Antimicrobial Efficacy of Nanoparticle-Coated Gutta-Percha: A Systematic Review

Samira Adnan[1], Abhishek Lal[2], Maham Muneeb Lone[1], Jamshed Ahmed[1], Isma Sajjad[1], Huma Shareef[3] and Muhammad Sohail Zafar[4,5]

[1]*Department of Operative Dentistry, Sindh Institute of Oral Health Sciences, Jinnah Sindh Medical University, Karachi, Pakistan*
[2]*Department of Medicine, The Aga Khan University, Karachi, Pakistan*
[3]*Department of Pharmacognosy, Institute of Pharmaceutical Sciences, Jinnah Sindh Medical University, Karachi, Pakistan*
[4]*Department of Clinical Sciences, College of Dentistry, Ajman University, Ajman, United Arab Emirates*
[5]*School of Dentistry, Jordan University, Amman, Jordan*

ABSTRACT

This systematic review aimed to evaluate the use of different nanoparticles for their ability to impart antimicrobial properties to conventionally inert gutta-percha when it is coated with nanoparticles. After registering with PROSPERO (CRD42023420939), the search was conducted using the PubMed, Cochrane, Scopus, CINAHL, and Google Scholar with predefined keywords. The research question was constructed on PICO as "Does gutta-percha coated with various nanoparticles provide antibacterial activity against root canal microbes compared to conventional gutta-percha points?". A total of five relevant *in-vitro* studies published in English were included. Nanoparticles used for coating included nanopropolis, silver-curcumin, chitosan, silver, and zinc oxide. Micro-organisms tested comprised of *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. Higher concentration of nanoparticles and prolonged coating duration resulted in increased antimicrobial activity, irrespective of the type of nanoparticle, as compared to conventional gutta-percha. The highest risk of bias was identified in reporting of blinding, randomisation, sampling techniques, and sample size calculation. Fewer studies, as well as heterogeneity of methodologies and results preclude the recommendation of a specific nanoparticle for coating gutta-percha.

Key Words: *Antimicrobial efficacy, Endodontics, Gutta-percha, Micro-organisms, Nanoparticles*.

How to cite this article: Adnan S, Lal A, Lone MM, Ahmed J, Sajjad I, Shareef H, *et al.* Antimicrobial Efficacy of Nanoparticle-Coated Gutta-Percha: A Systematic Review. *J Coll Physicians Surg Pak* 2025; **35(02)**:213-220.

INTRODUCTION

Nanoparticles (NP) are being utilised in specialities of healthcare, medicine, medical imaging, and dentistry, indicating the potential of this novel modality.[1-4] The term nanomaterial is defined by the European Commission's recommendation as a natural or manufactured material containing particles, in an unbound state, as an aggregate, or an agglomerate, in which ≥50% of the particles have external dimensions of 1-100 nm.[5] Nanomaterials exhibit diverse compositions and morphologies, including spheres, rods, tubes, and prisms.[6] Based on their composition, nanoparticles can typically be classified as naturally occurring (organic or inorganic) or man-made.[7] The enhanced efficacy of nanoparticles is derived from their capability to interact with living tissues at a molecular and subcellular level, to alter hydrogen bonding, unwind DNA, ultimately lead to cell death.[6] Nanoparticles enhance the physical properties of root dentine when used with photosensitisers.[8]

Correspondence to: Dr. Maham Muneeb Lone, Department of Operative Dentistry, Sindh Institute of Oral Health Sciences, Jinnah Sindh Medical University, Karachi, Pakistan
E-mail: maham.mlone@gmail.com

Received: March 29, 2024; Revised: July 07, 2024; Accepted: September 05, 2024
DOI: https://doi.org/10.29271/jcpsp.2025.02.213

In addition, they decrease the formation of biofilm and augment mineralisation of tooth structure by halting its de-mineralisation.[9] The innate antimicrobial property of nanoparticles can be utilised by incorporation into dental materials or by utilising them as surface coatings.[10,11]

In endodontics, proper cleaning and shaping of root canal space is essential to remove all infective tissue and micro-organisms before this space is obturated. Gutta percha (GP) is a commonly used material for root-canal filling because of its biocompatibility, affordability, and aseptic nature. Even though GP cones are made in an aseptic environment, investigations have shown that even recently opened boxes of GP have an increased chance of contamination, especially with *Staphylococcus* species, due to poor handling, storage, and aerosolisation.[11] Additionally, since gutta-percha cones have negligible antibacterial activity, they are ineffective in preventing microbial infections post-obturation.

Various strategies have been employed to help improve and enhance the antimicrobial properties of GP, including surface-modified and medicated gutta-percha. Calcium hydroxide, ZnO/chlorhexidine, and ZnO/iodine-polyvinylpyrrolidone containing gutta-percha cones failed to suppress endodontic infections in root canals despite being generally recognised as antimicrobial materials. As a result, finding the alternate coating mate-

rials for gutta-percha cones to boost their anti-bacterial efficiency is imperative for the improved prognosis of root canal therapy.[12]

The use of nanoparticles has been tested in obturating materials, sealers, restorative and regeneration materials, irrigation strategies, photodynamic therapy, and medicine delivery systems.[6,8,13] The most frequently used nanoparticles in endodontics are composed of silver, followed by those of polymeric origin.[14] However, the majority of the novel nanoparticles are still in the early stages of laboratory evaluation.[15]

The ability of nanoparticles to function effectively in preventing and treating dental infections caused by various microbes is an exciting dimension to be further explored.[16] The aim of this systematic review was to evaluate all current scientific literature available on the use of different nanoparticles for their ability to impart antimicrobial properties to the conventionally inert gutta percha when it is coated with them. This would help to comprehensively compile relevant current evidence, evaluate effectiveness, identify knowledge gaps, and provide guidance for clinical practice with the aim of improving the outcome of endodontic treatment.

METHODOLOGY

This study protocol was registered with PROSPERO (CRD4202 3420939), and PRISMA guidelines were followed for reporting. The research question was "Does gutta-percha coated with various nanoparticles provide antibacterial activity against root-canal microbes compared to conventional gutta-percha points?" The PICO was applied as; population: Gutta-percha, intervention: Coating of gutta-percha with any type of nanoparticles, comparator / control: Coating of gutta-percha with another type of nanoparticles, an antimicrobial agent or no coating, and outcomes: Antimicrobial efficacy against common root canal microbes as assessed through various methods.

The search strategy and article selection were completed by the first and second authors. All *in-vitro, in-vivo* or *ex-vivo* experimental studies where nanoparticles were used to coat the surface of gutta-percha and anti-bacterial properties of these nanoparticles were compared to other interventions, or one type of nanoparticle was compared to another type, published in the English language, were to be included. Studies where nanoparticles had been incorporated into the structure of gutta-percha instead of coating the gutta-percha points were excluded, as were case series, case reports, conference proceedings / articles, book chapters, dissertations / theses, reviews, ideas, opinions, and editorials. Articles with inaccessible full text were also not included. The period of publication was restricted from 2000 till June 2023. Databases including PubMed, Cochrane, Scopus, and CINAHL were used. Hand searching was not conducted; however, grey literature was searched by using Google Scholar. The electronic search was conducted on 1st July 2023 using keywords including endodontology, endodontics, root-canal therapy, root-canal treatment, gutta-percha, nanoparticle, nanomaterial, bacteria, micro-organisms, *Enterococcus faecalis*, *Staphylococcus aureus*, *Candida albicans*, *Escherichia coli*, antibacterial, antifungal, biocompatibility, cytotoxicity, and disinfection. All retrieved records were imported into EndNote, followed by the removal of duplicates.

The third, fourth, and fifth authors independently screened articles by assessing their titles and abstracts to determine their potential eligibility. After initial screening, full texts of articles were further screened by these three reviewers. The bibliographies and citations of the included studies were searched manually to make sure all the relevant studies were included, in case they had been missed by the initial electronic search. Any discrepancies between the three reviewers were resolved by a fourth reviewer (HS). All data from included studies were to be entered into a specifically designed data extraction form on Microsoft Word and were based on all information pertinent to this systematic review.

Articles selected for inclusion were to be evaluated for risk of bias by two independent reviewers (HS, MSZ) using risk-assessment tools based on the type of included studies. A third reviewer (SA) was to be consulted about any disagreements in terms of data extraction and quality assessment between the two reviewers.

RESULTS

A total of 5,230 records were initially identified for screening from the selected databases and registers after the removal of duplicates, and five studies were finally included in this systematic review. The PRISMA flowchart for the included studies is shown in Figure 1.

The summary of methodologies used in the five *in-vitro* studies fulfilling the inclusion criteria, along with the procedure of coating gutta-percha with nanoparticles, is given in Table I. The included articles were published from the year 2018 till 2023. Nanoparticles used in the studies for coating gutta-percha were nanopropolis, silver-curcumin, chitosan, silver, and zinc oxide.

Micro-organisms against which nanoparticles were tested in these studies included *Enterococcus faecalis*, *Escherichia coli, Staphylococcus aureus*, and *Candida albicans*. In all studies, the primary method employed to evaluate the zone of inhibition formed around the nanoparticle-coated gutta-percha was the agar diffusion method. The zone of inhibition was measured 24 hours after exposure to nanoparticle-coated gutta-percha. Higher concentration of nanoparticles and prolonged duration of coating resulted in increased antimicrobial activity, irrespective of the type of the nanoparticle used for coating. Results reported in the included studies are summarised in Table II.

Apart from assessing antimicrobial efficacy by agar diffusion method, other methods used were live-dead assay, growth-curve analysis, colony-forming unit (CFU) assay, reactive oxygen species (ROS) assay,[17] live-dead staining and osteoblastic cytocompatibility,[18] and colony counting method and ROS assay.[19] Irrespective of the method used to assess antimicrobial efficacy, nano-particle-coated gutta-percha showed more antimicrobial activity than uncoated gutta-percha.

Table I: Summary of methodologies used in the included studies.

Author- year	Size / specification of GP	No. of GP	No. of groups	Nanoparticle used	Size of nano- particle used	Method of coating nanoparticle on GP
Mohan *et al.*[17] (2020)	60 / 2% taper	03 each	05 (untreated control- 1% Chitosan, 2% Chitosan, 1% Silver, 2% Silver)	Chitosan, Silver	30-40 nm (chitosan) 20-30 nm (Silver)	Dipped in solution and left to dry for 20 minutes
Alves *et al.*[18] (2018)	ISO 80	06	06 (Control; NaOCl; PT; ZnO; PT + ZnO; PT + ZnO + UV)	ZnO	Not mentioned	Physical vapour deposition by Magnetron sputtering
Monisha *et al.*[19] (2023)	Not mentioned	05	05 (untreated control; 30 AgGP, 60 AgGP, 60 + 60 AgGP; Azithromycin antibiotic discs)	Nano-silver	50-100 nm	*In-situ* route-nucleation and crystal growth
Hamid *et al.*[20] (2020)	40 / 6% taper	10 each	02 (Nanopropolis coated GP; Ag-Curcumin coated GP)	Nanopropolis, silver-curcumin	Not mentioned	Dipped in solution for 24 hours. Removed and left to dry for 24 hours
Panwar *et al.*[21] (2023)	30 / 4% taper	22 each	02 (control- uncoated GP; experimental- Nanocurcumin coated GP)	Nanocurcumin	2-40 nm	Manually

Table II: Summary of the results reported in the included studies.

Author- year	Micro-organisms tested	Method to assess antimicrobial efficacy	Time of testing	Results
Mohan *et al.*[17] (2020)	*E. faecalis* ATCC29212	Zone of inhibition measurement by agar diffusion method	24 hours	Concentration-dependent anti-bacterial activity. 2% AgNP-GP >1%AgNP-GP >2% Chit NP GP >1% Chit NP GP.
Alves *et al.*[18] (2018)	*E. faecalis;* *S. aureus*	Direct assay- sessile bacteria quantified by cultivable cell numbers	24 hours	PT >ZnO >ZnO alone *S. Aureus* >*E. fecalis*.
Monisha *et al.*[19] (2023)	*E. faecalis* ATCC29212; *E. coli* ATCC25922	Zone of inhibition measurement by agar diffusion method	24 hours	Increased coating duration a improves anti-bacterial activity a higher Ag loading. *E. faecalis* >*E. coli*.
Hamid *et al.*[20] (2020)	*E. faecalis* ATCC29212; *E. coli* ATCC 25922; *S. aureus* ATCC 6538; *C. albicans* ATCC 10231	Zone of inhibition measurement by agar diffusion method	24 hours	Silver-curcumin > nanopropolis. Inhibitory effect *S. aureus* = *C. albicans* >*E. coli* = *E. faecalis*.
Panwar *et al.*[21] (2023)	*E. faecalis* ATCC29212	Zone of inhibition measurement by agar diffusion method	24 hours	12.5 mm- experimental group (MIC-50 mg/ml) 2.5 mm- control group

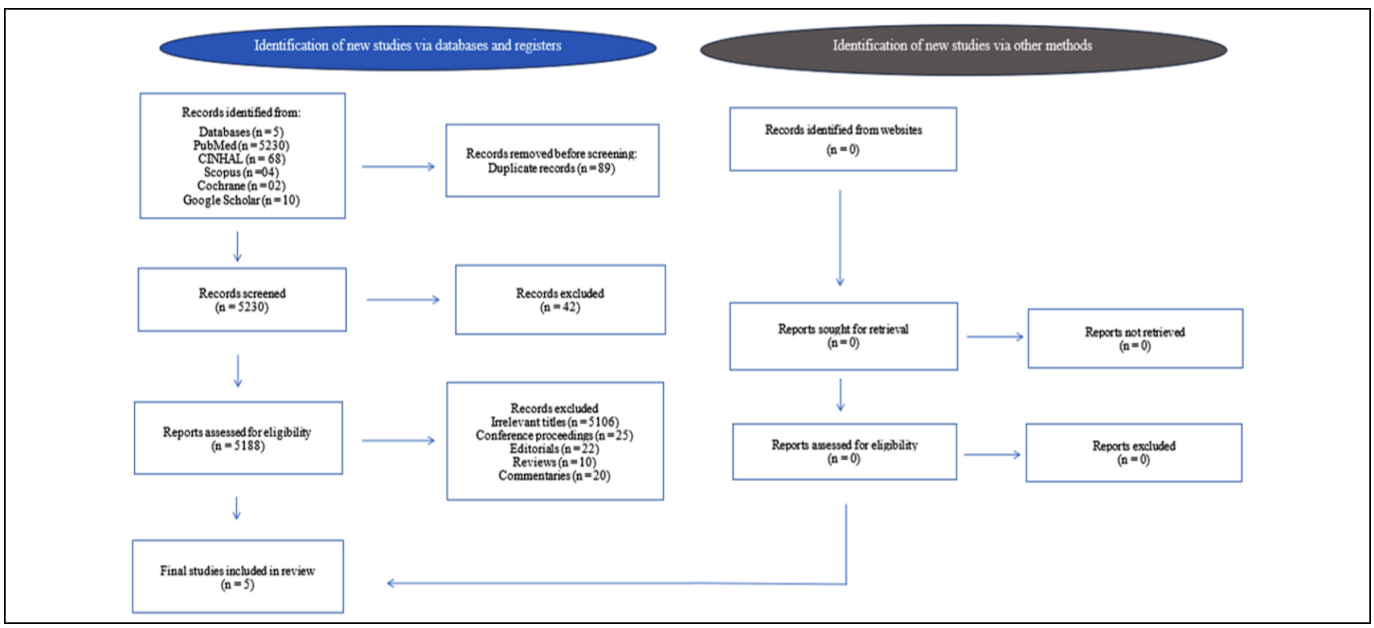

Figure 1: PRISMA flowchart for the included studies.

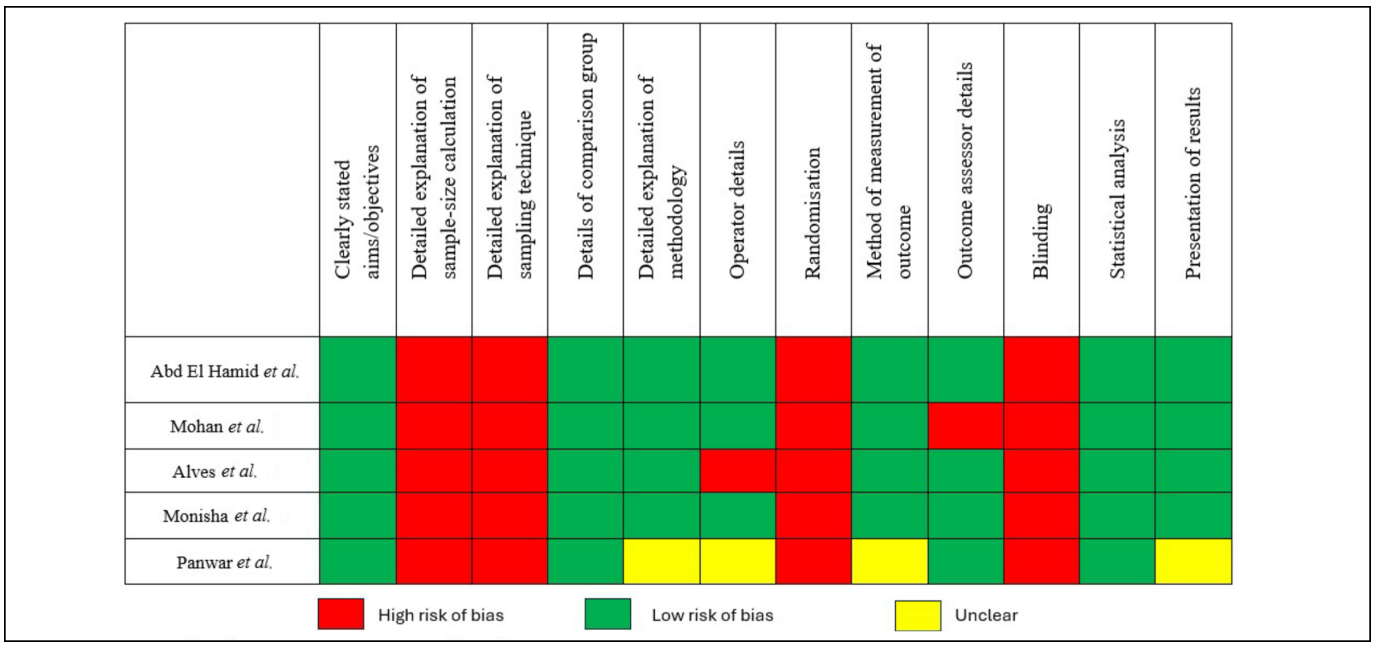

Figure 2: Risk of bias of each included study based on the QUIN tool.

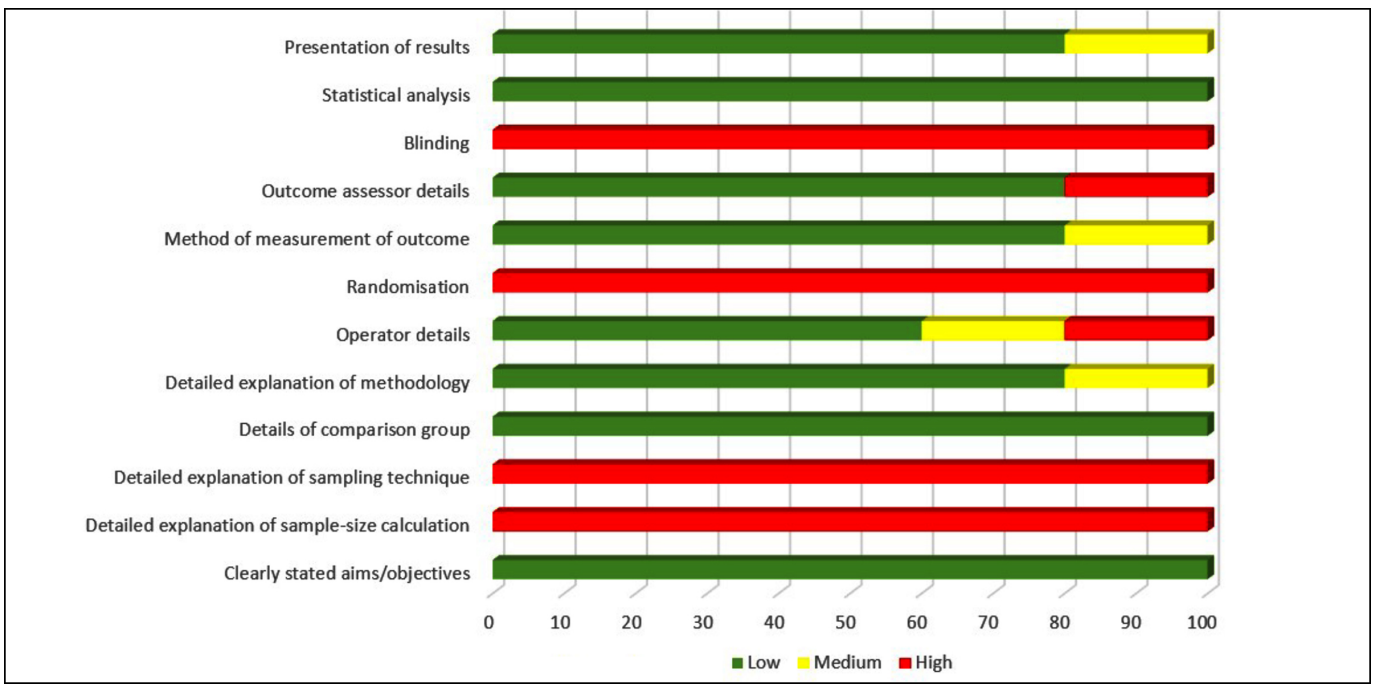

Figure 3: Overall risk of bias of the included studies.

As all included studies were *in-vitro*, therefore, the QUIN tool was used to assess and record the risk of bias and quality of studies.[22] Risk of bias in individual studies, as well as overall risk, is depicted in Figure 2 and 3, respectively. All studies were found to be in the "medium" category in terms of risk of bias, as these studies encompassed most of the relevant methodology information. The included studies had clearly stated aims and objectives, details of a comparison group, and detailed descriptions of the statistical analysis used. However, no study reported blinding, details of sampling technique, and sample size calculations. The majority of studies gave a detailed explanation of the methodology used,[17-20] a clear presentation of results,[17-20] methods used to measure the outcome,[17-20] and outcome assessor details.[18-21] Three studies gave clear operator details in their methodology.[17,19,20]

DISCUSSION

To increase the anti-bacterial effectiveness of root-canal obturation, coating gutta-percha with nanoparticles that exhibit antimicrobial properties has been investigated as a viable method. The objective of this systematic review was

to evaluate if gutta-percha coated with various nanoparticles provides antibacterial activity against root-canal microbes compared to the conventional gutta-percha points. All available literature was assessed that was based on the application of various nanoparticles and their capacity to impart inert gutta-percha with antimicrobial properties after coating it. Various methods have been advocated in literature for coating nanoparticles on gutta-percha surface. These include simple deposition of nanoparticles on gutta-percha by dipping in nanoparticles solution, depositing nanoparticles using a reactive magnetron sputtering, manually-coating gutta-percha points or using *in-situ* methods, among others.[17-20]

A comparative *in-vitro* study,[21] included in this review, was conducted on antimicrobial effectiveness of size 40 / 6% gutta-percha points coated with nanopropolis and with silver-curcumin nanoparticles on a variety of bacteria species, including *E. faecalis, E. coli, C. albicans*, and *S. aureus*. Besides *E. coli* and *E. faecalis*, it was found that Ag-curcumin nanoparticles inhibited the growth of *S. aureus* as well as *C. albicans.*[20] *C. albicans* and *S. aureus* were likewise inhibited by nanopropolis, followed by *E. coli* and *E. faecalis*. The antimicrobial effectiveness of silver-curcumin nanoparticles was more noticeable compared to nanopropolis when used as a coating on the gutta-percha points. Curcumin has the potential to disrupt bacterial biofilms by inhibiting bacterial quorum sensing, damaging cell walls and membranes, and interfering with cellular processes by targeting bacterial DNA. Curcumin has also been reported to down-regulate genes involved in oxidative stress response and adhesion in Candida biofilms, thereby disrupting the biofilm and improving its antifungal efficacy in the root-canal space.[23,24] Flavonoids in propolis bind to the DNA gyrase subunit of *E. coli*, hindering its activity, causing fractional bacterial lysis, and affecting cell proteins.[25] Hydrolytic enzymes in the cell membrane of gram-negative bacteria reduce the functionality of propolis, thus making it less effective against gram-negative in comparison to gram-positive bacteria. This may be a reason for its reduced antimicrobial effect in comparison to silver-curcumin.[25,26] Both materials have an inhibitory effect on a variety of bacteria, and gutta-percha cones coated with one or the other could be very effective against microbes that persist in the canal space post-obturation. This would minimise chances of flare-ups, subsequently lowering endodontic treatment failure rates,[20] since *E. faecalis* is reported to be resistant to many of the regularly used modalities used for its eradication.[27]

In another study,[18] authors reported a favourable antimicrobial effect of chitosan and silver nanoparticle-coated GP, evaluated by determining their ability to inhibit microbial growth by a modified technique i.e. induction of oxidative stress by reactive oxygen species (ROS) generation.[17] The authors recommend that silver nanoparticles coating exhibited greater antimicrobial activity compared to chitosan-coated gutta-percha points. The Ag^+ ions released by silver nanoparticles cause increased-cell wall permeability resulting in damage to bacterial DNA and its cytoplasm.[20] The positively charged NH_3^+ ion in the chitosan molecule binds electrostatically to bacterial membranes, thereby impairing vital bacterial activities and contributing to its antimicrobial mode of action.[28]

To evaluate the biocompatibility and anti-bacterial properties of nanostructured ZnO, an innovative technique of coating gutta-percha was reported in another study where untreated GP cones were compared with cones coated with a thin film of ZnO, which caused the growth of roughly 30% less *E. faecalis*.[19] This reduction was comparable to GP cones which were submerged in NaOCl. Contrary to uncoated gutta-percha and coating with ZnO without any surface treatment, ZnO-coated cones following plasma treatment (PT + ZnO) had considerably improved anti-bacterial activity. When additional UV activation was done on plasma-treated ZnO-coated cones, no additional anti-bacterial effect was reported. Anti-bacterial effect of PT + ZnO cones against *S. aureus* showed a similar pattern, but with greater microbial load reduction, compared to uncoated gutta-percha and ZnO cones. The bactericidal effect was also seen on PT + ZnO GP cones.[18] This novel approach of surface treatment with argon plasma prior to deposition of ZnO nanoparticles appears promising to help prevent the ingress of micro-organisms and formation of biofilms within the root-canal space.

Another *in-vitro* study analysed gutta-percha surfaces after disinfecting them with NaOCl and Ag nanoparticles using an atomic force microscope method.[21] The *ex-vivo* study evaluated the antimicrobial efficacy of silver nanoparticles (70 ug/ml AgNPs)-coated GP compared to NaOCl disinfected gutta-percha points.[21] AgNP demonstrated efficacy in killing micro-organisms equivalent to that of NaOCl.

The study by Monisha *et al.*[19] explored the relationship between internal endodontic failures brought on by bacterial infections and gutta-percha cones that have been coated with nano-Ag. A straightforward *in-situ* growing method was used to coat gutta-percha surface with AgNPs. Using zone of inhibition and colony-counting procedures, the anti-bacterial susceptibility of GP coated with AgNPs was evaluated against *E. faecalis* and *E. coli*. Results showed that there was a proportionate increase in the anti-bacterial activity of AgNPs with its growth on GP increased. This was supported by the increased diameter of the zone of inhibition and decreased colony counts.[19] Assays used to determine the mechanism underlying anti-bacterial action discovered that this effect was caused due to Ag+ ionic release from AgNPs on the GP surface. As a result of recent advancements in nano-technology, researchers have manufactured pure silver nanoparticles that have been shown to be more effective than silver ions. AgNPs hold interest because they offer a wide range of anti-bacterial effects and are harmless to humans at low concentrations.[29] Scientists have developed nano-silver-coated GP in an effort

to enhance the anti-bacterial function of GP. This AgNP-coated GP has shown a notable negative impact on the growth of *Candida albicans, Staphylococcus aureus, E. coli*, and *E. faecalis*. The positive charge on silver ions is essential to its anti-bacterial activity, as positively- charged nanoparticles are attracted to negatively-charged cell membranes of micro-organisms electrostatically. The bacterial membrane permeability is disturbed by nanoparticle accumulation, leading to cell death.[30] Because they exhibit a complex mechanism of action that is not constrained to a single channel, silver (Ag)NPs appear to possess the most promising anti-bacterial activity.

ZnO-coated gutta-percha cones maintained good cytocompatibility with human cells. Slight cell inhibition was reported in gutta-percha points pre-treated with argon-plasma but this was still well within non-toxic parameters set by ISO standards (ISO- 10993-5).[18] No other study included in this review assessed cytocompatibility of nanoparticles used for gutta-percha coating.

All included studies also evaluated and recorded the impact on gutta-percha surface topography after being coated with various nanoparticles. Scanning electron microscope (SEM) and field emission SEM were used for this purpose.[18-21] When the coating operation was carried out twice (60 + 60AgGP), a consistent coating of AgNPs was obtained on gutta-percha surface compared to a single coat of nanoparticles.[11] Panwar *et al.* reported greater surface irregularity when gutta-percha was disinfected with NaOCl, but less surface degradation in comparison when AgNPs were used for GP coating. NaOCl, even at 700 times lower concentrations than AgNPs, resulted in a 5x more irregular GP surface topography, and 10x more irregular surface compared to untreated GP.[21] In comparison, Hamid *et al.* compared two different nanoparticle coatings and reported homogenous and tightly adherent nanopropolis particles on GP cones compared to silver curcumin nanoparticles. For silver-curcumin nanoparticles to better adapt to GP cones without interacting with coating ingredients, a more stable adhesive needs to be developed.[20] Investigations can also be directed towards surface-treating gutta-percha points with argon plasma treatment before the deposition of nanoparticles to help enhance their bonding to the GP surface. Argon plasma treatment has been reported to remove the outer wax layer of gutta-percha, forming porosities for nanoparticle crystals to embed, forming a homogenous, nanostructured topography.[18]

Even though systematic reviews have been conducted on five or less studies, one limitation of this systematic review is that relatively less number of studies fulfilling the inclusion criteria were found, with only *in-vitro* studies available.[31] As a result of heterogeneity of methodologies and results reported in selected studies, no specific nanoparticle could be identified as having the most efficacious antimicrobial properties when coating gutta-percha. Hence, concrete clinical recommendations cannot be put forth, even though the antimicrobial efficacy of gutta-percha coated with various nanoparticles was clearly evident. Further individual studies should be conducted to evaluate and compare the antimicrobial activity of different nanoparticles using *in-vitro* and *in-vivo* methods, and relevant protocols developed.

It is crucial to note that the long-term effectiveness of nanoparticle-coated gutta-percha cannot be established unless clinical studies with an appropriate follow-up period are conducted on endodontically-treated teeth. The next step is identifying nanoparticles that perform optimally in clinical scenarios, based on their coating mechanism on the gutta-percha and their anti-microbial effectiveness. Other clinical factors that may have to be considered before endorsing coated gutta-percha include the impact of heat as used in continuous wave compaction or thermoplastic obturation techniques and the potential interaction of nanoparticles coated on gutta-percha with sealers used for obturation.

None of the studies included in this review mentioned methods to minimise selection bias i.e., randomisation and allocation concealment. Since blinding of the outcome assessor was not done, thereby detection bias was also not accounted for. Clinical studies, or more *in-vitro* studies considering these limitations, need to be conducted before clinical recommendations can be put forward for the use of these nanoparticles in endodontics. The long-term effects, biocompatibility, and potential side effects of using nanoparticles-coated gutta-percha in clinical practice need further investigation.

CONCLUSION

Gutta-percha coated with various nanoparticles demonstrated enhanced antibacterial activity against common root-canal microbes. Higher concentration of the nanoparticles and prolonged duration of coating resulted in increased antimicrobial activity irrespective of the type of nanoparticle used for coating. Propolis, silver-curcumin, chitosan, silver, and zinc oxide nanoparticles were suggested to possess promising antimicrobial effects against common root-canal microbes i.e., *E. faecalis*, *E. colis*, *S. aureus*, and *C. albicans*.

COMPETING INTEREST:
The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
SA: Conceptualisation.
SA, AL, MML, IS: Methodology.
SA, MML, JA, IS: Data analysis.
SA, MML, JA, HS: Writing of the manuscript and preparation of original draft.
HS, MML, AL: Writing, reviewing, and editing.
MSZ, IS: Methodology review and critical review of the manuscript.
All authors approved the final version of the manuscript to be published.

REFERENCES

1. Hayat F, Sabbir J, Khurshid Z, Zafar MS, Ghabbani HM, Shahbazi MA, *et al*. Nanoparticles in Endodontics. In: Khurshid Z, Zafar MS, Najeeb S, Eds. Biomaterials in endodontics. UK; Woodhead publishing; Shaston 2022. p. 195-209.
2. Aminu N, Zafar MS. Roles of nanomedicines in dental therapeutics. In: Shukla AK, Ed. Nanotechnology for dentistry applications. Bristol, UK; IOP publishing; 2021. p. 1-32. doi: 10.1088/978-0-7503-3671-0ch4.
3. Zafar MS, Alnazzawi AA, Alrahabi M, Fareed MA, Najeeb S, Khurshid Z. Nanotechnology and nanomaterials in dentistry. In: Khurshid Z, Najeeb S, Zafar MS, Sefat F, Eds. Advanced dental biomaterials: Woodhead publishing; Shaston, UK: 2019. p. 477-505. doi: 10.1016/B978-0-08-102476-8.00018-9.
4. Zafar MS, Khurshid Z, Najeeb S, Zohaib S, Rehman IU. Therapeutic applications of nanotechnology in Dentistry. In: Andronescu E, Grumezescu AM, Eds. ed 1st. Nanostructures for oral medicine: Oxford, UK; Elsevier; 2017. p. 833-62. doi: 10.1016/B978-0-323-47720-8.00027-4.
5. Nanomaterials definition matters. *Nat Nanotechnol* 2019; **14(3)**:193. doi: 10.1038/s41565-019-0412-3.
6. Raura N, Garg A, Arora A, Roma M. Nanoparticle technology and its implications in endodontics: A review. *Biomater Res* 2020; **24(1)**:21. doi: 10.1186/s40824-020-00198-z.
7. Baig N, Kammakakam I, Falath W. Nanomaterials: A review of synthesis methods, properties, recent progress, and challenges. *Mater Adv* 2021; **2(6)**:1821-71. doi: 10.1039/D0MA00807A.
8. Wong J, Zou T, Lee AHC, Zhang C. The potential translational applications of nanoparticles in endodontics. *Int J Nanomedicine* 2021; **16**:2087-106. doi: 10.2147/IJN.S293518.
9. Zakrzewski W, Dobrzynski M, Knefel AZ, Lubojanski A, Dobrzynski W, Janecki M, *et al*. Nanomaterials application in endodontics. *Materials (Basel)* 2021; **14(18)**:5296. doi: 10.3390/ma14185296.
10. Mirsasaani SS, Hemati M, Tavasoli T, Dehkord ES, Yazdi GT, Poshtiri DA. Nanotechnology and nanobiomaterials in dentistry. In: Subramani K, Ahmed W, Hartsfield JK, Eds. ed 1st. Nanobiomaterials in clinical dentistry: USA; William Andrew publish; Waltham; 2013. p. 17-33.
11. Mukka PK, Pola SSR, Kommineni NK, Pachalla MS, Karne AR, Labishetty K, *et al*. Comparative evaluation of three herbal solutions on the disinfection of guttapercha cones: An *in-vitro* study. *J Clin Diagn Res* 2017; **11(3)**:zc21-4. doi: 10.7860/JCDR/2017/25208.9487.
12. Estrela C, Pimenta FC, Ito IY, Bammann LL. Antimicrobial evaluation of calcium hydroxide in infected dentinal tubules. *J Endod* 1999; **25(6)**:416-8. doi: 10.1016/S0099-2399(99)80269-6.
13. Ozdemir O, Kopac T. Recent progress on the applications of nanomaterials and nano-characterisation techniques in endodontics: A review. *Materials (Basel)* 2022; **15(15)**:5109. doi: 10.3390/ma15155109.
14. Afkhami F, Forghan P, Gutmann JL, Kishen A. Silver nanoparticles and their therapeutic applications in endodontics: A narrative review. *Pharmaceutics* 2023; **15(3)**:715. doi: 10.3390/pharmaceutics15030715.
15. Altammar KA. A review on nanoparticles: Characteristics, synthesis, applications, and challenges. *Front Microbiol* 2023; **14**:1155622. doi: 10.3389/fmicb.2023.1155622.
16. Capuano N, Amato A, Annunziata FD, Giordano F, Folliero V, Di Spirito F, *et al*. Nanoparticles and their antibacterial application in endodontics. *Antibiotics (Basel)* 2023; **12(12)**:1690. doi: 10.3390/antibiotics12121690.
17. Mohan A, Dipallini S, Lata S, Mohanty S, Pradhan PK, Patel P, *et al*. Oxidative stress induced antimicrobial efficacy of chitosan and silver nanoparticles coated gutta-percha for endodontic applications. *Mater Today Chem* 2020; **17**:100299. doi: 10.1016/j.mtchem.2020.100299.
18. Alves MJ, Grenho L, Lopes C, Borges J, Vaz F, Vaz IP, *et al*. Antibacterial effect and biocompatibility of a novel nanostructured ZnO-coated gutta-percha cone for improved endodontic treatment. *Mater Sci Eng C Mater Biol Appl* 2018; **92**:840-8. doi: 10.1016/j.msec.2018.07.045.
19. Monisha K, Shilpa SA, Hikku GS. *In-situ* nano-Ag coated gutta-percha cones to counteract internal endodontic failures through bacterial infections. *Mater Chem Phys* 2023; **301**:127532. doi: 10.1016/j.matchemphys.2023.127532.
20. Hamid HAE, Aziz MB, Naeem FA. Antimicrobial efficacy of nanopropolis coated *vs* silver-curcumin nanoparticles coated gutta-percha points on various microbial species: A comparative *in-vitro* study. *Egypt Dent J* 2020; **66**:1893-902. doi: 10.21608/edj.2020.31338.1132.
21. 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(2)**:160-4. doi: 10.4103/jcd.jcd_ 512_22.
22. Sheth VH, Shah NP, Jain R, Bhanushali N, Bhatnagar V. Development and validation of a risk-of-bias tool for assessing *in-vitro* studies conducted in dentistry: The QUIN. *J Prosthet Dent* 2024; **131(6)**:1038-42. doi: 10.1016/j.prosdent.2022.05.019.
23. Ma J, Shi H, Sun H, Li J, Bai Y. Antifungal effect of photodynamic therapy mediated by curcumin on Candida albicans biofilms *in-vitro*. *Photodiagnosis Photodyn Ther* 2019; **27**:280-7. doi: 10.1016/j.pdpdt.2019.06.015.
24. Trigo-Gutierrez JK, Vega-Chacon Y, Soares AB, Mima EGO. Antimicrobial activity of curcumin in nanoformulations: A comprehensive review. *Int J Mol Sci* 2021; **22(13)**:7130.
25. Almuhayawi MS. Propolis as a novel antibacterial agent. *Saudi J Biol Sci* 2020; **27(11)**:3079-86. doi: 10.1016/j.sjbs.2020.09.016.
26. Afrouzan H, Tahghighi A, Zakeri S, Es-Haghi A. Chemical composition and antimicrobial activities of iranian propolis. *Iran Biomed J* 2018; **22(1)**:50-65. doi: 10.22034/ibj.22.1.50.
27. Rosen E, Kolodkin-Gal I, Tsesis I. Challenges in the eradication of enterococcus faecalis and its implications on health. *Curr Oral Health Rep* 2018; **5(1)**:70-7. doi: 10.1007/s40496-018-0172-4.

28. Harugade A, Sherje AP, Pethe A. Chitosan: A review on properties, biological activities and recent progress in biomedical applications. *React Funct Polym* 2023; **191**:105634. doi: 10.1016/j.reactfunctpolym.2023.105634.
29. Xu L, Wang YY, Huang J, Chen CY, Wang ZX, Xie H. Silver nanoparticles: Synthesis, medical applications and biosafety. *Theranostics* 2020; **10(20)**:8996-9031. doi: 10.7150/thno.45413.
30. Jandt KD, Watts DC. Nanotechnology in dentistry: Present and future perspectives on dental nanomaterials. *Dent Mater* 2020; **36(11)**:1365-78. doi: 10.1016/j.dental.2020.08.006.
31. Minja IK, Lwoga ET. Evidence based dentistry among dentists in low and middle income countries: A systematic review. *East Afr Health Res J* 2021; **5(2)**:129-36. doi: 10.24248/eahrj.v5i2.662.