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

**ABSTRACT**

**Background:** Graphene, with multifunctional potential in dentistry, is being explored in a variety of fields, including endodontics, due to its antibacterial properties. However, it is not yet a widely accepted reality in endodontics. As a result, a scoping review was undertaken to throw light on studies in the literature that combine graphene and its antimicrobial impact in various ways in endodontic therapy, with the goal of identifying the major developments and uses of graphene, as well as its potential applications in endodontics. **Methods:** A scoping review was done to identify graphene's primary achievements and usage, as well as potential endodontic applications. Our protocol was created 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 regard to publication year or language: PubMed, Scopus, Embase, and Web of Science. As inclusion requirements, the study must use a qualitative or quantitative antimicrobial test model to evaluate the substance, and at least one group must be tested with an endodontic agent or material for clinical use. **Results:** The evaluation comprised nine research that used graphene oxide, reduced graphene oxide, and nano-graphene oxide in in vitro and ex vivo experiments. Graphene has been employed in biofilm investigations as an irrigant, filler, intracanal medicament, and photosensitizer. **Conclusion:** Although studies show positive results with graphene, standardization of research, manner of usage, and concentration is required for direct comparison of studies and a prospective 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 prompted the appearance of novel phenomena, opening possibilities in physics, chemistry, and biomedicine. The graphene family of nanomaterials includes many other forms of this material, including graphene oxide2–4 and reduced graphene oxide. 5

 Based on breakthroughs in the application of graphene in numerous sectors, the material has showed multifunctional potential in dentistry, and is being researched in tissue engineering,6 orthodontics,7 oral surgery,8 restorative dentistry,9 endodontics,10 caries management,11 and periodontology.12

 One of the characteristics of graphene is its antibacterial impact, which has been extensively studied,13–15 providing new possibilities in dentistry, particularly in endodontics, where intracanal antimicrobial control is critical for endodontic therapy success. However, graphene is not yet a widely accepted fact in endodontics. As a result, a scoping review was undertaken to throw light on studies in the literature that combine graphene and its antimicrobial impact in various ways in endodontic therapy, with the goal of identifying the major developments and uses of graphene, as well as its potential applications in endodontics.

**1.2 Objectives**

A scoping review was conducted to systematically map existing research in the literature employing graphene in its many forms for antimicrobial purposes in potential materials and agents for endodontic usage, as well as to identify 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. There were no restrictions on paper publication dates, languages, or study models (i*n vivo*, *ex vivo*, or *in vitro*). Exclusion criteria were established, including failure to describe the type 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 comprises information on the papers (authors and publication dates), aims, and study design. The study summarized the graphene shape, bacteria strains, antimicrobial efficacy assessment, treatment groups, and findings.

**3. RESULTS**

**3.1 Selection of sources of evidence**

The number of evidence sources selected, assessed for eligibility, and included in the review17 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**

 In this review, we focused on research that attempted to clinically use the produced or modified material or agent and used group comparisons with traditional materials or agents in clinical dentistry practice. Graphene was found in numerous forms in the research evaluated, such as a potential photodynamic treatment agent, 18,19 intracanal medication,20,21 filling material, 22,23 irrigants or adjuvants.24–26 The possibilities for employing these materials or agents were determined by the study's purpose, as previously demonstrated, the method by which it was applied, and the research groups.

Antimicrobial activity can be determined using a variety of approaches, both quantitative and qualitative, and typically one sort of test complements the other. The test used will be determined by the experimental design, experimental groups, and variable to be evaluated.

In photodynamic treatment (PDT), the light source appears to influence antibacterial activity. When nano-graphene oxide was combined with indocyanine green and a laser, the colony-forming unit count was significantly reduced compared to the untreated group; the crystal violet test also revealed a similar reduction.18 The combination of reduced graphene oxide and curcumin resulted in a minimum biofilm inhibitory concentration of 250 µg/mL in an MTT reduction experiment. Furthermore, when paired with LED, the minimum biofilm inhibitory concentration was lowered, as evidenced by scanning electron microscopy.19

Double antibiotic paste (DAP), an intracanal medicine containing metronidazole and ciprofloxacin, demonstrated increased antibacterial activity when combined with graphene oxide and nano-graphene oxide. The connection allowed for a decrease in the minimum inhibitory concentrations and colony-forming unit count. The results were superior when compared to DAP alone. Although graphene oxide and nano-graphene oxide did not produce the greatest results, a concentration-dependent effect was discovered.20,21

Modifying well-known materials might be an intriguing method. Gutta-percha, a filler material, was compared against several polymer formulae including 1% reduced graphene oxide. Depending on the polymer concentration, the antibacterial property outperformed gutta-percha.22 In another study, the mineral trioxide aggregate (MTA) was treated with graphene oxide at 1%, 3%, and 5% concentrations, and the modified materials were compared to the MTA and Biodentine cement that had not been modified. It was discovered that the higher the concentration of graphene oxide, the greater the bacterial inhibition over unmodified materials.23

 There were also further studies that used irrigating compounds like chlorhexidine and sodium hypochlorite (NaOCl). In an ex vivo model, the irrigants and irrigation method were investigated utilizing graphene oxide functionalized with silver (Ag-GO) to irrigate the main and lateral canals of teeth. Ag-GO activity varied depending on location and irrigation method; however, the biofilms of the groups treated with Ag-GO were analyzed using confocal laser scanning microscopy, and the biovolume in the three irrigation methods was significantly lower than 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 found that the combination of 0.2% NaOCl, electrical energy, and graphene oxide outperformed them alone or simply NaOCl with electrical energy. Confocal laser scanning microscopy and transmission electron microscopy were used to demonstrate antibacterial activity, which revealed a considerable reduction in the biovolume of biofilms as well as destruction of bacterial cells via graphene oxide penetration.25

 Gene expression can be used to assess genes linked with biofilm development. The RT-qPCR test revealed a significant reduction in the expression of *walR*, *ace*, *gel*, *epal*, and *epaOX* in the presence of graphene oxide (GO-PEI-AS*walR*), which increased chlorhexidine's antibacterial activity.26 In another study,19 groups were treated with photodynamic treatment and curcumin functionalized with reduced graphene oxide (rGO-Cur), resulting in a significant reduction in the *efa*, *esp*, *gel*, and *fsr* genes.

 Studies demonstrate that graphene has a variety of applications and variants. Furthermore, the antibacterial activity found may vary depending on the concentration and medium in which it is put. Although this analysis focuses solely on antibacterial activity, the physicochemical properties of a material or agent in the development phase must be explored in order to fully characterize it and determine its suitability for clinical use.

**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**

Graphene has been employed against biofilms in a variety of applications as potential endodontic agents and materials. However, more research is needed to provide direct comparisons with agents and materials in common clinical usage, allowing comparisons to be made closer to clinical reality, and then addressing the benefits and drawbacks of a novel method of combating microorganisms in endodontics.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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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 ofmetronidazole 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 ofmetronidazole 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.