ANTIMICROBIAL EFFICACY OF NANOPARTICLES AS AN INTRACANAL MEDICAMENT: A SYSTEMATIC REVIEW

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ABSTRACT

Nanoparticles have attracted attention as modern antimicrobial, especially in the complicated oral cavity environment for diagnosis and treatment, intending to improve comprehensive oral health. In endodontics, the development of nanomaterials is focused on steps that would improve antimicrobial efficacy. The current study undertakes a systematic review to assess the effectiveness of different nanoparticles as an intracanal medicament to improve the antibiofilm efficacy in the root canal. A wide electronic database search was done restricting to the past ten years. Articles that are published in the English language were included for the review. Data were extracted, and the risk of bias was assessed. Of the 351 studies identified and screened according to the inclusion criteria, nine in vitro studies were included. The results indicate that nanoparticle-based intracanal medicament in the form of solution or suspension has greater antimicrobial efficacy. Thus, the increasing outcome from this review highlights the need for sound research-based scientific and clinical collaborations to emphasize the future potential of nanoparticles as an intracanal medicament.

INTRODUCTION

Endodontic infection is a bacterial biofilm surviving within the anatomic complexities and uninstrumented portions of the root canal system. Bacteria present inside the dentinal tubules hinder host defence cells and molecules' effects and systemically administered antibiotics. Conventionally, antimicrobial chemo-mechanical preparation is used for an effective microbial reduction before obturating the canal with an inert material. Unfortunately, despite advancements in antibiofilm strategies developed for disinfection, the current level of evidence shows the rate of failure of treatment has not decreased below 20-28% for the past four decades. [(2)] Owing to the shortcomings in disinfection strategies and to achieve promising results in endodontics, the application of antimicrobial nanoparticles will provide a new paradigm shift in dentistry.

Nanomaterials are natural or synthetic materials, having a molecular size that does not exceed 100 nm. As nanoparticles are less stable and exhibit weaker bonding and interaction with other molecules, they provide greater antimicrobial benefits (3) like the disruption of bacterial cell wall synthesis, inhibition of enzyme activity, release of reactive oxygen species, metal ion homeostasis, and regeneration stem cells. The virtue of its exclusive physicochemical and biological properties, [(1)] the authors suggest that it can be widely used in infection control strategies, especially in the complex oral cavity environment by its intensive penetrative capacity and microbicidal effect as a sealer, obturating, and intracanal medicament or disinfectants.

However, to understand the effectiveness of nanoparticles as an intracanal medicament in combating endodontic microbes, a systematic review of the literature available was conducted. Hence the objective of the present review was to systematically review the antimicrobial potential of nanoparticle-based root canal intracanal medicament with other medicaments on microbial load reduction during root canal disinfection.

MATERIALS AND METHODS

According to the Preferred Reporting Items for Systematic Reviews (PROSPERO) and Meta-Analyses PRISMA guidelines, a detailed protocol was developed for this systematic review.

Search strategy

The electronic search of the literature was conducted on the 'Pubmed, Web of Science, Embase and Google Scholar' databases with the following MESH-terms: "nanoparticles OR nanomaterials OR nanomixtures AND antimicrobial AND endodontics OR root canal infection AND intracanal medicament OR intracanal dressing". In addition, manually searched data were also scanned to identify additional documents that had been missed. Only papers published in English were used. The electronic searches were conducted in October 2021, restricting papers published on nanoparticles for the past ten years.

Population Intervention Control Outcome Question

Focus Question: The research question addressed by this review was "Can nanoparticles be used as an efficient antimicrobial intracanal medicament?"

Eligibility criteria

The following inclusion criteria were adopted to refine further the search: Studies assessing the antimicrobial activity of various nanoparticles reporting outcomes of reduction in microbial load when used as an intracanal medicament.

Inclusion Criteria: The PICOS format was used to include studies for review.

- **Population (P)** was Inoculated root canals of extracted teeth with relevant microbial species /standard inoculums.
- **Intervention (I)** was the use of nanoparticles alone in the form of a solution or a suspension or gel as an intracanal medicament
- **Comparison (C)** was done with a control group that has an intracanal medicament
- **Outcome (O)** was antibacterial activity measured based on eradication of microbes or persistence in the acceptable concentration level using a confocal laser scanning microscope or bacterial culture.
- **Studies (S)** that assessed the association between the use of nanoparticles and antibacterial activity ex vivo were included. Only articles published in English were included in this systematic review.

Exclusion Criteria: Narrative and systematic reviews, case/reports, letters to the editor, experimental/exploratory studies, opinion pieces, conference abstracts were excluded from the review. Articles did not quantify the antimicrobial effect of silver nanoparticles or assess the general activity of antimicrobial nanoparticles against microbial species non-relevant to root canal infection or assessed the antimicrobial behaviour of nanoparticles with no potential application in the dental root canal or assessing the efficiency of nanoparticles as an irritant or its impact on bond strength of various cement or tooth colour were excluded.

Data extraction

The authors thoroughly studied all the included studies, independently collected the data, and assessed the risk of bias.

All retrieved titles and abstracts from the three described databases were screened to assess eligibility. Then the abstracts of the selected studies were reviewed if they met the selection criteria. Any articles that did not match the standards were excluded. And after full-text articles were assessed for eligibility, some of them were eliminated because of nonconformity to the criteria

The study characteristics extracted were author, specimen used, microbial species test material, nanoparticle size, other groups, method of detection.

Quality assessment of selected studies

The authors thoroughly studied all the included studies, independently collected the data, and assessed the risk of bias. The risk of bias for each study was assessed using Checklist for Reporting *in vitro* Studies (CRIS guidelines) according to the article's description of the following parameters:

- Research question/objective clearly stated
- Study groups clearly defined
- Sample size justification
- Standardization in sample preparation
- Use of controls
- Mention of particle size
- Allocation sequence, Randomization, and blinding

If the authors reported the parameter, the article had a "yes" on that specific parameter; if it was impossible to find the information, the article received a "no." Articles that reported one to two items were classified as having a high risk of bias, three to four items as a moderate risk of bias, and above five items as a low risk of bias

RESULTS

The selection process is summarized in the Prisma Flow Chart shown in Figure 1. A total of 351 titles and abstracts were identified after an electronic database and manual search using the specific combination of terms and keywords. Out of 351 studies, 224 studies were excluded, as they did not meet inclusion criteria. No clinical reports concerning the application of antimicrobial nanoparticles in endodontics were found. Thus, the review was restricted to in vitro studies. In addition, 60 articles were examined for full-text screening; of these, 9 articles were identified and selected for full-text review as per the selection criteria for further analysis. The remaining articles were not considered as they did not meet the eligibility criteria.

Figure 1 – PRISMA flowchart depicting the workflow of the systematic review

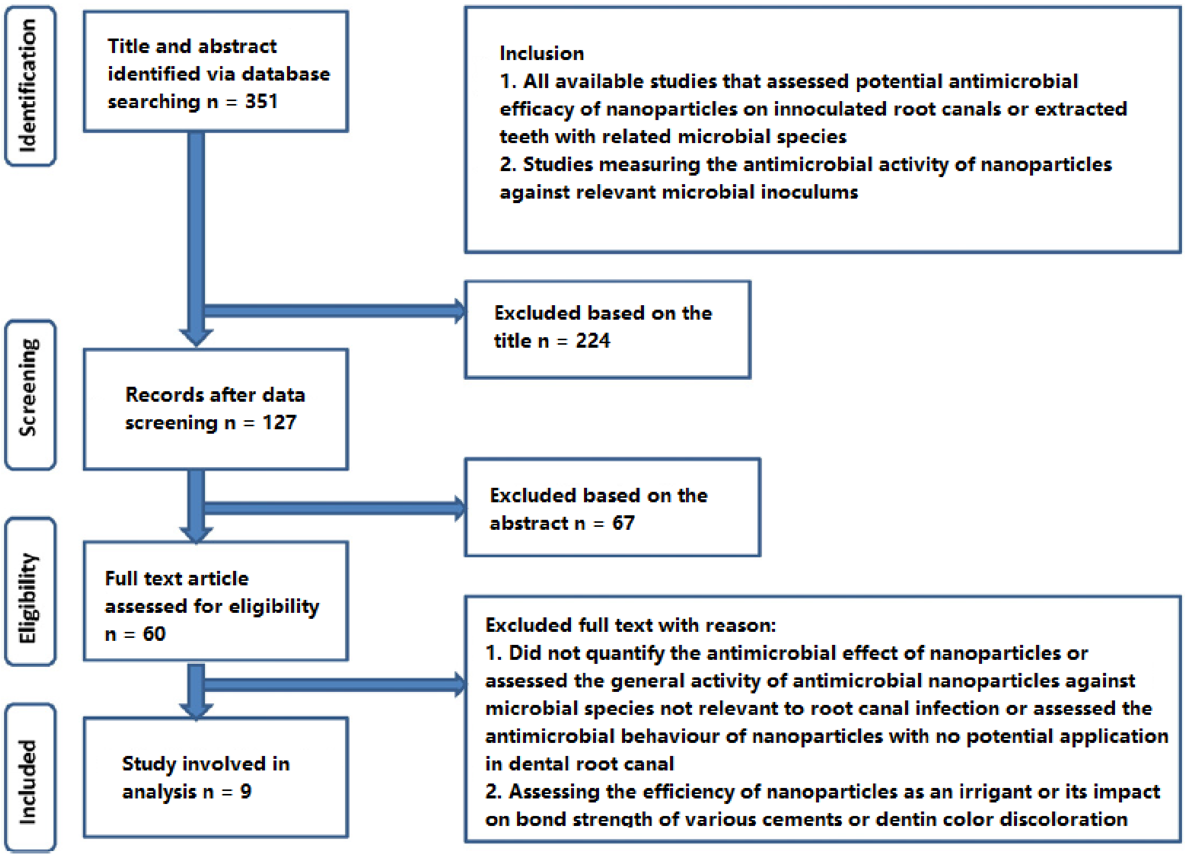

Table 1 – Summary of study characteristics included in the systematic review

Study	Type of study	Specimen used	Microbial species	Test material	Nanoparticle size	Other groups	Method of detection	Results	Inference
Parolia et al (14)	In-vitro	240 extracted human teeth	E.faecalis	chitosan-propolis nanoparticle (CPN)	100 µg/ml and 250 µg/ml	Saline, chitosan, propolis, calcium hydroxide, and 2% chlorhexidine	CFU levels with SEM and CLSM analysis	Significant reduction in colony count and SEM & CLSM images demonstrated that the no.deal cells present in dentin was high in CPN 250 cases compar	Seven days following the usage of CPN 250 and CPN100 as iintracanal medicament, there was reduced E.Faecalis count compared to Day 1 & 3.

								ed to CPN 100(>40%)	
Pankajakshan et al (15)	In vitro	24 extracted human teeth	A. naeslundii and E. faecalis	TAP polydioxanone fibres	Not mentioned	Saline, TAP	Bacterial viability assessed using LIVE/DEAD assay and CLSM	Significant bacterial death and enhanced adhesion of dental pulp stem cells on dentin specimens	triple antibiotic–containing nanofibers shows significant bacterial death and did not affect DPSC attachment and proliferation on dentin
Rojas et al (16)	Ex vivo	80 human extracted teeth	S.mutans, E.feacalis	Copper nanoparticles	Range 20-60nm	Calcium hydroxide	Bacterial culture	256 µg/mL of CuNP showed more antimicrobial	Presence of immediate and overtime antimicrobial

								activity ie decrease of more than 3 logarithms	effect of CuNP on a biofilm of E. faecalis and S. mutans.
Wu et al (9)	In vitro	Bacterial inoculum in dentin sections	E. faecalis	Silver nanoparticles	Not mentioned	Calcium hydroxide	Bacterial viability assessed using LIVE/DEAD assay and CLSM	Biofilms treated with AgNP gel as medicament significantly disrupted the structural integrity of the biofilm. These resulted in reduced number of	AgNPs used as a medicament demonstrated efficiency to eleiminate the bacterial biofilms during Root Canal disinfection.

								post-treatment viable E-Faecalis cells	
Halkai et al (17)	In vitro	30 extracted human teeth	E. faecalis	The varied concentration of fungi Fusarium semitectum derived silver nanoparticles	Not mentioned	Chlorhexidine, calcium hydroxide	Bacterial culture	AgNPs (100 ml) showed highest zone of inhibition 19.5 mm against E. faecalis. CHX (0.2%) 14.52 mm	biosynthesized AgNPs derived from fungi exhibit efficient antibacterial activity as a medicament for root canal disinfection
Dianat et al (18)	In vitro	23 extracted human teeth	E. faecalis	Calcium hydroxide nanoparticles	Not mentioned	Calcium hydroxide, distilled water	Bacterial culture	1/16 dilution of nanoparticle group (6.25	Calcium hydroxide nanoparticles have

								mg/mL) showed no microbial growth	superior antimicrobial activity against E. faecalis compared to conventional calcium hydroxide in culture media as well as dentinal tubules.
Mozayeni et al (19)	In vitro	81 extracted human teeth	Candida albicans	Nanosilver gel	One g of 50ppm nanosilver solution	Calcium hydroxide, chlorhexidine	Fungal culture	CFU was low in C.H. and CHX group compared to nanosilver gel	Antifungal activity of C.H. and CHX gels was significantly higher than

									that of nanosilver gel
Samiei et al (20)	In vitro	132 extracted human teeth	E.faecalis	Zinc oxide nanoparticles, Zinc oxide silver nanoparticles,	50ppm of zinc oxide nano gel 1ppm of silver	Calcium hydroxide mixed with 0.12% chlorhexidine	Bacterial culture	Antibacterial effect - zinc oxide and zinc oxide/silver nanoparticles 69.01 % and 69.19 % and mixture of calcium hydroxide and CHX 89.65 %	low antibacterial effect of nanoparticles in the form of gel
Pourhashemi et al (21)	In vitro	54 extracted huma	E.faecalis	calcium hydroxide with silver nanopar	20nm	Calcium hydroxide with saline and	Bacterial culture	Significant reduction in biofilm	AgNPs have a good potentia

		n teeth		ticles suspension		calcium hydroxide with chlorhexidine		treated with calcium hydroxide using AgNP as vehicle	l to be used as an appropriate vehicle of $Ca(OH)_2$ to eliminate *E. faecalis* bacteria from human dentine within one week.

Figure 2 – Assessment of Risk of Bias

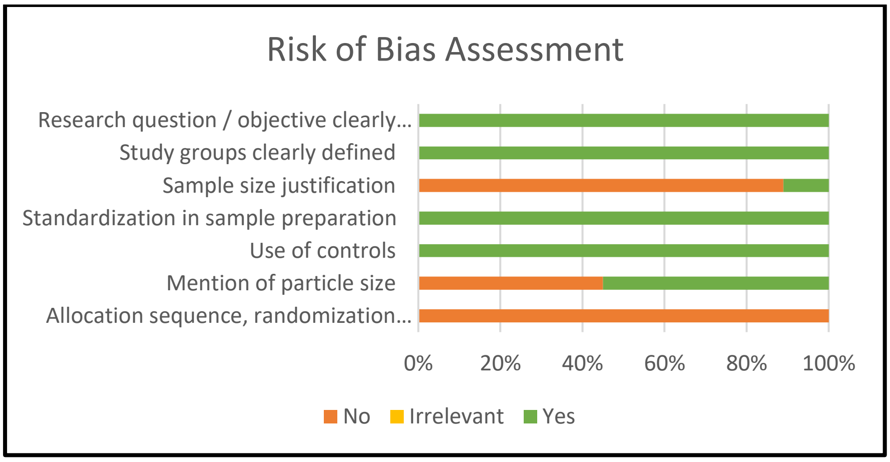

Table 2 – Assessment of Risk of Bias

Quality assessment criteria	Parolia et al	Pankajakshan et al	Rojas et al	Wu et al	Halkai et al	Dianat et al	Mozayeni et	Samiei et al	Pourhashemi
Research question/objective clearly stated	yes	yes	yes	yes	yes	yes	yes	yes	yes
Study groups clearly defined	yes	yes	yes	yes	yes	yes	yes	yes	yes
Sample size justification	no	no	no	no	no	no	no	yes	no
Standardization in sample preparation	yes	yes	yes	yes	yes	yes	yes	yes	yes
Use of controls	yes	yes	yes	yes	yes	yes	yes	yes	yes
Mention of particle size	yes	no	yes	no	no	no	yes	yes	yes
Allocation sequence, Randomization and blinding	no	no	no	no	no	no	no	no	no
Risk of bias	Low	Moderate	Low	Moderate	Moderate	Moderate	Low	Low	Low

After examination of the results, studies that fulfilled the inclusion criteria were added to the review. The detailed data was collected from the selected studies. **Table 1** gives the characteristics of the included studies. The risk of bias is summarized in **Table 2 & Figure 2**. Of nine included studies, 5 had a low risk of bias, and 4 had a moderate risk of bias.

DISCUSSION

In the field of endodontics, recurring flare-ups due to the persistence of E.faecalis in the root canal peripherals resisting high pH (pH – 11.5) [(13)] by its cytoplasmic buffering capacity causes increased resistance to antimicrobial agents, possibly leading to failure of treatment. Del Carpio-Perochena *et al.* found that incorporating nanoparticles could be beneficial, especially as an interappointment intracanal medication, because of their ability to kill bacteria in short- and long-term exposure. [(4)] Furthermore, antibacterial effectiveness is attributed to factors such as zeta potential, polydispersity index, encapsulation of nanoparticles, and rate of release of the active ingredients. Typically, stabilized nanoparticles have zeta potential (surface electrostatic potential) of ± 30 mV and a polydispersity index of <0.1 for homogenous particle size distribution. [(5)] For toxicity testing of metal nanoparticles on stem cell viability, the dose released (i.e., a mass of nanoparticles per volume of suspension) should be considered rather than only the dose administered (initial mass concentration of nanoparticles). [(7)] In vitro dose-response outcomes depend on stimulation of cellular response pathways involved in nanoparticle uptake, the nanoparticle/cell association ratio, and multiple physicochemical parameters that influence nanoparticle sedimentation and internalization.

With advances in nanotechnology in endodontics, 9 articles were selected for descriptive analysis. Based on the analysis made, clinical trials are in progress, with results yet to be published. So, in this systematic review, we have included both in vitro and ex-vivo studies. The studies included in this review utilized nanoparticles as an intracanal medicament in root canal disinfection. Since the aim was to evaluate whether nanoparticles can be used as an efficient antimicrobial intracanal medicament either alone or as an additive to conventional medicament, those studies that used nanoparticles as another adjunctive material is excluded as it can be a possible confounding factor. Customized criteria was formulated to assess the risk of bias of the studies and also to determine the strength of the study protocol and whether the conditions being tested were standardized. PICOS format was used to devise the research question for the review. PRISMA protocol was followed for screening and selection of studies.

Based on evidence extracted from the scientific literature, it is clear that metal nanoparticles have unique characteristics bring used alone or in combination with the conventional intracanal medicaments for endodontic treatment. Studies suggest that these metal nanoparticles create pits in the bacterial cell wall (11), leading to the accumulation of nanoparticles in the membrane, increasing permeability and cellular damage by indirectly altering DNA or protein synthesis, enzyme inactivation and generation of hydrogen peroxide. (10) Finally, they interact with –S.H. groups (atoms of hydrogen), which inturn can cause protein denaturation. All these elements extremely reduce the possibility of selecting resistant strains.

The authors observed that most of the included studies (86%) used mono-species biofilm. *E.* faecalis, in which human dentine was the most frequently used substratum with different incubation times ranging from one to fourteen days. Using the dentin model enlightened that standard controls like calcium hydroxide rapidly kill the bacteria in the test tubes. Only incomplete disinfection can be achieved in the surface wall of the root canal, so they are relatively ineffective. (12) Furthermore, bacterial culturing was the most common quantification method used, followed by the proportion of live and dead assay determined by fluorescent staining, similar to the protocol done by Dawood et al.

Both these methods showed different results. Those studies which evaluated the bacterial viability using LIVE/DEAD assay and CLSM showed that nanoparticles have an immediate and an over-time effect, gradually reaching their highest efficacy on the 7th day compared to the control, suggesting that they could access tiny dentinal tubules with an average size of 5 micrometres and would penetrate dentinal tubules of 200 µm and 400 µm deep. (16) On the other hand, using cultural methods, some studies favour the use, but others gave contradictory findings too.

The findings of Wu et al. study (9) suggested that the antibiofilm efficacy of AgNPs depends on the mode of application. Their formulation plays an important role in eliminating residual bacterial biofilms during root canal disinfection. The efficiency of nanoparticles was 88% (representing the drug-carrying capacity) compared to pure drug solution, (6) showing reduced bacterial load and residual biofilm thickness on the root canal dentin determined by SEM and CLSM image analysis. This suggests that drug formulation in solution and suspension has a high antibacterial effect compared to gel form. Similarly, nanoparticles biosynthesized from fungi as antibacterial agents against *E. faecalis* exhibit efficient antibacterial activity as a medicament for root canal disinfection

These studies reinforced that nanoparticle-based medicament can be used as a substitute to medicament and not just as an added material to irrigant. The fact that these nano antimicrobial agents are not only biocompatible at low antibacterial effective doses but also has cytotoxic effect after more than 6 h of exposure. (8) In comparison with the conventional medicament preparations, the action of gold standard drugs was higher on day 1 compared to day 7; suggesting that shorter medication periods with superior effectiveness(22) in root canal treatments can reduce selection pressure within the root canal system. Significantly the quantitatively analyzed studies suggest that antimicrobial nanostructures are better as an intracanal medicament than as an irrigant(23,24). Therefore, N.P.s seem to have a good potential to be used as an appropriate vehicle of conventional intracanal medicament in order to eliminate *E. faecalis* biofilm from human dentine in the short term.

CONCLUSION

Nanoparticles-based root canal medicament can be considered an alternative disinfectant or combined with other disinfectants to exert an antimicrobial effect. However, no clinical trials exist on the topic; randomized controlled trials need to be performed to extrapolate the results to the clinical scenario.

Quality of Evidence

All are in vitro studies; thus, the level of evidence is low.

Implications for practice

The nanoparticles-based intracanal medicament can be used as an antimicrobial agent either solely or in combination for root canal treatment.

Implications for Research

In the future, research should utilise these nanoparticles-based root canal disinfectants in animal and human populations to exert their associations with other products.

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