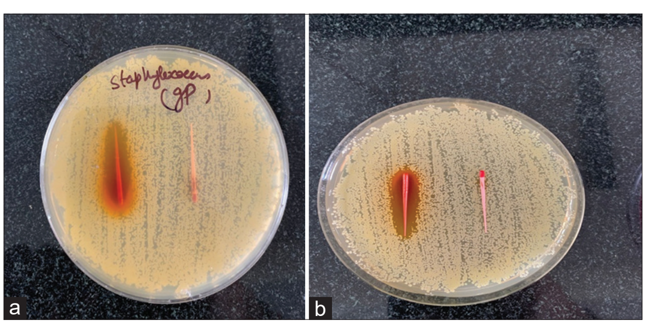

Figure 1: Zone of inhibition of nanocurcumin-coated gutta-percha and conventional gutta-percha, (a) external view, (b) internal view

Scanning electron microscopy study

Scanning electron microscopy findings showed that the coating of nanocurcumin that was formed on the surface of gutta-percha cones was uniform and closely adherent, which is clear from Figure 2a and b.

Statistical analysis

The comparison of the zone of inhibition of coated gutta-percha cones with that of noncoated gutta-percha cones was evaluated using an independent *t*-test. Since the *P* value came out to be <0.0001 [Table 1], the results were extremely significant, suggesting that for *S. aureus*, the mean value of the zone of inhibition of nanocurcumin-coated gutta-percha was significantly higher than that of nonnanocurcumin-coated gutta-percha.

DISCUSSION

Endodontic treatment aims to put an end to microbial flora responsible for infecting root canals, but the failure of endodontic cases occurs as many bacteria survive chemical and mechanical cleaning of the root canal and hence persist in the canal.[18] Endodontically treated or obturated teeth are nine times more likely to harbor microorganisms in comparison to nonroot canal-filled teeth,[19] so there is a need for a better methodological approach to address these residual microbes and microbes that may cause new infections after obturation is done.

This study was designed to compare the antimicrobial effect of curcumin-loaded chitosan nanoparticle-coated gutta-percha and its comparison with conventional

Table 1: Statistical Test

Independent *t*-test				
	t	Degree of freedom	*P*	95% CI of this difference
Zone of inhibition	134.76	4	0.0001	From 11.98 to 12.48

Descriptive statistics		
Groups	Coated gutta-percha	Noncoated gutta-percha
Mean	13.150	0.013
SD	0.036	0.005
SEM	0.020	0.003
n	3	3

CI: Confidence interval, SD: Standard deviation, SEM: Standard error of mean

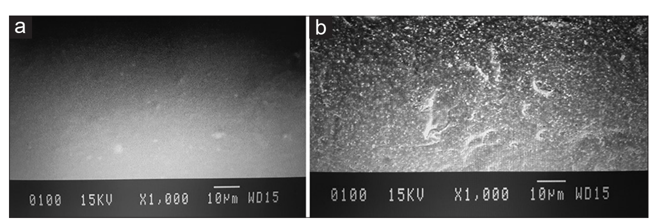

Figure 2: (a) Scanning electron microscopy (SEM) image of nanocurcumin-coated gutta-percha at ×1000, (b) SEM image of noncoated gutta-percha at ×1000

gutta-percha. Conventionally used gutta-percha points have been shown to have antibacterial effect on a few bacterial species.[20] The results of our study are not in accordance with this study which showed that conventional gutta-percha points did not have any antibacterial activity against *S. aureus* as checked by the diffusion method. Melker *et al*.[21] in their studies showed the failure of conventional gutta-percha in killing the various endodontic microorganisms, and hence, the results of our study are in accordance with those done by them. Our study is also in consensus with Moghadas *et al*.,[22] who showed that nanosilver had antimicrobial activity against bacteria such as *E. faecalis* and *S. aureus*. A study by Tyagi *et al*.[14] demonstrated the antimicrobial effect of curcumin against *E. faecalis*, *S. aureus,* and *E. coli*. Yet, another study by Mandroli and Bhat[23] showed the antimicrobial potential of curcumin against endodontic bacteria.

This study demonstrated enhanced antimicrobial activity of curcumin-loaded chitosan nanoparticles coated with gutta-percha. These results of our studies are in concordance with several other studies that show that coated gutta-percha has an increased antimicrobial effect.[24] Yet, another study by Moorer and Genet[25] enhanced the effect of gutta-percha after being coated with zinc oxide.

CONCLUSIONS

A coating of thin film of curcumin-loaded chitosan nanoparticle on gutta-percha cones is beneficial as it increases its antibacterial activity against *S. aureus*. To encounter the residual microbes in the treated as well as failed root canal cases, this can be of immense use. Further, this will also provide a solution to reduce the use of chemicals and their side effects, including toxicity. This will be helpful in overcoming the challenge of antimicrobial resistance. The results are encouraging, yet additional work is recommended to appraise the physical and mechanical properties of these coated gutta-percha cones.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Siqueira JF Jr. Aetiology of root canal treatment failure: Why well-treated teeth can fail. Int Endod J 2001;34:1-10.
2. Siqueira JF Jr., Rôças IN, Favieri A, Abad EC, Castro AJ, Gahyva SM. Bacterial leakage in coronally unsealed root canals obturated with 3 different techniques. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000;90:647-50.
3. Sjögren U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. Int Endod J 1997;30:297-306.
4. Sundqvist G, Figdor D, Persson S, Sjögren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85:86-93.
5. Dadgostar P. Antimicrobial resistance: Implications and costs. Infect Drug Resist 2019;12:3903-10.
6. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: Problems and promises. Mol Pharm 2007;4:807-18.
7. Jyoti BB. Phytotherapeutics in conservative dentistry & endodontics – A review. J Conserv Dent 2005;8:31.
8. Shaikh J, Ankola DD, Beniwal V, Singh D, Kumar MN. Nanoparticle encapsulation improves oral bioavailability of curcumin by at least 9-fold when compared to curcumin administered with piperine as absorption enhancer. Eur J Pharm Sci 2009;37:223-30.
9. Singh BN, Singh BR, Gupta VK, Kharwar RN, Pecoraro L. Coating with microbial hydrophobins: A novel approach to develop smart drug nanoparticles. Trends Biotechnol 2018;36:1103-6.
10. Ratrey P, Dalvi SV, Mishra A. Enhancing aqueous solubility and antibacterial activity of curcumin by complexing with cell-penetrating octaarginine. ACS Omega 2020;5:19004-13.
11. Sanidad KZ, Sukamtoh E, Xiao H, McClements DJ, Zhang G. Curcumin: Recent advances in the development of strategies to improve oral bioavailability. Annu Rev Food Sci Technol 2019;10:597-617.
12. Bhawana, Basniwal RK, Buttar HS, Jain VK, Jain N. Curcumin nanoparticles: Preparation, characterization, and antimicrobial study. J Agric Food Chem 2011;59:2056-61.
13. Panwar D, Sidhu K, Bhushan J, Kakkar V, Mehta M, Sharma J. Evaluation of antimicrobial efficacy of nanocurcumin-coated gutta-percha against *Enterococcus faecalis*: An *in vitro* study. J Conserv Dent 2023;26:160-4.
14. Tyagi P, Singh M, Kumari H, Kumari A, Mukhopadhyay K. Bactericidal activity of curcumin I is associated with damaging of bacterial membrane. PLoS One 2015;10:e0121313.
15. Nair RS, Morris A, Billa N, Leong CO. An evaluation of curcumin-encapsulated chitosan nanoparticles for transdermal delivery. AAPS PharmSciTech 2019;20:69.
16. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. CLSI Approved Standard M100-S15. Wayne: Clinical and Laboratory Standards Institute; 2018.
17. Afanasyev SS, Kychkina TV, Savvinova LN. Scanning electron microscope (advantages and disadvantages). Research Paper on Medical Technology. Colloq J 2019;2:2019;2:25-7.
18. Nair PN. On the causes of persistent apical periodontitis: A review. Int Endod J 2006;39:249-81.
19. Siqueira JF Jr., Rôças IN. Diversity of endodontic microbiota revisited. J Dent Res 2009;88:969-81.
20. Turner SR, Love RM, Lyons KM. An *in-vitro* investigation of the antibacterial effect of nisin in root canals and canal wall radicular dentine. Int Endod J 2004;37:664-71.
21. Melker KB, Vertucci FJ, Rojas MF, Progulske-Fox A, Bélanger M. Antimicrobial efficacy of medicated root canal filling materials. J Endod 2006;32:148-51.
22. Moghadas L, Shahmoradi M, Narimani T. Antimicrobial activity of a new nanobased endodontic irrigation solution: *In vitro* study. Dent Hypotheses 2012;3:142-6.
23. Mandroli P, Bhat K. An *in-vitro* evaluation of antibacterial activity of curcumin against common endodontic bacteria. JAPS 2013;3:106-8.
24. Jain VM, Karibasappa GN, Dodamani AS, Vishwakarma PK, Mali GV. Comparative assessment of antimicrobial efficacy of different antibiotic coated gutta-percha cones on *Enterococcus faecalis* an *in vitro* study. J Clin Diagn Res 2016;10:C65-8.
25. Moorer WR, Genet JM. Antibacterial activity of gutta-percha cones attributed to the zinc oxide component. Oral Surg Oral Med Oral Pathol 1982;53:508-17.