

**EVALUATION OF MODIFIED BIO-CERAMIC ENDODONTIC
SEALERS FOR ANTIMICROBIAL EFFICACY:
SYSTEMATIC REVIEW AND
META-ANALYSIS**

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ABSTRACT

Endodontic treatments are significantly improved by the efficacy of root canal sealers against microbial invasion. This systematic review and meta-analysis focus on comparing the antimicrobial efficacy of nanoparticle-modified bio-ceramic endodontic sealers with conventional bio-ceramic sealers. This review adhered to PRISMA guidelines and included both in vitro and ex vivo studies after a comprehensive search in PubMed, Embase, and Web of Science. The antimicrobial property of the bioceramic sealers was analyzed through the colony forming units (CFU), the diameter of inhibition zone and microbial viability. Eleven studies were used for the analysis. Overall, results from the meta-analysis indicated that modified bio-ceramic sealers exhibit an enhanced antibacterial effect compared to their conventional counterparts. A standardized mean difference was computed across 11 studies and 28 sub study groups, revealing a significant antimicrobial effect size in favor of the nanoparticle-modified bio-ceramic sealer. However, high heterogeneity among studies was observed. Chitosan, silver and bioactive glass were among the common nanoparticles used to modify bio-ceramic sealers. A 2% concentration of chitosan nanoparticles showed the highest antimicrobial effect size. Despite the heterogeneity found in the study, the findings suggest that the modification of bio-ceramic sealers with nanoparticles possesses the potential to advance endodontic treatments. The promising direction of these nanoparticle enhancements indicates a valuable progression in endodontics, advocating for additional research and development to optimize the use of nanoparticles in clinical settings.

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CHAPTER 1

INTRODUCTION

1.1 Introduction to Endodontics

Endodontics is a specialized area of dentistry that focuses on the diagnosis and treatment of dental pulp and its surrounding tissue.¹ Root canal treatment, also known as endodontic treatment, involves removing the infected or damaged pulp from inside a tooth, followed by thorough cleaning and sealing of the root canal system using dental biomaterials.¹ Teeth with irreversible pulpitis, traumatically or mechanically exposed pulps, as well as non-vital pulps may require root canal therapy.² After removing the infection, the use of irrigation solutions such as sodium hypochlorite helps to remove any remaining microbes and debris after cleaning and shaping.¹ Obturation materials are used to fill the root canal system to prevent further entry of fluids and microorganisms.² These steps in the root canal procedure are necessary to facilitate the healing and repair of periradicular tissues to restore tooth function.²

Many technological advancements in endodontics, such as nickel-titanium files, advanced apex locators, dental operating microscopes, and alternative filling materials have enhanced the success rate of root canal treatment over time.³ While the expected success rate ranges between 86-98%, effectively eliminating microorganisms from the intricate root canal system remains challenging.³ The complex anatomy of the root canals and the porous nature of dentin provide hiding places for these microorganisms.¹ These microbes have the potential to cause reinfection and challenge the success of the endodontic treatment. A research analysis of 493 root examinations highlighted primary reasons of failure of endodontically treated teeth and microbial leakage was found to be the most prevalent cause (Figure 1.1).⁴ Additional factors contributing to failure included

inadequate mechanical cleaning procedures, poor quality of obturation, as well as under or overfilling of root canals with filling material.³

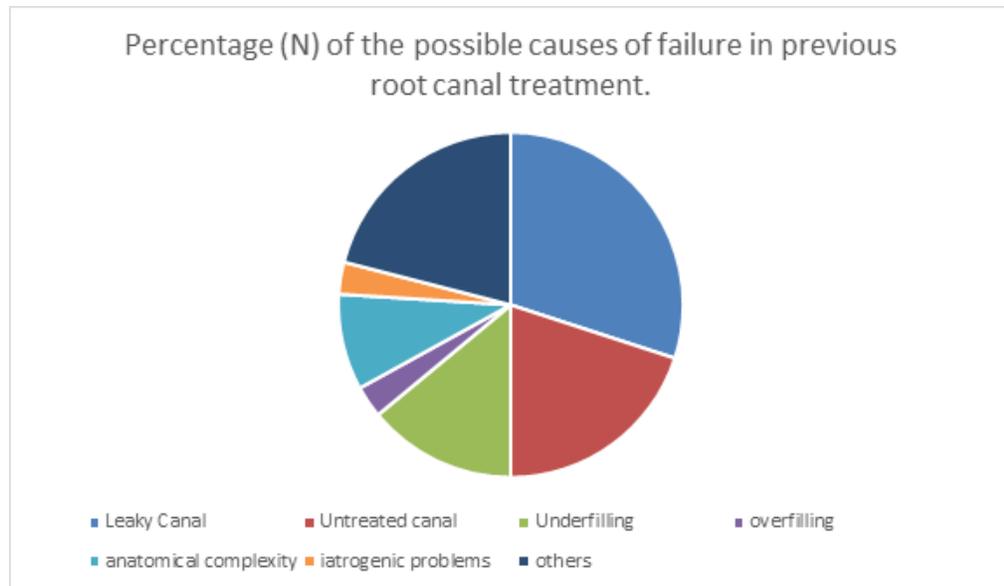


Figure 1.1. Percentage of causes of failure of endodontically treated teeth⁴

Other factors also challenge the success of endodontic treatment. A research study conducted on 1001 teeth that had undergone endodontic treatment revealed the quality of root canal treatment affected the clinical outcome.⁵ Another study showed that endodontic failure occurred in 65% of cases due to poor obturated material, while 42% was attributed to untreated canals.⁶ It is found that overextended obturation is four times more likely to result in failure compared to under-obturated canals.⁷ Furthermore, a meta-analysis demonstrated a higher success rate for endodontically treated teeth with good quality restorations.³ Lin et al conducted research on 236 cases and found that a higher number of bacteria within the root canal is associated with larger periapical lesions following failed root canal treatment.⁸

1.2 Endodontic Infection and *Enterococcus Faecalis*

The primary cause of infection within the root canal is the persistence of microbial presence.⁹ However, chemical and physical factors can lead to inflammation and damage of the dental pulp and surrounding tissues. In the inflamed area, microorganisms can defend themselves against host phagocytic cells, antibodies, and the complement system. The defensive action against these microorganisms is carried out by host polymorphonuclear neutrophils.⁹ This constant shift in equilibrium leads to chronic inflammation of the pulp, where persistent inflammation and pulp damage favor microorganism survival while tissue repair and healing facilitate host defense mechanisms.⁹ Virulence factors such as lipopolysaccharides, capsular components, enzymes, and metabolites in microorganisms contribute to their pathogenicity. There is a rich diversity of microorganisms found in endodontic infections.¹⁰ Common microbial species identified in endodontic infections include *E. faecalis*, *Streptococcus*, *Prevotella*, *Porphyromonas*, *Tannerella*, *Fusobacterium*, *Actinomyces*, *Candida albicans* and *Cytomegalovirus (CMV)* and *Epstein Barr virus (EBV)* amongst *Herpes simplex* species identified through molecular methods like polymerase chain reactions (PCR) or next generation sequencing (NGS).¹¹ Varied microbial species prevail in different endodontic conditions including unsuccessful endodontic treatment, endodontic flare ups and extra-radicular infection.¹⁰

E. faecalis is recognized as the most prevalent pathogen found in unsuccessful root canal procedures.¹² The mechanism of endodontic infection caused by *E. faecalis* is illustrated in Figure 1.2. It is a spherical-shaped facultative anaerobic gram-positive bacterium capable of surviving in low-nutrient environments and at pH levels exceeding

11.5.¹² Additionally, *E. faecalis* can adapt its genetic activity to endure elevated pH levels and develop biofilm inside treated canals.¹² Lipoteichoic acid, gelatinase, and hyaluronidase are influential factors responsible for the attachment and growth of *E. faecalis*.¹² Deterring the presence of these microbes inside the root canal may improve the clinical effectiveness of endodontic therapy.

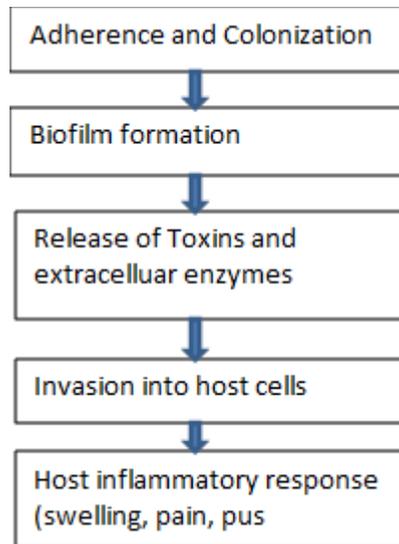


Figure 1.2. Pathogenesis of *E. faecalis* infection¹³

1.3 Endodontic Sealers

During the process of filling the root canal system, gutta-percha cones and endodontic sealers are utilized to seal the canal.¹⁴ The dense paste-like endodontic sealers function as a lubricant to secure the gutta percha points and fill in gaps, lateral canals, and accessory canals where the gutta percha is unable to reach.¹⁴ Ideally, these sealers should be adhesive when mixed, create a tight seal without shrinking or discoloring the tooth, possess antibacterial properties, easily dissolve in fluids while being biocompatible with peri-radicular tissues.¹⁵ They should also be radiopaque with smaller particle size for easy removal during retreatment of root canal therapy.¹⁵ Sealers are broadly categorized based on their main ingredients such as resin-based sealers, calcium hydroxide-based sealers,

silicone-based sealers, calcium silicate- based sealers, zinc oxide eugenol -based sealers, and MTA based sealers.¹⁶ Some commercially available sealers in each category are detailed in Table 1.1.

Table1.1 Type of endodontic sealers based on composition¹⁶

ROOT CANAL SEALER	BRAND NAME
1. Zinc oxide eugenol sealers	Roth sealer Kerr PCS Proco seal Endomethsone
2. Epoxy resin-based sealers	AH Plus AH 26 Top Seal 2-Seal
3. Silicon based sealers	Roekoseal Guttaflow
4. MTA based sealers	Endo-CPM-Sealer MTA Obtura ProRoot EndoSealer MTA Fillapex
5. Calcium-silicate-phosphate sealers	Endosequence/iRootSP iRoot BP Bioaggregate
6. Methacrylate resin-based sealers	First generation-Hydron Second generation- EndoREZ, Realseal Third generation- Epiphany, Fibrefill Fourth Generation- Realseal SE, Metaseal SE, Smartseal
7. Calcium-phosphate based sealers	Capseal

The ADA and ISO standards assess endodontic sealers based on various criteria including flow, setting time, solubility, sealing effectiveness, biocompatibility, and antimicrobial properties.¹⁴ Pro Root Endo Sealer is a calcium silicate sealer that exhibits comparable sealing performance to epoxy resin-based sealers and superior sealing capability compared to ZOE-based sealers.¹⁶ AH 26, an epoxy resin-based sealer can

release formaldehyde, leading to potential adverse reactions.¹⁶ Gutta flow demonstrates excellent flow characteristics, while Endomethasone does not meet the ISO standards for flow testing.¹⁶ Endosequence exhibits higher bond strength with dentinal walls.¹⁶ Epiphany has superior bond strength compared to AH plus.¹⁶ Different sealers display varying levels of antimicrobial effects. ZOE-based sealers have shown improved antimicrobial properties when compared to epoxy resin-based sealers, whereas glass ionomer-based sealers exhibit minimal antimicrobial activity.¹⁴ Additionally, silicone-based sealers do not demonstrate any antimicrobial activity against *E. faecalis*; on the other hand, calcium silicate-based sealants show favorable antimicrobial effects.¹⁴

1.4 Bio-ceramic Endodontic Sealers

Bio-ceramic sealers have been recently introduced to the field of dentistry and medicine, comprising of a variety of substances including zirconia, alumina, bioactive glass, glass ceramic, hydroxyapatite, and calcium phosphate particles.¹⁷ Bio-ceramic endodontic sealers set hydraulically, forming a tight seal within root canals. Their superior sealing ability, antibacterial properties, and biocompatibility make them stand out, promoting tissue healing and long-term stability in root canal treatments. There are subcategories within bio-ceramic sealants such as calcium silicate-based sealers, MTA based sealers and calcium phosphate-based sealers.¹⁷ These can be further classified into bioactive and bioinert types based on their interaction with surrounding periradicular tissues.¹⁸ For instance, glass and calcium phosphate act as bioactive agents while zirconia and alumina act as bioinert agents.¹⁹ Some examples of commercially available bio-ceramic sealants include BioRoot RCS, Endosequence, Total Fill BC, and MTA Fill

apex.¹⁷ Table 1.2 shows some examples and components present in each bio-ceramic sealers.

The properties of the different bio-ceramic sealers may be different. MTA Fillapex exhibits moderate cytotoxicity upon initial mixing and demonstrates reduced cytotoxicity over time in in vitro testing.²⁰ Endosequence and iRoot SP sealer can set in the presence of moisture within dentinal tubules, with a setting time of 4 hours.²⁰ Both Endosequence and MTA Fillapex show radiopacity attributed to the presence of bismuth trioxide. In comparison to AH plus and Epiphany, MTA Fillapex and iRoot SP exhibit higher resistance to dislodgement.²⁰ Bio-ceramic sealers are utilized for pulp capping in primary and permanent teeth, as well as for root end filling, due to their biocompatible nature.²¹

Table 1.2. Commonly used bio-ceramic sealers and their components²⁰

TYPE	BRAND NAME	COMPONENTS
Calcium silicate-based sealers	iRoot SP Endosequence	Zirconium oxide, calcium silicate, calcium phosphate, calcium hydroxide, filler, thickening agent
MTA based sealer	MTA Fillapex ProRoot Endo	MTA, bismuth trioxide, salicylate resin, silica, pigments Tricalcium silicate, calcium sulphate, bismuth trioxide, calcium phosphate

1.5 Application of Nanoparticles in Endodontics

Nanoparticles are tiny particles, smaller than 100 nm in size, that can occur naturally or be synthesized.¹⁸ They come in various forms such as particles, tubules, spheres, and rods.¹⁸ Due to their large surface area compared to their mass and increased

chemical reactivity, they have the advantage of interacting with the human body at a subcellular and molecular level.¹⁹ This makes them highly effective for therapeutic purposes with minimal side effects.¹⁹ In the field of biomedicine, nanomaterials are utilized for drug delivery, tissue regeneration, and antimicrobial applications.²² Additionally, nanoparticles have also been used in dentistry for addressing issues related to dentin hypersensitivity, biofilm removal and tissue regeneration.²² The potential use of nanoparticles in endodontics is presented in Figure 1.3.

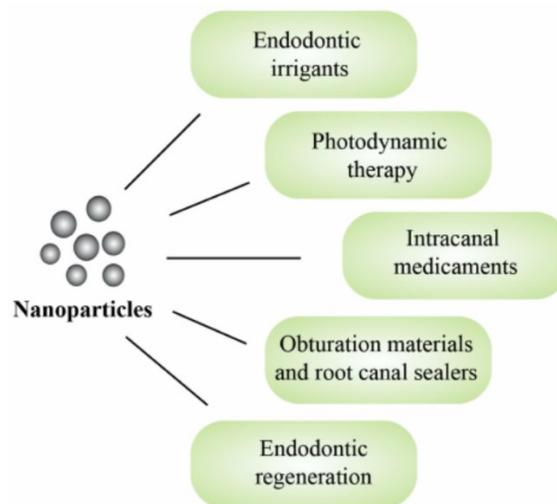


Figure 1.3. Potential translational application of nanoparticles in endodontics²³

Various types of nanoparticles are classified based on their composition, such as inorganic, organic, and composite variations.¹⁹ For example, chitosan nanoparticles are obtained from chitin and belong to the category of organic particles; bioactive glass nanoparticles support healing and regeneration as inorganic particles; quaternary ammonium polyethylenimine nanoparticles can be considered composite due to the

amalgamation of organic polyethylenimine with the inorganic quaternary ammonium group.¹⁹

A variety of nanoparticles, including graphene, silver, chitosan, hydroxyapatite, iron compounds, zirconia, a poly glycolic acid and bioactive glass nanoparticles are employed in endodontics.²⁴ Silver nanoparticles have strong antimicrobial properties and are integrated into irrigants to sterilize the root canal system effectively and prevent bacterial infections.²⁴ Graphene nanoparticles contribute to strengthening endodontic sealers due to their exceptional durability and electrical conductivity.²⁴ Chitosan nanoparticles act as carriers for drug delivery while being biocompatible.²⁴ Hydroxyapatite nanoparticles imitate the mineral component found in bones; they are used for bone regeneration applications as well as dentin remineralization with potential benefits in reducing dentin hypersensitivity by sealing tubules.²³ Titanium dioxide nanoparticles demonstrate photocatalytic activity under UV light which provides antibacterial effects that can be utilized for disinfecting root canals effectively.²³ Calcium oxide and copper oxide nanoparticles help regulate acidic conditions in root canals, promoting mineralization and offering antibacterial properties.²³ These advancements contribute to improvements in endodontic treatments. Additionally, the use of nanoparticles in scaffolds such as poly-lactic-coglycolic acid, polyglutamic acid, polycaprolactone/gelatin/nanohydroxyapatite, and poly-L-lactic acid benefits regenerative endodontics by serving as carriers for bioactive molecules that aid tissue regeneration.²³ This enhances scaffold characteristics and encourages cell adhesion and differentiation.²³

1.6 Antimicrobial Mechanism of Action of Nanoparticles

Recent studies have incorporated nanoparticles into endodontic sealers including quaternary ammonium polyethyleneimine (QAPE), zinc oxide, silver, chitosan or combination of these nanoparticles for improved antimicrobial activity within the canal.²³ QAPE is a polycationic disinfectant that demonstrates antimicrobial activity via electrostatic interaction with bacterial cell membrane. It provides prolonged effect due to its insoluble nature.²³ QAPE NP is strain dependent on *E. faecalis* when added to an epoxy based sealer, and zinc oxide-based sealer.²³ Silver nanoparticles exhibit strong antimicrobial effect when used in higher concentration.²³ Zinc oxide NP combined with chitosan NP show improved antimicrobial effect against *E. faecalis* on Apexit Plus, calcium hydroxide-based sealer.²³

Nanoparticles can effectively combat microorganisms through various methods, such as disrupting cell membranes and vital cellular processes, generating reactive oxygen species that damage crucial cellular elements, disturbing the balance of essential metal ions for survival, and impeding key bacterial enzymes needed for DNA replication and cell wall formation.²³ Together, these actions create an inhospitable environment that is unfavorable for bacterial survival and growth.²³ Chitosan nanoparticles work by interacting with bacterial cell membranes, creating an electrostatic attraction between the positive charge of chitosan and the negative charge of the membranes.¹⁸ This interaction alters cell wall permeability, potentially resulting in cellular rupture and the release of intracellular components. In fungi, it is believed that chitosan enters cells, binds to DNA, obstructs RNA and protein synthesis, disrupts cellular processes, and induces cell death.¹⁸

1.7 Knowledge Gap

Selecting the right endodontic sealer is crucial as it can significantly impact the outcomes of endodontic treatment.¹⁴ Bio-ceramic sealers may exhibit some degree of antimicrobial effects through the alkalinity of the calcium silicate and calcium phosphate-based components.²⁵ However, the effects do not long for extended periods and have questionable action against *E. faecalis*. The nanoparticles added to bio-ceramic sealers could enhance antimicrobial efficacy by extending the duration of ion release from these sealers. The aim of this research was to investigate whether the incorporation of nanoparticles into conventional bio-ceramic endodontic sealers can improve their antibacterial efficacy. Additionally, this study will provide a thorough examination of the various nanoparticles that have been incorporated into bio-ceramic endodontic sealers, along with the specific concentrations at which they have been used.

CHAPTER 2

METHODS

The systematic review followed the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) guidelines²⁶ and was registered in the Open Science Framework (OSF).²⁷

2.1 Eligibility Criteria

All relevant in-vitro studies or ex-vivo models with endodontic sealers were included. Animal studies were not considered. Studies that added antibacterial nanoparticles to bio-ceramic sealers were included. We excluded studies that worked with other types of endodontic sealers (e.g., epoxy resin based, zinc oxide based), nanoparticles added to gutta-percha, nanomaterials like natural oil added to bio-ceramic sealers (e.g., origanum vulgare oil), antifungal drugs incorporated in bio-ceramic sealers (e.g., fluconazole and ketoconazole) or irrigation solution (e.g., sodium hypochlorite, EDTA, saline). The outcome of interest was colony forming Unit (CFU), diameter of inhibition zone and microbial viability. CFU count provides an estimate of the number of viable microbial cells or microorganisms present in a sample. The diameter of inhibition zone refers to the measurement of the clear area surrounding an antimicrobial disk or other antimicrobial source on an agar plate. Confocal Laser Scanning Microscopy (CLSM) with a live stain was used to assess microbial viability. Microbial Viability refers to the ability of microorganisms to be able to survive, reproduce and grow. This technique allows for high-resolution visualization of microbial cells and their viability. Any studies conducted in narrative review, scoping review, literature review, systematic review, randomized clinical trials or ex-vivo study in animal teeth were excluded.

Population: endodontically treated teeth.

Interventions: added nanoparticles or nanomaterials with antimicrobial effects.

Comparators: bio-ceramic endodontic sealers without nanoparticles.

Outcomes: Antimicrobial efficacy in terms of CFU, inhibition zone and microbial viability.

Setting: In-vitro and ex-vivo studies.

2.2 Search strategy

The searches were conducted in the following databases: PubMed, Embase and Web of Science. The mesh terms or subject terms for search strategy for each database are described in Table 2.1. Dates searched were inception until 20th January 2024. No publication type restrictions were applied. The studies searched were in English language. The duplicated research articles were removed through Rayyan software (AI tool).

Table 2.1. Search Strategy of PubMed, Embase and Web of Science

DATABASE	SEARCH STRATEGY
PubMed	("Root Canal Filling Materials"[MeSH Terms] OR ("Calcium silicate-based sealer"[All Fields] OR "bioceramic sealer"[All Fields] OR "endodontic sealer*" [All Fields] OR "root canal sealer*" [All Fields]) OR (("endodontal"[All Fields] OR "endodontic"[All Fields] OR "endodontical"[All Fields] OR "endodontically"[All Fields] OR "endodontics"[MeSH Terms] OR "endodontics"[All Fields]) AND ("sealer"[All Fields] OR "sealers"[All Fields]))) AND ("colony count, microbial"[MeSH Terms] OR "Microbial Viability"[MeSH Terms] OR ("colony count, microbial"[MeSH Terms] OR "microbial colony count"[Text Word]))
Embase	('bacterial count'/exp OR 'microbial viability'/exp) AND ('root canal filling material'/exp OR 'calcium silicate-based sealer' OR 'bioceramic sealer' OR 'endodontic sealer*' OR 'root canal sealer*')

Table 2.1 (continued)

Web of Science	(TS=(antimicrobial property)) AND TS=(endodontic sealer) and Preprint Citation Index (Exclude – Database)
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2.3 Screening

Initially, 306 studies were screened with title and abstract. Out of which, 289 studies were excluded for not meeting the inclusion and exclusion criteria. Some of the reasons for exclusion were wrong study design, irrelevant background, and ineligible comparison groups. Later, full-text screening of 69 studies were done, out of which 19 studies met the selected criteria. All 19 studies were retrievable. Eight studies were further excluded as they were non-reliable antibacterial test method reported, no nanoparticles added, natural oil represented as nanoparticle, and subgingival plaque used as sample. Finally, 11 studies were included in the systematic review. The screening process was summarized in PRISMA flow chart (Figure 2.1). There were 28 sub-study groups present in 11 included studies. The sub-study groups are shown in Table 2.2.

Table 2.2. List of included studies and their sub study groups

Study Name, Year (Total 11)	Sub-study groups (Total 28)
1. Carvalho, N K et al, 2021	1. 2% CHX
	2. 5%CHX
2. Del Carpio-Perochena et al, 2022	3. 10% CS-HA
3. Marashdeh, M et al, 2021	4. 1% DSP
	5. 2% DSP
4. Yang, J et al, 2023	6. 10% CS (CFU)
	7. 5% CS (CFU)

Table 2.2 (continued)

	8. 5% CS (%LIVE)
	9. 10% CS (%LIVE)
5. Mediboyinam A et al, 2023	10. Fillapex + 1%SiTiO2 NPs
	11. Fillapex + 2%SiTiO2 NPs
	12. BioC + 1%SiTiO2 NPs
	13. BioC + 2%SiTiO2 NPs
	14. MTA Fillapex + 1% SiTiO2 NPs
	15. MTA Fillapex + 2% SiTiO2 NPs
	16. Bio-C + 1% SiTiO2 NPs
	17. Bio-C + 2% SiTiO2 NPs
6. Marashdeh et al,2021(IVM)	18. 1% DSP(CFU)
	19. 1% DSP(% LIVE)
7. Jung et al, 2022	20. 0.5% bioactive glass
	21. 1% bioactive glass
8. Rajisha et al, 2019	22. 2% CS NP
9. Loyola-Rodriguez JP et al, 2019	23. 10% silver NP VS Endosequence
	24. 0.3% Chitosan NP vs MTA
	25. 10% silver NP vs MTA
	26. 0.3% CS NP vs Endosequence
10. Pattanaik S et al, 2019	27. 2% CS NP
11. Primathena I et al, 2021	28. 20% bismuth trioxide

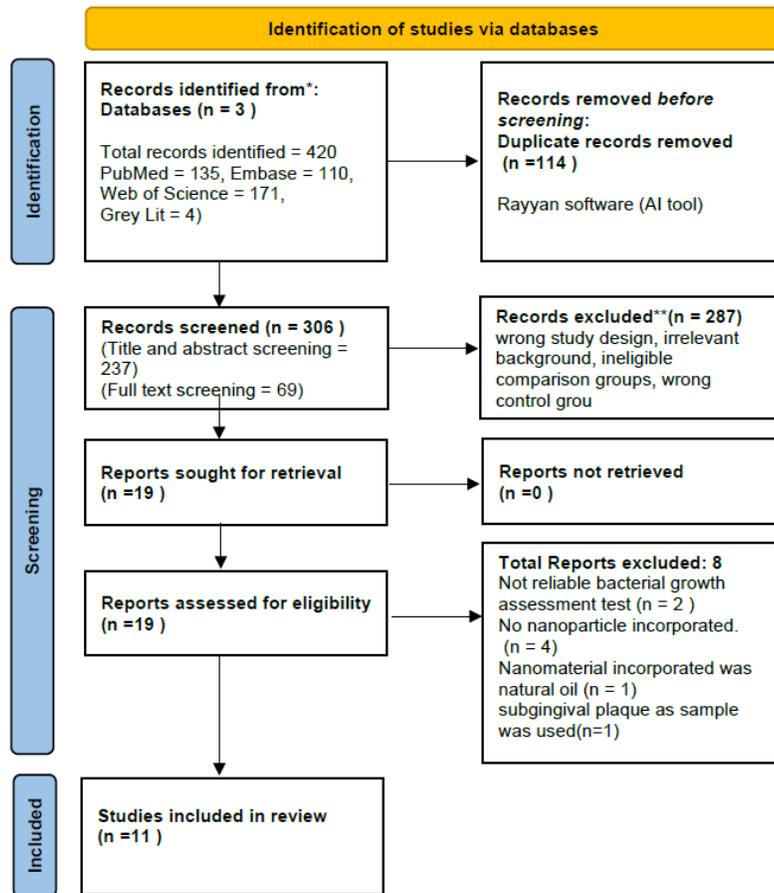


Figure 2.1. PRISMA flow chart²⁶.

2.4 Data Extraction

A standardized form (initially piloted on six included studies) was used for data extraction of characteristics of studies, outcomes, and risk of bias. The following data was extracted from included studies:

- Types of studies: in-vitro studies
- Methods that the study used: study authors; year; study design; storage conditions of sample; positive or negative control group, if any; broth used; bacterial growth

assessment test; time points, if any; bacterial growth conditions, aerobic or anaerobic; nanoparticle synthesized or mixed directly; followed manufacturer's instructions; pathogen or microorganism tested.

- The intervention/s of interest: type of nanoparticle incorporated, the concentration of nanoparticle added, and the comparator/s used (commercial name of calcium silicate endodontic sealer, if provided).
- Any reported mean and standard deviation of outcomes (CFU count, diameter of inhibition zone, viability) reported.

2.5 Assessment of the Risk of Bias

The risk of bias was assessed using the Joanna Briggs Institute (JBI) Critical Appraisal tool for quasi-experimental studies.²⁸ The questions were modified to represent more relevant questions for this systematic review. The ROBVIS traffic light plot (data visualization tool) was used to represent the findings.²⁹

2.6 Measurement of Antimicrobial Effect

The treatment/intervention effect was measured by Comprehensive Meta-Analysis Software V.4 software (Biostat, Inc. Englewood, NJ, USA).³⁰ The effect of the intervention was expressed as standardized mean difference. We undertook meta-analyses for 11 studies based on a random effects model. A forest plot was provided for all findings, and an overall measure of effect was computed along with the respective confidence intervals.³⁰

2.7 Assessment of Heterogeneity

The heterogeneity was assessed using the Cochran Q statistic and degree of freedom (df).³⁰ This test was used to assess the difference in antimicrobial effect size across each study to the overall antimicrobial effect size for all included studies. Degree of Freedom should be one minus the Q-value to show consistent results across all studies with p-value of less than 0.05 for statistical significance. I-square value assessed the true difference in antimicrobial effect size across the studies to control for the varied sample size differences across the studies. Higher value indicates higher heterogeneity.

2.8 Assessment of Publication Biases

The publication bias/small study effect was assessed using a Funnel plot. A graph was plotted against standardized difference in means for each study and standard of error. Each study was presented as a hexagonal point as shown in Figure 3.2. In absence of publication bias, larger studies cluster in the top of funnel and smaller studies scatter in the bottom of the funnel. An asymmetrical funnel would suggest smaller studies with non-significant results were missing from the analysis. Publication bias was conducted with Duval and Tweedie's trim and fill method³⁰. Potentially missing studies in an asymmetrical funnel were added to make the funnel symmetrical. Revaluation antimicrobial effect size and confidence interval of imputed studies against observed studies.

2.9 Sensitivity analysis

The study with highest effect size was removed to observe if there would be any significant changes to overall antimicrobial effect size intervention for sensitivity analysis³⁰.

2.10 Subgroup analysis

The presence of chitosan nanoparticles was noted and presented in categorical data. Subgroup analysis was conducted to examine the effect of chitosan nanoparticles on the outcomes of interest³⁰. Additionally, subgroup analysis was done for concentration of chitosan particles (2%, 5% and 10%) to explore the potential dose-dependent response.

2.11 Meta regression

The percentage of concentration of nanoparticles was recorded for each study included from a scale of 1-20%. Meta-regression analysis was performed to explore the relationship between percentage of nanoparticle concentration and antimicrobial effect size of nanoparticle.³⁰ This will allow us to generalize the results across varied bio-ceramic sealers.

CHAPTER 3

RESULTS

3.1 Description of Individual Studies

Table 3.1 summarizes the outcome of interest, statistical analysis and result of each individual study. Table 2.2 previously included the sub-study groups depending on different types and concentration of nanoparticles present in the individual study.

Table 3.1. Descriptive data of each individual study

Study,Year	Statistical Analysis used	Outcome of interest	Result
Carvalho N et al , 2021	Descriptive data presented in mean, SD. Statistical test used was Shapiro-Wilks test and Tukey's test for post hoc analysis for comparisons between groups	microbial colony unit	MTA fillapex with CHX-HMP at 2% and 5% concentration showed significantly greater antibacterial effect compared to unmodified sealer.
Del Carpio-Perochena	ANOVA and tukey's test were used for multiple comparisons of data from antibacterial assays, (significance level $p < 0.05$)	microbial colony unit	Bacterial loads reduced significantly when Chitosan hydroxyapatite nanocomplexes were added.
Jung MK et al, 2022	All data were represented in mean and SD. Stat test:one way ANOVA followed by Tukey's post hoc test with significance level of 0.05 after performing Shapiro Wilk test to confirm normality.	microbial colony unit	As the BGN concentration increased, the number of E. faecalis decreased
Loyola-Rodriguez JP et al, 2019	All data were expressed in mean, SD, and range. Kruskal-Wallis and Dunn's multiple comparison test were used for statistical analysis with significant p values less than 0.05.	Inhibition zone diameter	All groups show higher inhibition zone when chitosan nanoparticle were added
Marashdeh et al, 2021	ANOVA and Tukey post hoc test were used for comparisons between groups. significance level $p < 0.05$	microbial colony unit	All materials (with or without DSP)killed planktonic bacteria initially. BC +1% DSP AND BC +2%DSP maintained antibacterial activity over 30 day period.
Marashdeh et al, 2021(in vitro tooth model)	ANOVA and Tukey post hoc test were used for comparisons between groups. significance level $p < 0.05$	microbial colony count and %LIVE/DEAD bacteria	Both groups show significant reduction in CFU at all time points compared to control. Addition of DSP to BC sealer significantly reduced CFU compared to unmodified BC sealer

Table 3.1. (continued)

Primathena I et al, 2021	not mentioned	Inhibition zone diameter	bacterial inhibition zone for both the groups were at similar measured diameter
Yang, J et al, 2023	The data was expressed as mean,SD. Statistical differences in the results were evaluated using one-way ANOVA with significance 0.05.The tukey post hoc test was used for multiple comparisons with 95% confidence level.	microbial colony unit and %LIVE/DEAD bacteria	The addition of CS significantly reduced the viable cells (log(CFU/mL)) (CS-5%:5.3, CS-10%:4.6) compared with the BC (5.9) and positive control (7.2).
Rajisha K R et al , 2019	Student T test, One way Analysis (ANOVA) and Tukey test were used to compare the antimicrobial efficacy between groups.	microbial colony unit and inhibition zone	The antibacterial tests demonstrated significant <i>E. faecalis</i> growth inhibition in the DCT with the sealer samples incorporated with chitosan nanoparticles
Mediboyinam A et al, 2023	One-way ANOVA was used for the analysis, and Tukey's post-hoc test was used to compare groups. The level of significance was set at (p-value <0.05).	microbial colony unit and %LIVE/DEAD bacteria	The addition of SiTiO ₂ NPs to Bio-C and MTA Fillapex significantly reduced the bacteria, compared to an unmodified sealer

Overall, the collected studies examined the antimicrobial effects of bio-ceramic sealers against the pathogens *E. faecalis* and *C. albicans*. Various antimicrobial agents, including chitosan, bioactive glass, SiTiO₂, and bismuth trioxide, were incorporated into the sealers at concentrations ranging from 1-20%. The review included 3 ex-vivo studies on extracted teeth and 8 in-vitro studies. The antimicrobial efficacy of the sealers was evaluated over time periods ranging from 7 to 30 days. The commonly used commercial bio-ceramic sealers in the studies were MTA Fillapex, Endosequence, Bright Endo MTA, ProRoot MTA, and BC Sealer.

3.2 Meta-Analysis

The mean, standard deviation and sample size of intervention and comparator group were used to conduct meta-analysis for 28 subgroup studies from the 11 included studies as shown in Table 3.2. The CFU outcome involved 15 subgroup studies,

%live/dead bacteria involved 7 subgroup studies, and the inhibition zone analysis included 6 subgroup studies.

Table 3.2. Data of intervention and comparator group used for meta-analysis

Study Name, Year	Out come	BC sealer Group MEAN	BC Sealer Group SD	BC Sealer Group Sample Size	Modifie d BC sealer Group MEAN	Modifie d BC sealer group SD	Modifie d BC sealer Group Sample Size	Chitosan (Y/N)	% of NP
Carvalho, N K et al, 2021, a	CFU	4.88	0.03	5	4.54	0.04	5	No	2%
Carvalho, N K et al, 2021, b	CFU	5.38	0.53	5	0.5	0.5	5	No	5%
Del Carpio-Perochena et al, 2022	CFU	1.24	0.77	8	0.98	0.57	8	Yes	10%
Marashdeh, M et al, 2021, a	CFU	5.62	0.6	3	0.5	0.5	3	No	1%
Marashdeh, M et al, 2021, b	CFU	4.2	0.2	3	2.25	0.17	3	No	2%
Yang, J et al, 2023, a	CFU	6	0.25	6	4.33	0.33	6	Yes	10%
Yang, J et al, 2023, b	CFU	6	0.25	6	5.05	0.2	6	Yes	5%
Yang, J et al, 2023, c	MV	62.06	29.42	6	45.09	22.51	6	Yes	5%
Yang, J et al, 2023, d	MV	62.06	29.42	6	22.67	18.01	6	Yes	10%
Mediboyinam A et al, 2023, a	CFU	7.46	1.174	10	4.73	0.66	10	No	1%
Mediboyinam A et al, 2023, b	CFU	7.46	1.174	10	2.32	0.804	10	No	2%
Mediboyinam A et al, 2023, c	CFU	3.46	0.614	10	1.68	0.277	10	No	1%
Mediboyinam A et al, 2023, d	CFU	3.46	0.614	10	0.48	0.672	10	No	2%
Mediboyinam A et al, 2023, e	MV	49.6	5.89	10	59	31.25	10	No	1%
Mediboyinam A et al, 2023, f	MV	49.6	5.89	10	21.4	4.5	10	No	2%
Mediboyinam A et al, 2023, g	MV	45.2	26.89	10	30	34.02	10	No	1%
Mediboyinam A et al, 2023, h	MV	45.2	26.89	10	4	1.64	10	No	2%
Marashdeh et al,2021(IVM), a	CFU	4.5	0.5	4	2.5	0.5	4	No	1%
Marashdeh et al,2021(IVM), b	MV	28.67	7.78	4	12.97	5.8	4	No	1%
Jung et al, 2022, a	CFU	12.5	0.5	5	9.5	0.5	5	No	0.50 %
Jung et al, 2022, b	CFU	12.5	0.5	5	6.5	0.5	5	No	1%
Rajisha et al, 2019	CFU	17.56	0.05	3	13.09	1.39	3	Yes	2%

Table 3.2. (continued)

Loyola-Rodriguez JP et al, 2019, a	IZ	8.6	0.9	3	9.5	1.3	3	No	10%
Loyola-Rodriguez JP et al, 2019, b	IZ	8.7	0.9	3	10.4	0.9	3	Yes	0.30 %
Loyola-Rodriguez JP et al, 2019, c	IZ	8.7	0.9	3	9.5	1.3	3	No	10%
Loyola-Rodriguez JP et al, 2019, d	IZ	8.6	0.9	3	9.5	0.8	3	Yes	0.30 %
Pattanaik S et al, 2019	IZ	3.8	0.71	10	5.8	0.54	10	Yes	2%
Primathena I et al, 2021	IZ	15.86	0.5	3	15.83	0.5	3	No	20%

The effect size index used in this analysis was the standardized difference in means (d). The standardized difference in means with upper and lower 95% confidence interval is shown in Table 3.3. A random effect model was employed for the analysis. The mean antimicrobial effect size was 3.162 favoring the nanoparticle intervention group. The antimicrobial effect size with nanoparticles incorporated showed lower limit of effect size as 2.332 and upper limit of effect size as 4.002 as shown in Forest plot (Figure 3.1). The Z-value is a standardized measure of effect size, indicating the distance of standard deviation from the null value. The Z-value is 7.376 with $p < 0.001$. Using a criterion alpha of 0.050, we reject the null hypothesis and conclude the mean antimicrobial effect size significantly larger than null value (zero). The incorporation of nanoparticles to bio-ceramic sealers showed three times more antimicrobial efficacy than traditional bio-ceramic sealers. However, the magnitude of antimicrobial effect varied across the included studies. The antimicrobial effect size for intervention with nanoparticles and comparator group without nanoparticles can fall under -0.970 to 7.294.

Individual subgroup studies carried relative weights ranging from 1.3 to 4.6, and all subgroup studies impact the antimicrobial effect size estimation (Table 3.3).

Table 3.3. Individual sub-study standardized difference in means, confidence intervals, and relative weight

Study Name		Std diff in means	Lower 95%CI	Upper 95%CI	Relative weight
Carvalho, N K et al, 2021, a		9.6	5.2	14	2.1
Carvalho, N K et al, 2021, b		9.4	5.1	13.8	2.1
Del Carpio-Perochena et al		0.3	-0.6	1.3	4.6
Marashdeh, M et al, 2021, a		9.2	3.7	14.7	1.6
Marashdeh, M et al, 2021, b		10.5	4.3	16.6	1.3
Yang, J et al, 2023, a		1.6	0.3	2.9	4.4
Yang, J et al, 2023, b		0.6	0.5	1.8	4.5
Yang, J et al, 2023, c		4.1	2.1	6.2	3.8
Yang, J et al, 2023, d		5.7	3.1	8.2	3.4
Mediboyinam A et al, 2023, a		3.7	2.2	5.1	4.3
Mediboyinam A et al, 2023, b		4.6	2.9	6.3	4.1
Mediboyinam A et al, 2023, c		2.8	1.6	4.1	4.4
Mediboyinam A et al, 2023, d		5.1	3.2	6.9	4
Mediboyinam A et al, 2023, e		2.1	1	3.2	4.5
Mediboyinam A et al, 2023, f		0.4	-0.3	1.3	4.6
Mediboyinam A et al, 2023, g		-0.4	-1.3	0.4	4.6
Mediboyinam A et al, 2023, h		5.3	3.4	7.2	3.9
Marashdeh et al,2021(IVM), a		4	1.6	6.4	4
Marashdeh et al,2021(IVM), b		2.2	0.5	4	4
Jung et al, 2022, a		6	3	8.9	3.1
Jung et al, 2022, b		12	6.5	17.4	1.6
Rajisha et al, 2019		4.5	1.5	7.5	3
Loyola-Rodriguez et al, 2019, a		1	-0.6	2.7	4.1
Loyola-Rodriguez et al, 2019, b		0.8	-0.8	2.4	4.1
Loyola-Rodriguez et al, 2019, c		0.7	-0.9	2.3	4.1
Loyola-Rodriguez et al, 2019, d		1.8	0	3.8	3.9
Pattanaik S et al, 2019		3.1	1.8	4.4	4.4
Primathena I et al, 2021		0	-1.5	1.6	4.2

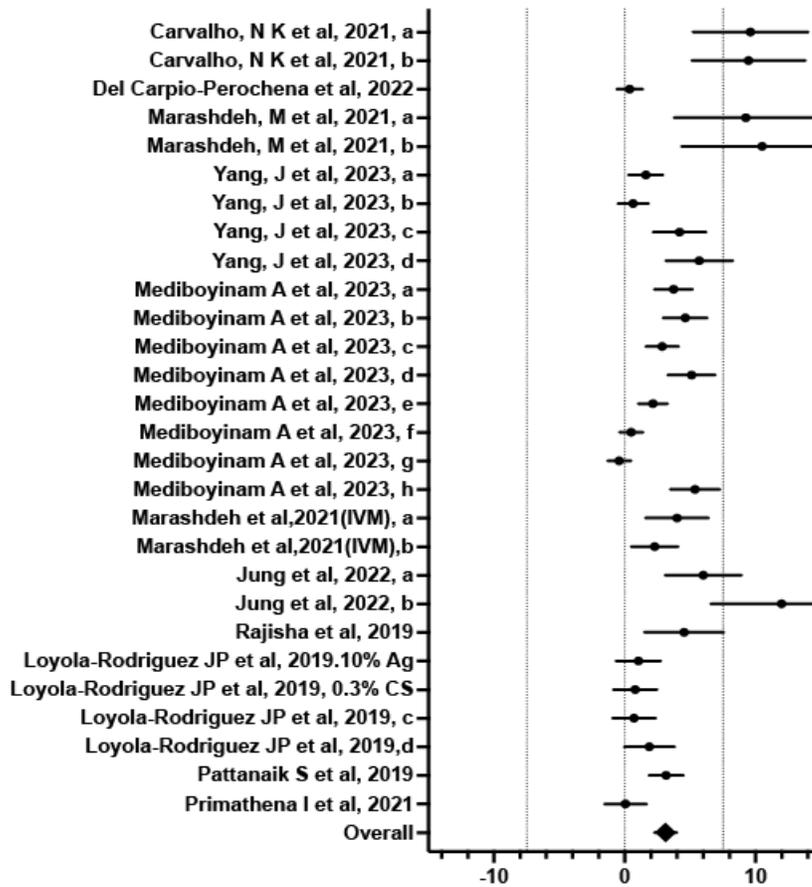


Figure 3.1. Forest Plot

3.3 Heterogeneity

The Q-statistic provides a test of the null hypothesis that all studies in the analysis had similar antimicrobial effect size. The Q-value is 185.406 with 27 degrees of freedom (df) and $p < 0.001$. We reject the null hypothesis and conclude all studies had varied antimicrobial effect size. Although the included studies demonstrate considerable heterogeneity likely due to variances in the nanoparticles incorporated, the concentrations of nanoparticles used, and the antimicrobial testing methods employed, the findings consistently indicate that the incorporation of nanoparticles into bio-ceramic sealers results in improved antimicrobial efficacy. The I-squared statistic is 85%, which tells us that some 85% of the variance in observed effects reflects variance in true effects rather

than sampling error. The tau-squared, the variance of true effect sizes, is 3.857 in d units. Tau, the standard deviation of true effect sizes, is 1.964 in d units.

3.4 Publication Bias

After adjusting for potential publication bias with an additional set of ten studies, the adjusted antimicrobial effect size reduced to 1.6, still suggesting a positive outcome despite possible missing studies (Figure 3.2).

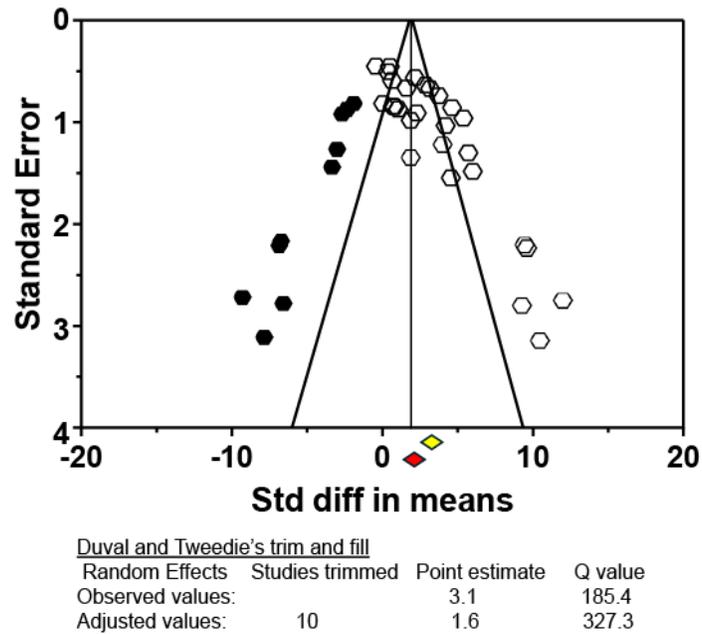


Figure 3.2. Funnel Plot

3.5 Risk of Bias

Overall, there was low risk of bias across all included studies as seen in Figure 3.3. The green color indicates low risk of bias and red color indicates high risk of bias. The key inconsistency across the studies was the evaluation of antimicrobial outcomes at different time points, and none of the studies followed up on the antimicrobial outcome beyond 30 days. Apart from this, the studies exhibited consistency in terms of the

microbial agents used for the intervention and comparator groups, the antimicrobial testing methods applied to both groups, and the statistical analyses employed.

Study	D1	D2	D3	D4	D5	D6	D7	D8	D9	Overall	Question
	Carvalho N et al , 2021	+	+	+	+	+	?	+	+	+	+
Del Carpio-Perochena	+	+	+	+	X	?	+	+	+	+	Q2 Were the microbial strain included in any comparisons similar?
Jung MK et al, 2022	+	+	+	+	X	?	+	+	+	+	Q3 Were the microbial colonies exposed to similar sealer without addition of another sealer, other than the exposure of nanomaterial incorporated sealer?
Loyola-Rodríguez JP et al, 2019	+	+	+	-	X	?	+	+	+	+	Q4 Was there a control group?
Marashdeh et al, 2021	+	+	+	+	+	?	+	+	+	+	Q5 Were there multiple measurements of the outcome post the intervention/exposure?(different timepoints)
Marashdeh et al, 2021(in vitro tooth model)	+	+	+	+	+	?	+	+	+	+	Q6 was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analyzed?
Pattanaik S et al, 2019	+	+	+	+	X	?	+	+	+	+	Q7 Were the outcome measured in similar way for comparison groups?
Primathena I et al, 2021	+	+	+	-	X	?	+	+	+	+	Q8 Were outcomes measured in reliable way?
Yang, J et al, 2023	+	+	+	+	X	?	+	+	+	+	Q9 Was appropriate statistical analysis used?
Rajisha K R et al , 2019	+	+	+	+	+	?	+	+	+	+	
Mediboyinam A et al, 2023	+	+	+	-	X	?	+	+	+	+	

D1: Q1
D2: Q2
D3: Q3
D4: Q4
D5: Q5
D6: Q6
D7: Q7
D8: Q8
D9: Q9

Judgement

- High
- Unclear
- Low
- No information

Figure 3.3. ROBVIS risk of bias assessment tool

3.6 Subgroup Analysis and Meta Regression

A subgroup analysis of 7 subgroup studies on chitosan nanoparticle concentrations (2%, 5%, or 10%) found that the 2% concentration had the highest antimicrobial effect size of 3.3, which was statistically significant (Figure 3.4). Additionally, an overall antimicrobial effect size across all chitosan concentrations was statistically significant. Incorporating 2% chitosan into bio-ceramic sealers demonstrated approximately three times greater antimicrobial efficacy compared to traditional bio-ceramic sealers. Furthermore, bio-ceramic sealers containing 5% and 10% chitosan exhibited around twice the antimicrobial activity compared to traditional bio-ceramic sealers.

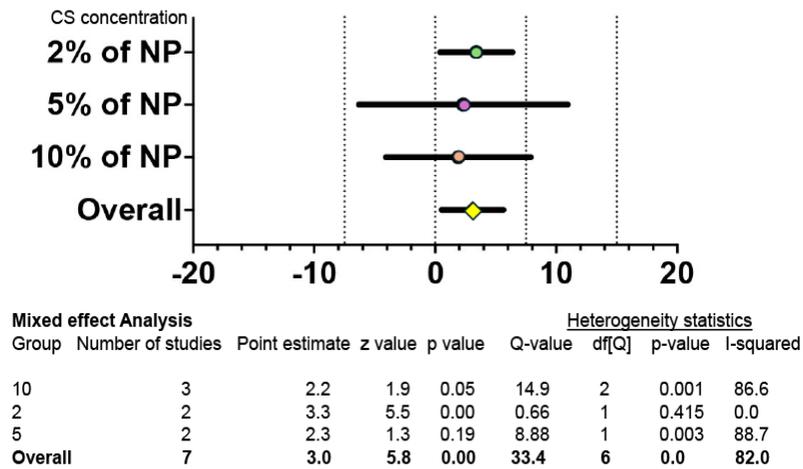


Figure 3.4. Subgroup analysis of chitosan nanoparticles

Although an increase in nanoparticle concentration corresponded to a reduction in antimicrobial effect size (Figure 3.5), this finding did not reach statistical significance and accounted for only a small variation (1%) in effect size due to diverse experimental conditions and sample sizes across studies. The varied concentration of nanoparticles incorporated into bio-ceramic sealers appears to have limited impact on their antimicrobial efficacy, accounting for 1% of the observed effect.

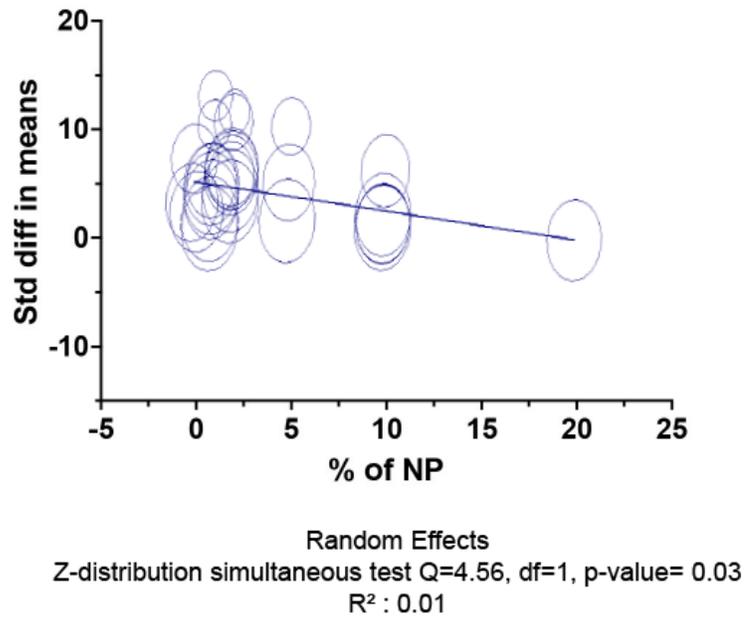


Figure 3.5. Meta regression of standardized difference in means on percentage of NP

CHAPTER 4

DISCUSSION

This systematic review and meta-analysis analyzed the antimicrobial efficacy of bio-ceramic sealers when incorporated with nanoparticles offering additional antibacterial effects. Adding nanoparticles to bio-ceramic sealers showed three times more favorable results for antimicrobial efficacy compared to traditional sealers. The antimicrobial effect size varies across the included studies. None of the studies showed favorable antimicrobial effect towards the traditional bio-ceramic sealer. There were five sub studies that showed that antimicrobial efficacy was similar for both traditional bio-ceramic sealers and nanoparticle incorporated to bio-ceramic sealer groups. Hence, overall, these results were favorable for modified bio-ceramic sealers independent of microorganism tested, type of nanoparticle and concentration of nanoparticle.

While there was a high heterogeneity found across the included studies, one of the factors contributing to heterogeneity was the use of different nanoparticles agents and varying concentrations in the studies. Chitosan was identified as the primary nanoparticle incorporated into the bio-ceramic sealers. While the number of studies evaluating chitosan at concentrations of 2%, 5%, and 10% was limited, all of these concentrations demonstrated favorable results for the nanoparticle-incorporated bio-ceramic sealers, with the 2% concentration exhibiting the highest antimicrobial efficacy. Most of the studies had concentration of nanoparticles at 1-5%. While the results show with increase in concentration of nanoparticles the antimicrobial efficacy decreases, it cannot be unnoticed that most the studies incorporated nanoparticles at 1% and 2%. There were only a few studies at 5% and 10% of nanoparticle incorporated to bio-ceramic sealers and one study for 20% of nanoparticle incorporated to bio-ceramic sealers. In this case, 20%

of bismuth trioxide was added to improve the radiopacity of the modified bio-ceramic sealers. Additionally, it was shown that concentration of nanoparticle contributes to 1% of antimicrobial efficacy to bio-ceramic sealers. While it was known about the heterogeneity of studies during data extraction for this review, the heterogeneity can be reduced by focusing on subgroups like specific nanoparticles, specific outcome of interest, specific subgroup of bio-ceramic sealers if more studies are conducted. The substantial variability observed across studies does not necessarily lead to the conclusion that the reported results are unreliable.

Similar high heterogeneity was found in the study by Iqbal et al.³¹ There are several methodological diversities among the included studies. The studies included varied outcomes for testing antimicrobial efficacy, the types of commercial sealers used, the days and environment conditions tested, different concentration and composition of nanoparticles. Iqbal et al mentioned about the antimicrobial effect induced by nanosized particles is dose-dependent, with an expected increase in effectiveness as nanoparticle concentrations increase. Yang et al, mentioned the increase in concentration of chitosan nanoparticle affects the flow of endodontic sealer and its capacity to penetrate in dentinal tubules.³² Hence, while conducting research for nanoparticles incorporated in bio ceramic, it is important to understand the concentration at which the physiochemical properties of bio-ceramic sealers remain unchanged. The nanoparticle incorporated sealers could show improved antimicrobial efficacy than traditional bio-ceramic sealer because traditional bio-ceramic sealer shows antimicrobial efficacy until their setting time. The nanoparticles are smaller in size and can penetrate more into the tubules releasing particles from sealers for an extended period of time.

The publication of this paper may be subject to bias, as the reported results favor the incorporation of nanoparticles into bio-ceramic sealers, even when potentially including studies with small sample sizes and non-significant findings. Additionally, there is a possibility that relevant studies published in languages other than English may be missing from the analysis. There was consistency in the outcomes and low risk of bias provided by the included studies, but there was lack of information and inconsistency for the timepoints when the study was conducted for some of the included studies.

In this systematic review, various nanoparticles incorporated into the bio-ceramic sealers with chitosan were present in 5 out of 11 studies included. Carvalho, N. K. et al.,³³ studied incorporation of chlorhexidine metaphosphate. Carpio-Perochena, A. del. et al.,³⁴ Loyola-Rodríguez J. P. et al.,³⁵ Pattanaik, S. et al.,³⁶ Yang, J. et al.,³² Rajisha, R. et al.,³⁷ studied the incorporation of chitosan nanoparticles. Jung, M. et al.,²⁵ studied the incorporation of bioactive glass. Two studies of Marashadeh, M. et al.,³⁸ studied incorporation of drug silica particles. Mediboyina, A. et al.,³⁹ studied incorporation of SiTiO₂ while Primathena, I. et al.,⁴⁰ studied bismuth trioxide. All included studies mentioned if the nanoparticles were synthesized or mixed in a laboratory. Most of the studies mentioned the storage conditions for samples, presence of the control group and broth used for the experiment. A few studies, Carvalho, N. K. et al.,³³ Marashadeh, M. et al.,⁴¹ and Rajisha.R. et al.,³⁷ mentioned the time points of 24 hr., 7 days, 28-30days (about 4 and a half weeks) when the antibacterial tests were analyzed.

The all included studies examined the effectiveness against the *E. faecalis* strain except for Pattanaik, S et al.,³⁶ which focused on *Candida albicans*. Various sealers have demonstrated different levels of antifungal effects. Endodontic sealers like AH Plus and

iRoot SP exhibit high antimicrobial activity not just against *E. faecalis*, but also *C. albicans* and *S. aureus*.⁴² Freshly mixed AH Plus, iRoot SP, and MTA Fillapex have exhibited antifungal efficacy, although this effect diminishes over time. Guttaflow has been shown to be less effective against *C. albicans*.⁴³ *E. faecalis* is an anaerobic microorganism, but Loyola-Rodríguez, J. P. et al.,³⁵ and Jung, M. et al.,²⁵ conducted experiments in an aerobic conditions. Seven included studies didn't mention environmental conditions tested for the microorganism. The included research studies, Marashdeh, M. et al.,³⁸ Rajisha, R. et al.,³⁷ Jung, M. et al.,²⁵ Mediboyina, A. et al.,³⁹ Carpio-Perochena, A. del et al.,³⁴ and Carvalho, N. K. et al.,³³ employed direct contact as a method for antimicrobial testing to measure colony forming units. Additionally, confocal laser scanning microscopy was utilized in the assessment of microbial viability by Marashadeh, M. et al.,³⁸ Rajisha, R. et al.,³⁷ and Mediboyina, A. et al.³⁹ Furthermore, Loyola-Rodríguez, J. P. et al.,³⁵ Pattanaik, S. et al.,³⁶ and Primathena, I. et al.,⁴⁰ used the agar diffusion tests to evaluate the diameter of inhibition zones. All the studies that were included conducted suitable statistical analysis, except for Primathena, I. et al.,⁴⁰ that did not mention any statistical analysis.

CHAPTER 5

LIMITATION AND FUTURE WORK

The research was primarily limited to in vitro studies on extracted teeth and did not include any clinical trials involving human patients. The meta-analysis does not show the impact of individual nanoparticle added to endodontic sealer. It does not provide the comparison of effect size between commonly used nanoparticles as there was variation in concentration of each nanoparticle used as well. The systematic review had studies of different nanoparticles at varying concentrations and differences in commercial brands of bio-ceramic sealers. Bio-ceramic sealer is a relatively new material. More research is needed on bio-ceramic sealers, especially focusing on less-studied nanoparticles like chitosan, bioactive glass and silver NP with a larger sample size. It is important for researchers to explore the development of new nanoparticles for enhancing the antimicrobial effectiveness of bio-ceramic sealers. In future one could do meta-regression for studies with only 1-2% concentration of nanoparticles and verify if the results remain the same or unchanged.

CHAPTER 6

CONCLUSION

Nanoparticle-modified bio-ceramic sealers show enhanced antimicrobial effectiveness compared to conventional counterparts, which has profound implications for endodontic treatments. Despite heterogeneity and publication bias in the studies included, consistent favorable outcomes underline the potential of these modified sealers in clinical applications. The promising nanoparticles found were chitosan, silver nanoparticles, bioactive glass with hydroxyapatite nanoparticles. The concentration between 2-5% shows majority of the studies with improved antimicrobial efficacy without disturbing the physio mechanical properties of the material. This is important as the overall goal is to establish a material which shows better sealing ability, antimicrobial efficacy and improved physio mechanical properties to prevent reoccurring infections.

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