Antimicrobial properties of graphene applied in endodontic agents and materials: a scoping review

**ABSTRACT**

**Background:** Graphene, with multifunctional potential in dentistry, is being studied in several areas, including endodontics, due to its antimicrobial effect. However, it is not yet a popular reality in endodontics. Therefore, a scoping review was conducted to shed light on studies present in the literature that combine graphene and its antimicrobial effect in different ways in endodontic treatment, seeking to identify the main advances and uses of graphene and its possible applications in endodontics. **Methods:** A scoping review was conducted to identify graphene's main advances and uses and its possible applications in endodontics. Our protocol was drafted using the Preferred Reporting Items for Systematic Reviews and Meta-analysis Extension for Scoping Reviews (PRISMA-ScR) and the following bibliographic databases were searched, without limitation on the year of publication and language: PubMed, Scopus, Embase, and Web of Science. As inclusion criteria, a qualitative or quantitative antimicrobial test model must be used in the study to evaluate the material and at least one group must be used in the evaluation with some endodontic agent or material for clinical use. **Results:** After screening, 9 studies were included in the review, including the use of graphene oxide, reduced graphene oxide, and nano-graphene oxide in in vitro and ex vivo studies. Graphene was used in studies against biofilms as an irrigant, filler, intracanal medication or photosensitizer. **Conclusion:** Even though studies present positive results in the use of graphene, standardization of experiments, form of use and concentration is necessary for a direct comparison of studies and a possible meta-analysis.

Keywords: Graphene. Endodontics. Antimicrobial activity. Biofilms. Dentistry.

**1. INTRODUCTION**

**1.1 Rationale**

Graphene, a single layer of carbon atoms forming a two-dimensional (2D) honeycomb lettice (1), has triggered the emergence of new phenomena, creating opportunities in physics, chemistry, and biomedical fields. The graphene family of nanomaterials contains a wide variety of this material, such as graphene oxide (2–4) and reduced graphene oxide (5).

Based on advances in the application of graphene in several areas, the material has presented multifunctional potential in dentistry, being studied in tissue engineering as scaffolds (6), orthodontics (7), oral surgery (8), restorative dentistry (9), endodontics (10), caries management (11), and periodontology (12).

The antimicrobial effect, one of the effects that graphene presents, has been widely explored (13–15), bringing innovative potential in dentistry, especially in endodontics, where intracanal antimicrobial control needs to be rigorous for the success of endodontic therapy.

However, graphene is not yet a popular reality in endodontics. Therefore, a scoping review was conducted to shed light on studies present in the literature that combine graphene and its antimicrobial effect in different ways in endodontic treatment, seeking to identify the main advances and uses of graphene and its possible applications in endodontics.

**1.2 Objectives**

A scoping review was carried out to systematically map existing investigations in the literature using graphene in its various forms, with antimicrobial purposes in possible materials and agents for endodontic use, as well as identifying any existing gaps. The following research question was formulated: “Does graphene have antimicrobial action when evaluated as a possible endodontic agent or material against bacteria of clinical interest?”

**2. METHODS**

**2.1 Protocol and registration**

Our protocol was drafted using the Preferred Reporting Items for Systematic Reviews and Meta-analysis Extension for Scoping Reviews (PRISMA-ScR), which was revised by the research team. The final protocol was registered on the Open Science Framework on 29 December 2023 (https://osf.io/m2tzp/).

**2.2 Eligibility criteria**

To be included, the articles needed to develop a study with materials and agents to be used in endodontic antimicrobial therapy with graphene, especially as irrigants, filling materials, or intracanal medications, a qualitative or quantitative antimicrobial assay model to evaluate the material and at least one group in the evaluation with some endodontic agent or material for clinical use, such as cements, irrigants (chlorhexidine or sodium hypochlorite), intracanal medications or photosensitizers. For the inclusion of articles, there was no limitation on the publication period, language, or study model (*in vivo*, *ex vivo,* or *in vitro*). Exclusion criteria were defined: not specifying the form of graphene used and review articles.

**2.3 Information sources**

To identify relevant documents, the following bibliographic databases were searched, without limitation on the year of publication and language: PubMed, Scopus, Embase, and Web of Science. The search strategy was discussed and refined among the researchers and the search was carried out in January/2024. The results of the final search were exported to the StArt software (v. 3.3 Beta 03) (16) to identify duplicates and then select documents for subsequent data extraction.

**2.4 Search**

Research strategies were developed by the research team and refined through team discussions. The final search strategy for PubMed can be found in Table 1.

**2.5 Selection of sources of evidence**

To maintain consistency, all reviewers screened all publications. The screening was carried out by three reviewers, evaluating the title, abstract, and then the entire text when identified as a potentially relevant publication. In case of disagreement, a discussion with a third reviewer to reach a consensus could be held if necessary.

**2.6 Data charting process**

A data-charting form was developed by the three reviewers to extract the necessary data. The reviewers independently extracted the data, and, after a discussion of the results, the data were updated as necessary.

**2.7 Data items and synthesis of results**

The data-charting form contains characteristics of the articles (Authors and publication date), objectives, and study design. We summarized the form of graphene used, the bacteria strains, the assessment of antimicrobial effect, treatment groups, and results.

**3. RESULTS**

**3.1 Selection of sources of evidence**

The number of evidence sources selected, assessed for eligibility, and included in the review (17) are available in Figure 1.

**3.2 Characteristics of sources of evidence**

The main characteristics of the sources of evidence are described in Table 2.

**3.4 Results of individual sources of evidence**

Table 3 presents relevant data from studies related to the objective of the review.

**3.5 Synthesis of results**

Table 4 presents only the results related to graphene found in the studies.

**4. DISCUSSION**

**4.1 Summary of evidence**

**4.1.1 The application possibilities of graphene**

In this review, we focused on studies that aimed to clinically use the material or agent created or adapted and that used group comparisons with conventional materials or agents in clinical dental practice.

From the studies analyzed, graphene was present in its various forms as a possible photodynamic therapy agent (18,19), intracanal medicine (20,21), filling material (22,23), and irrigants or adjuvants (24–26). The possibilities of using these materials or agents were based on the objective of the study written by the authors themselves, as previously shown, by the way it was applied, and the groups used in the research.

When creating or adapting a material, the characterization and identification of its main characteristics, such as Minimum Inhibitory Concentration, is extremely important. However, comparing the object of study with substances, materials, or agents for clinical use, the results allow the research group and the reader of the article, who is looking for information, to make direct comparisons and answer a likely pre-existing question: "Could it be better? Should I adapt analyzes in my study? Do comparison groups make sense?" This justifies why studies that did not provide clear clinical applicability, through comparisons between groups, were not included. How is it possible to identify the magnitude of antimicrobial activity if it was not possible to compare it with, for example, sodium hypochlorite or chlorhexidine if the objective of the study is application as an irrigant?

**4.1.1 The antimicrobial activity of graphene**

Antimicrobial activity can be verified using different methods, quantitative or qualitative, and often one type of test complements the other. The choice of test will depend on the experimental design, the experimental groups, and the variable to be analyzed.

In photodynamic therapy (PDT), the light source appears to play a role in antimicrobial activity. When adding nano-graphene oxide to indocyanine green in combination with a laser, it was possible to observe a significant reduction in the colony-forming unit count compared to the untreated group, in addition, the crystal violet test showed a similar reduction.(18) Reduced graphene oxide was also evaluated together with curcumin, identifying its minimum biofilm inhibitory concentration of 250 µg/mL in an MTT reduction assay. Furthermore, when combined with LED, the minimum biofilm inhibitory concentration was reduced, which was confirmed by scanning electron microscopy.(19)

Double antibiotic paste (DAP), an intracanal medication prepared with metronidazole and ciprofloxacin, showed an improvement in antimicrobial activity when associated with graphene oxide and nano-graphene oxide. The association allowed a reduction in the minimum inhibitory concentrations and the colony-forming unit count. The results were superior when compared with DAP alone. However, although graphene oxide and nano-graphene oxide alone did not present the best results, a concentration-dependent effect was observed (20,21).

Modifying well-established materials can be an interesting strategy. Gutta-percha, a filling material, had its characteristics compared to different polymer formulas containing a single concentration of reduced graphene oxide (1%). Of the properties evaluated, the antimicrobial property was superior to gutta-percha depending on the concentrations of the polymers (22). In another study, the mineral trioxide aggregate (MTA) was modified with graphene oxide at concentrations of 1%, 3%, and 5%, comparing the modified materials with the MTA itself and Biodentine cement without modification. It was seen that the higher the concentration of graphene oxide, the greater the bacterial inhibition compared to unmodified materials (23).

Some studies using irrigating substances, such as chlorhexidine and sodium hypochlorite (NaOCl), were also carried out. The irrigants and the irrigation method were analyzed using graphene oxide functionalized with silver (Ag-GO) in the irrigation of the main canal and lateral canals of teeth in an ex vivo model. Ag-GO activity varied according to the location and irrigation method, however, the biofilms of the groups treated with Ag-GO were analyzed by confocal laser scanning microscopy, and the biovolume in the three irrigation methods were significantly lower compared to EDTA 17%, chlorhexidine 2% and NaOCl 1% (24). One study used electrical energy in combination with 0.2% NaOCl with and without graphene oxide for 2 minutes and the combination of 0.2% NaOCl, electrical energy, and graphene oxide was superior when compared to them alone or just NaOCl with electrical energy. The antimicrobial activity was confirmed by confocal laser scanning microscopy and transmission electron microscopy, observing a significant reduction in the biovolume of biofilms and disruption of bacterial cells by penetration of graphene oxide (25).

Gene expression can be a strategy to evaluate genes associated with biofilm formation. The RT-qPCR test showed a significant reduction in the expression of *walR*, *ace*, *gel*, *epal,* and *epaOX* in the presence of graphene oxide (GO-PEI-AS*walR*), increasing the antimicrobial activity of chlorhexidine (26). In another study (19), groups treated with photodynamic therapy and curcumin functionalized with reduced graphene oxide (rGO-Cur) caused a significant reduction in the *efa*, *esp*, *gel,* and *fsr* genes.

Studies show us several possibilities for applying graphene and its possible variations. Furthermore, the antimicrobial action observed may vary according to its concentration and the medium in which it is inserted. Although this review only addresses antimicrobial activity, the physicochemical properties of a material or agent in the development phase must be investigated for complete characterization and then infer the possibility of inclusion in clinical reality.

**4.2 Limitations**

Gray literature has not been explored widely. However, articles found manually in reviews found by the search, outside the databases proposed for this study, were added.

Due to heteroscedasticity and the low number of studies, there is difficulty in directly comparing the results.

**5. CONCLUSIONS**

The use of graphene against biofilms has been used in several ways as possible endodontic agents and materials. However, more studies need to provide direct comparisons with agents and materials in frequent clinical use, allowing comparisons closer to a clinical reality to be made, and then, addressing the advantages and disadvantages of a new way of combating microorganisms in endodontics.

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**TABLES:**

**Table 1: Final search strategy for PubMed**

|  |
| --- |
| Pubmed |
| **#1** ((Anti-Infective Agents[MeSH Terms]) OR (Biofilms[MeSH Terms])) OR (Agent\*, Anti-Infective[Title/Abstract] OR Anti Infective Agent\*[Title/Abstract] OR Antiinfective Agent\*[Title/Abstract] OR Agent\*, Antiinfective[Title/Abstract] OR Anti-Infective Agent\*[Title/Abstract] OR Microbicide\*[Title/Abstract] OR Anti-Microbial Agent\*[Title/Abstract] OR Agent\*, Anti-Microbial[Title/Abstract] OR Anti Microbial Agent\*[Title/Abstract] OR Antimicrobial Agent\*[Title/Abstract] OR Agent\*, Antimicrobial[Title/Abstract] OR Anti Microbial Agents[Title/Abstract] OR Microbicide[Title/Abstract] OR Antimicrobial Agent[Title/Abstract] OR Agent, Antimicrobial[Title/Abstract] OR Biofilm\*[Title/Abstract] OR bio-film\*[Title/Abstract] OR biofilm\* growth[Title/Abstract] OR biofilm\* prevention[Title/Abstract] OR anti bacterial agent\*[Title/Abstract] OR anti-bacterial agent\*[Title/Abstract] OR antibacterial\*[Title/Abstract] OR antibacterial agent\*[Title/Abstract] OR antibacterial drug\*[Title/Abstract] OR antibacterial spectrum[Title/Abstract] OR antimicrobial compound\*[Title/Abstract] OR antimicrobial factor\*[Title/Abstract] OR antiseptic\*[Title/Abstract] OR antiseptic agent\*[Title/Abstract] OR Antimicrobial Activit\*[Title/Abstract])  **#2** ((Silver graphene[Supplementary Concept]) OR (graphene oxide[Supplementary Concept])) OR (Graphene[Title/Abstract] OR silver graphene[Title/Abstract] OR graphene oxide[Title/Abstract] OR nanographene[Title/Abstract] OR nano-graphene[Title/Abstract])  **#3** ((((((((Root Canal Irrigants[MeSH Terms]) OR (Dental Cements[MeSH Terms])) OR (Root Canal Filling Materials[MeSH Terms])) OR (Root Canal Therapy[MeSH Terms])) OR (Dental Pulp Cavity[MeSH Terms])) OR (Endodontics[MeSH Terms])) OR (Dentin[MeSH Terms])) OR (canals sealer[Supplementary Concept])) OR (Cement\*, Dental[Title/Abstract] OR Dental Cement\*[Title/Abstract] OR Sealer\*[Title/Abstract] OR root canal sealer\*[Title/Abstract] OR canals sealer[Title/Abstract] OR canal\* sealer\*[Title/Abstract] OR root canal\* repair\*[Title/Abstract] OR canal\* repair\*[Title/Abstract] OR tooth cement\*[Title/Abstract] OR Root Canal Filling Material\*[Title/Abstract] OR Root Canal Sealant\*[Title/Abstract] OR Sealant\*, Root Canal[Title/Abstract] OR Canal Sealant\*, Root[Title/Abstract] OR root filling material\*[Title/Abstract] OR tooth root canal sealing agent\*[Title/Abstract] OR Canal Irrigant, Root[Title/Abstract] OR Canal Irrigants, Root[Title/Abstract] OR Irrigant, Root Canal[Title/Abstract] OR Irrigants, Root Canal[Title/Abstract] OR Root Canal Irrigant[Title/Abstract] OR Root Canal Medicament[Title/Abstract] OR Root Canal Medicaments[Title/Abstract] OR Canal Medicament, Root[Title/Abstract] OR Canal Medicaments, Root[Title/Abstract] OR Medicament, Root Canal[Title/Abstract] OR Medicaments, Root Canal[Title/Abstract] OR Canal Therap\*, Root[Title/Abstract] OR Root Canal Therap\*[Title/Abstract] OR Therap\*, Root Canal[Title/Abstract] OR Cavit\*, Dental Pulp[Title/Abstract] OR Pulp Cavit\*, Dental[Title/Abstract] OR Dental Pulp Cavit\*[Title/Abstract] OR Pulp Chamber\*[Title/Abstract] OR Chamber\*, Pulp[Title/Abstract] OR Pulp Canal\*[Title/Abstract] OR Canal\*, Pulp[Title/Abstract] OR Root Canal\*[Title/Abstract] OR Canal\*, Root[Title/Abstract] OR Endodontology[Title/Abstract] OR Endodontics[Title/Abstract] OR Dentin\*[Title/Abstract])  **#4** ((#1) AND (#2)) AND (#3) |

**Table 2.** Characteristics of the sources of evidence

|  |  |  |
| --- | --- | --- |
| **First Author,**  **Year** | **Study Design** | **Objectives** |
| Akbari,  2017 | In vitro | Incorporation of indocyanine green (ICG) into nano-graphene oxide (NGO) to produce a new photosensitizer and assess the antimicrobial effects against E. faecalis after photodynamic therapy. |
| Eskandari,  2023a | In vitro | Comparison of the antibacterial activity of double antibiotic paste and graphene oxide, both individually and in combination, against E. faecalis. |
| Eskandari,  2023b | In vitro | Investigation of the antibacterial and antifungal efficacy of nano-graphene oxide, double antibiotic paste, both individually and in combination against microorganisms. |
| Ghorbanzadeh,  2020 | Ex vivo | Investigation of the anti-biofilm and anti-virulence activities of curcumin-functionalized reduced graphene oxide, following irradiation with a light-emitting diode (LED), as a new disinfection method against an ex-vivo biofilm model of *E. faecalis*. |
| Ioannidis,  2019 | Ex vivo | Examination of the antimicrobial efficacy of silver nanoparticles synthesized on an aqueous graphene oxide matrix, using different irrigant delivery methods to enhance the disinfection regimen, in a novel ex vivo infected tooth model. |
| Lee,  2023 | In vitro | Investigation of the effects of electrical energy and its synergistic activity with graphene oxide on *E. faecalis* biofilms. |
| Singh,  2021 | In vitro | Development of a new polymer composite formulated with methacrylic acid and ethylene glycol dimethacrylate, embedded with graphene nanoplatelets, and comparison of its mechanical properties and antimicrobial activity with gutta-percha cones. |
| Somaie,  2023 | In vitro | Evaluation of the setting time, compressive strength, pH, calcium ion release, and antibacterial activity of mineral trioxide aggregate (MTA) modified with three different concentrations of nano-graphene oxide powder, compared to unmodified biodentine as a commercial control. |
| Wu,  2020 | In vitro | Investigation of the antimicrobial effects of a novel graphene oxide-polyethylenimine-based antisense *walR* (AS*walR*) on the inhibition of *E. faecalis* biofilm and its susceptibility to chlorhexidine. |

**Table 3.** Relevant data from studies

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **First Author,**  **Year** | **Form of Graphene** | **Bacteria Strains** | **Treatments Groups\*** | **Assessment of Antimicrobial Effect** |
| Akbari,  2017 | Nano-GO | **A.** *Enterococcus faecalis*  (ATCC 29212) | **A.** Indocyanine Green (1000 μg/mL)  **B.** Indocyanine Green + Laser (1000 μg/mL; 31.2 J/cm2)  **C.** Nano-Graphene Oxide- Indocyanine Green loaded (200 μg/mL)  **D.** Nano-Graphene Oxide- Indocyanine Green loaded + Laser (200 μg/mL; 31.2 J/cm2) | **A.** Colony count assessment (broth micro-dilution method)  **B.** Crystal violet assay |
| Eskandari,  2023a | GO | **A.** *Enterococcus faecalis* | **A.** Double Antibiotic Paste (500 mg of  metronidazole and 500 mg of ciprofloxacin)  **B.** GO  **C.** Graphene Oxide – Double Antibiotic Paste  **D.** Saline | **A.** Colony-forming unit (CFU) count |
| Eskandari,  2023b | Nano-GO | **A.** *E. coli* (ATCC 11.700)  **B.** *Salmonella typhi* (ATCC 13.311)  **C.** *Enterococcus faecalis* (ATCC 25.922)  **D.** *Staphylococcus aureus* (ATCC 25.923)  **E.** *Candida albicans* (ATCC 10.231) | **A.** Double Antibiotic Paste (500 mg of  metronidazole and 500 mg of ciprofloxacin)  **B.** Nano-GO  **C.** Nano-GO – Double Antibiotic Paste | **A.** Minimum inhibitory concentration (MIC) |
| Ghorbanzadeh,  2020 | rGO | **A.** *Enterococcus faecalis*  (ATCC 29212) | **A:** rGO-Curcumin (200, 100, 50, 25, 12.5, 6.25, 3.12, 1.5, 0.8, 0.4, 0.2 μg/mL)  **B:** LED (360 J/cm²)  **C:** rGO-Curcumin-Photodynamic Inactivation (200, 100, 50, 25, 12.5, 6.25, 3.12, 1.5, 0.8, 0.4, 0.2 μg/mL) and 360 J/cm² (energy density)  **D:** NaOCl (2.5%) | **A.** MBIC in an MTT-based assay  **B.** Gene expression analysis  **C.** Scanning electron microscopy (SEM)  **D.** Measurement of intracellular ROS |
| Ioannidis,  2019 | GO | **A.** *Propionibacerium acnes*,  **B.**  *Actinomyces radicidentis*,  **C.**  *Staphylococcus epidermidis*,  **D.**  *Streptococcus mitis*,  **E.** *Enterococcus faecalis*  (OMGS 3202) | **A:** NaCl (0,9%)  **B:** EDTA (17%)  **C:** NaOCl (1%)  **D:** NaOCl (2,5%)  **E:** Chlorhexidine (2%)  **F:** Ag-GO (2,5 mg/mL) | **A.** Colony-forming unit (CFU) count  **B.** Confocal laser scanning microscopy (CLSM) |
| Lee,  2023 | GO | **A*.*** *Enterococcus faecalis* OG1RF  (ATCC 47077) | **A:** NaOCl (0.1, 0.2 and 0.5%)  **B:** NaOCl: (0.2%) and 20 V electric energy  **C:** NaOCl (0.2%), GO (100 μg/mL) and 20 V electric energy | **A****.** Colony-forming unit (CFU) count  **B.** Crystal violet assay  **C.** Confocal laser scanning microscopy (CLSM)  **D.** Scanning electron microscopy (SEM)  **E.** Transmission electron microscopy (TEM) |
| Singh,  2021 | rGO | **A*.*** *Escherichia coli*  **B.** *Staphylococcus aureus* | **A.** A series of GNPs were prepared with different concentrations of monomers, methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), and rGO.  **B.** Commercial gutta-percha | **A.** Zone of inhibition  **B.** Cell growth of bactéria (Turbidity) |
| Somaie,  2023 | Nano-GO | **A.** *Streptococcus mutans* | **A.** Rootdent MTA  **B.** Rootdent MTA + GO powder (1%, 3% and 5%)  **C.** Biodentine (Unmodified) | **A.** Colony-forming unit (CFU) count |
| Wu,  2020 | Nano-GO | **A.** *Enterococcus faecalis* V583 | **A.** Chlorhexidine (2%)  **B.** AS*walR* + Chlorhexidine (2%)  **C.** GO- Polyethylenimine-AS*walR* + Chlorhexidine (2%) | **A.** Colony-forming unit (CFU) Count  **B.** Crystal violet assay  **C.** Confocal laser scanning microscopy (CLSM) |

**\*** Groups that had no treatments or were just bacterial suspension were not considered for the table

**Abbreviations for Graphene forms:** GO: graphene oxide; rGO: reduced graphene oxide; Nano-GO: nano-graphene oxide.

**Table 4.** Main results

|  |  |
| --- | --- |
| **First Author,**  **Year** | **Results** |
| Akbari,  2017 | **A.** The NGO-ICG-PDT group significantly reduced *E. faecalis* cell viability and bacterial count compared to the untreated control group in bacterial count. There was no significant reduction in the bacterial population in NGO-ICG and ICG alone.  **B.** NGO-ICG-PDT showed a significant reduction in biofilm formation capacity compared to the untreated control group.  **C.** The anti-biofilm potential of NGO-ICG-PDT (ICG 200 μg/mL) was 1.3x greater than that of ICG-PDT (ICG 1000 μg/mL) |
| Eskandari,  2023a | **A.** At all times, GO-DAP was superior against *E. faecalis* than GO.  **B.** There was a significant reduction in CFU counts in the GO and GO-DAP groups after 7 and 14 days.  **C.** Statistically significant reduction of CFU count after 1 day was only seen in the GO-DAP group. |
| Eskandari,  2023b | **A.** All treatments significantly increased the killing percentage at all concentrations compared with the control group.  **B.** Intergroup comparisons showed that functionalization of nGO by DAP significantly elevated its antimicrobial efficacy compared to nGO and DAP.  **C.** The antimicrobial effect of nGO-DAP was higher than nGO and DAP in some concentrations, with the difference being greater at lower concentrations. |
| Ghorbanzadeh, 2020 | **A.** rGO-Cur caused a reduction in cellular viability of *E. faecalis* in biofilms in a dose-dependent manner.  **B.** MBIC value of rGO-Cur-PDI was much reduced compared to the individual MBIC values of rGO-Cur and LED for *E. faecalis* biofilms.  **C.** rGO-Cur tested at 1 × MBIC had antibiofilm activity but did not completely eradicate the biofilms. rGO-Cur at 1/2 × MBIC combined with the LED irradiation time left no intact biofilm structures visible in several fields.  **D.** Under rGO-Cur-PDI and rGO-Cur treatment conditions, the expression of *esp*, *gel*, *efa*, and *fsr* were significantly lower than basal.  **E.** The intracellular ROS assay indicated a significant increase in rGO-Cur-PDI compared to the control. |
| Ioannidis,  2019 | **A.** Entire canal: Ag-GO was significantly higher than sterile saline and EDTA 17%. The application of UAI or XPEF did not significantly affect the number of detectable viable counts compared to CI.  **B.** Middle lateral canal: Ag-GO (UAI), UAI enhanced microbial killing efficacy compared to the rest of the experimental groups. The application of Ag-GO with XPEF showed no statistically significant difference compared to the sterile saline and positive control groups.  **C.** Apical lateral canal: The application of UAI significantly improved the microbial killing efficacy of Ag-GO compared to CI and XPEF.  **D.** All treatment groups presented significantly less total biovolume compared to the positive control group (no treatment). Ag-GO presented a significant reduction of total biovolumes compared to 17% EDTA, 2% CHX, and 1% NaOCl, regardless of the irrigation/agitation method. The application of UAI enhanced its biofilm disruption capacity, which was statistically significant compared to CI and XPEF. |
| Lee,  2023 | **A.** Graphene Oxide 100 μg/mL in combination with 0.2% NaOCl and electric energy (0.2- E-GO), the results were similar to those of 0.5% NaOCl treated group. Biofilm biomass of *E. faecalis* decreased in a dose-dependent manner, and the 0.2-E-GO group showed a similar effect to the 0.5% NaOCl-treated group.  **B.** When electric energy and GO were applied with 0.2% NaOCl (0.2-E-GO), the biovolumes of live and dead bacteria were similar to those of the 0.5% NaOCl treated group. The 0.2-E and 0.2-E-GO groups had less amount of bacteria than the 0.2% NaOCl only treated group. The 0.2-E-GO group had a similar amount of bacteria compared to the 0.5% NaOCl treated group.  **C.** *E. faecalis* which had no electrical energy treatment showed intact cell walls and membranes, while the bacteria treated with electric energy showed partially damaged cell walls and membranes. GO showed additional distinctive damage. |
| Singh,  2021 | **A.** The inhibition zone for gutta-percha was 70% lower than the GNP-2. The inhibition zone representing the GNP-6 was larger than that of GNP-2 and GNP-4, where the rGO loading had a significant impact on antimicrobial activity.  **B.** The bacterial growth is faster in control as compared to the polymers. |
| Somaie,  2023 | **A.** The increase of GO to MTA resulted in a significant decrease in bacterial count for the different concentrations used compared to MTA control. |
| Wu,  2020 | **A.** RT-qPCR test showed that the expression of *walR*, *ace*, *gel*, *epal,* and *epaOX* genes significantly decreased, and were lower in GO-PEI-AS*walR* *E. faecalis* biofilms.  **B.** GO-PEI-AS*walR* enhanced the antibacterial activity of CHX to *E. faecalis* biofilm, with the lowest percentage of live bacteria.  **C.** After being treated with 2% CHX, the GO-PEI-AS*walR* strain exhibited an EPS-bacterial biomass volume significantly lower than other groups. |

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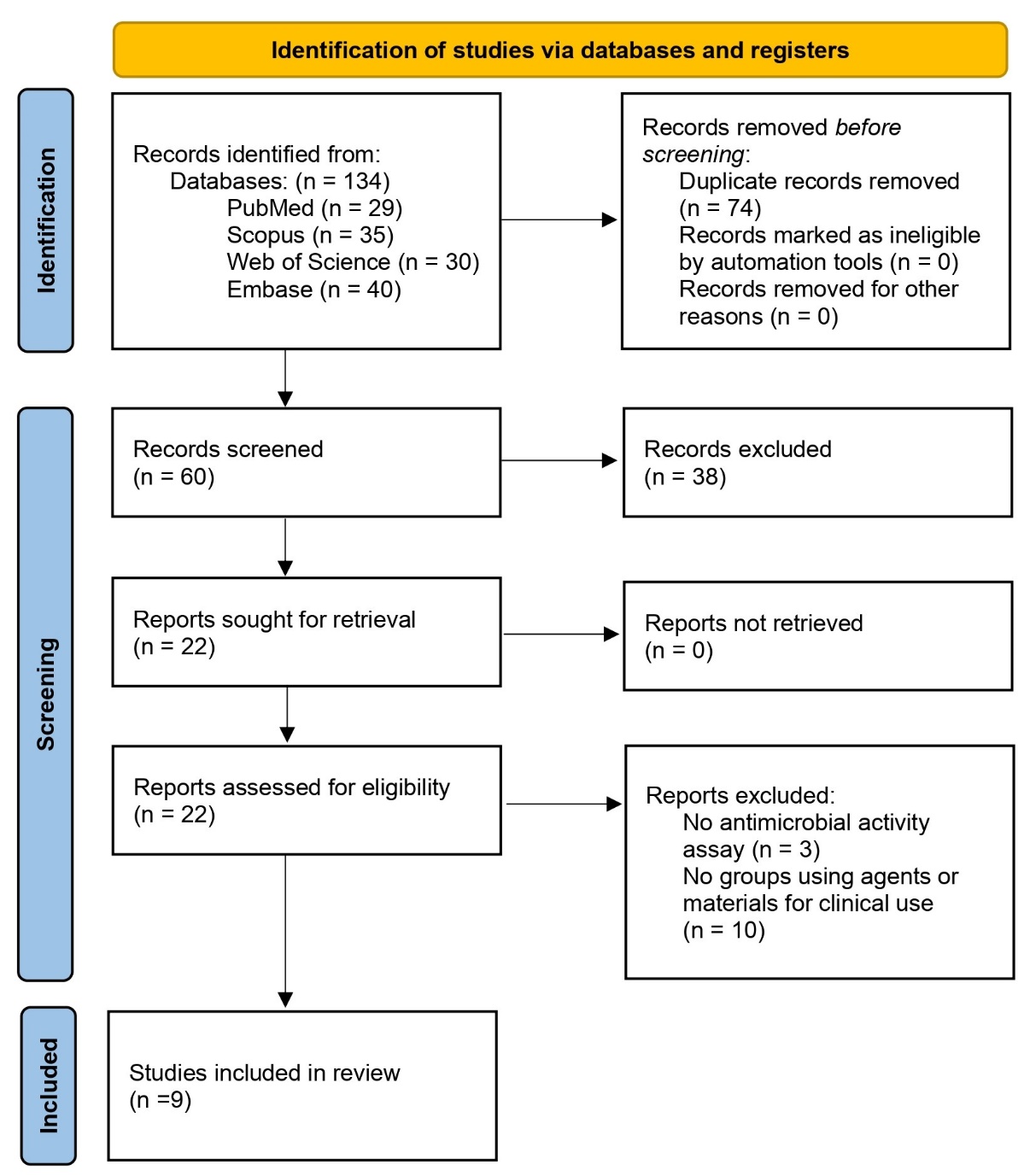


Fig 1- Identification of studies via databases and registers.