

Antibacterial Effects Of Graphene In Endodontics: A Systematic Review

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ABSTRACT

This systematic review critically evaluates the antibacterial properties of graphene-based materials in endodontics, emphasizing their potential to improve root canal disinfection. Graphene, a two-dimensional carbon allotrope, is noted for its exceptional surface area, electrical conductivity, and mechanical strength, making it a promising candidate for antibacterial applications. The review explores the use of graphene, particularly graphene oxide (GO), in various endodontic contexts, including root canal sealers, irrigation solutions, and intracanal medicaments. The antibacterial mechanisms of graphene involve physical disruption of bacterial membranes, induction of oxidative stress, and inhibition of biofilm formation. A comprehensive literature search was conducted across multiple electronic databases, including COCHRANE LIBRARY, EMBASE, and MEDLINE, covering publications from 2003 to 2023. The search identified 2570 articles, which were screened for relevance. After removing duplicates and non-eligible studies, 10 articles met the inclusion criteria and were included in the review. The selection process focused on experimental studies that directly examined the antibacterial effects of graphene in endodontics, while excluding review articles, commentaries, and studies with insufficient methodological detail. The review highlights the enhanced antibacterial efficacy of graphene-based materials, particularly when combined with other antimicrobial agents like TiO₂ and silver nanoparticles. It also points out the versatility of graphene in various forms and applications within endodontics. However, the review notes the need for more standardized methodologies, in vivo studies, and clinical trials to fully validate the safety and effectiveness of graphene in endodontic practice. The findings suggest that graphene holds significant promise for improving endodontic treatment outcomes.

1. INTRODUCTION

Researchers are investigating new materials with improved qualities as a result of the unrelenting pursue for efficient antibacterial agents in endodontics. Among these materials, graphene, a two-dimensional carbon allotrope, has garnered considerable attention due to its unique physicochemical characteristics. This systematic review endeavors to comprehensively assess and synthesize the existing literature on the antibacterial properties of graphene in the realm of endodontics.

Microbial infections continue to pose a substantial challenge in endodontic procedures, necessitating the exploration of innovative solutions. Graphene, with its exceptional surface area, electrical conductivity, and mechanical strength, presents itself as a potential candidate for disrupting bacterial growth and biofilm formation within the root canal environment. However, the current knowledge base is dispersed, with variations in experimental methodologies and reported outcomes.

The primary objective of this systematic review is to critically analyze and consolidate the diverse body of evidence pertaining to the antibacterial effects of graphene in endodontics. Through a rigorous assessment of study designs, methodologies, and outcomes, we aim to provide a nuanced understanding of the current state of research in this domain.

Furthermore, this review will address gaps in existing knowledge and propose directions for future research endeavors. The final objective is to provide insightful information that guide researchers and clinicians towards evidence-based strategies for leveraging graphene's antibacterial potential in endodontic practice, with a broader aim of advancing the field and improving patient outcomes.

2. BACKGROUND

One persistent challenge in the field of endodontics is the management of microbial infections within the intricate root canal system. Despite advancements in antimicrobial agents, achieving optimal disinfection remains elusive, necessitating the exploration of alternative materials with potent antibacterial properties.

Graphene, a single layer of carbon atoms arranged in a two-dimensional lattice, has captivated researchers across diverse scientific disciplines due to its exceptional physical and chemical attributes. Graphene, a single layer of carbon atoms arranged in a two-dimensional honeycomb lattice, has garnered significant attention for its potential applications in various fields, including biomedicine and dentistry ^[1]. Its high surface area, excellent electrical conductivity, and remarkable mechanical strength make graphene an intriguing candidate for applications in dentistry.

In endodontics, where bacterial infection is a primary concern, the antimicrobial properties of graphene present an intriguing avenue for exploration ^[2]. Previous studies have demonstrated the ability of graphene-based materials to exhibit potent antibacterial activity against a wide range of microorganisms, including those commonly found in endodontic infections ^[3,4]. However, despite the promising findings in other biomedical applications, the specific antibacterial efficacy of graphene in endodontics remains to be fully elucidated ^[5].

These observations prompt a systematic exploration and synthesis of the existing literature to comprehensively evaluate the viability of graphene as an antibacterial agent in endodontic applications.

This study seeks to build upon the current understanding of graphene's antibacterial properties by conducting a systematic review of the available literature. Through a rigorous analysis of experimental methodologies, reported outcomes, and potential limitations.

3. OBJECTIVES

The purpose of this study was to evaluate the antibacterial property of graphene in endodontics.

Methodology

Study inclusion/exclusion criteria

Randomized controlled trials (RCTs) on the subject were included in this review. Studies wherein the antibacterial property of graphene were checked and included.

Types of outcome measures

Antibacterial property were assessed through

1. Bacterial Viability
2. Inhibition of bacterial growth
3. Cell membrane integrity
4. Antimicrobial spectrum
5. Minimum inhibitory concentration

Literature search and screening strategy

The search strategy was developed using medical subject headings (MeSH terms) as well as free text terms.

All publication years up to 2003 to 2023 were included.

An initial electronic search of six databases including COCHRANE LIBRARY EMBASE, EBSCOHOST, GOOGLE SCHOLAR, MEDLINE using the search terms (alone or in combination) *graphene*, *antibacterial*, *endodontics* were carried out. All relevant titles, abstracts were identified and retrieved by the author. Potentially relevant reports identified from the reference lists of relevant studies, review articles and chapters were hand searched. The University websites were accessed to obtain relevant unpublished dissertations.

As the primary step 2570 articles were identified during a literature search from different sources. At the second level, since multiple databases were searched, a total of 1052 abstracts were identified as duplicates and were excluded which brought down the number of articles screened to 1518. Further, 952 abstracts were excluded as they were clinical case reports or literature reviews which left 566 articles. A total of 85 records were further screened. In the next level, 75 articles were excluded to obtain 10 full-text articles.

At this level, a total of ten records were considered potentially eligible and sought for further assessment and full-length articles were retrieved. Eventually, ten records were selected to be included in this review and sought for qualitative synthesis in this systematic review.

4. REVIEW OF LITERATURE

1. Liu et al. (2011): Antibacterial activity of graphite, graphite oxide, graphene oxide, and reduced graphene oxide: membrane and oxidative stress. *ACS Nano*, 5(9), 6971-80.^[6]

Liu and colleagues conducted a seminal study to explore the antibacterial activity of various graphene-based materials, including graphite, graphite oxide, graphene oxide, and reduced graphene oxide. Their research delves into the mechanisms underlying the antibacterial effects, specifically focusing on membrane disruption and oxidative stress. The study provides foundational insights into the potential applications of graphene derivatives as antibacterial agents, with implications for addressing microbial challenges in diverse contexts.

2. Correa et al. (2017): Antimicrobial activity from polymeric composites-based polydimethylsiloxane/TiO₂/GO: evaluation of filler synthesis and surface morphology. *Polymer Bulletin*, 74, 2379-90.^[7]

Correa and co-authors investigated the antimicrobial activity of polymeric composites based on polydimethylsiloxane/TiO₂/GO. Their work explores the synthesis of fillers and their impact on surface morphology, shedding light on the potential of these composite materials for antimicrobial applications. This research contributes to the understanding of polymeric composites as effective agents against microbial proliferation.

3. Martini et al. (2020): Antimicrobial and antibiofilm properties of graphene oxide on *Enterococcus faecalis*. *Antibiotics*, 9(10), 692.^[8]

Martini and colleagues focused on elucidating the antimicrobial and antibiofilm properties of graphene oxide specifically against *Enterococcus faecalis*. The study explores the potential of graphene oxide as an effective agent against this clinically relevant pathogen, contributing valuable insights to the field of antibacterial research.

4. Perreault et al. (2015): Antimicrobial properties of graphene oxide nanosheets: why size matters. *ACS Nano*, 9(7), 7226-36.^[9]

Perreault and team delved into the antimicrobial properties of graphene oxide nanosheets, emphasizing the importance of size in determining efficacy. Their findings underscore the nuanced relationship between graphene oxide size and antimicrobial effects, providing crucial considerations for the design of graphene-based antimicrobial materials.

5. Nasim et al. (2022): Antioxidant and anti-inflammatory activity of a nanoparticle-based intracanal drug. *Bioinformation*, 18(5), 450.^[10]

Nasim and colleagues explored the antioxidant and anti-inflammatory activities of a nanoparticle-based intracanal drug. This study expands the scope of antibacterial research by considering the broader immunomodulatory and anti-inflammatory effects of nanoparticle-based interventions in endodontic applications.

6. Olczak et al. (2023): Bactericidal Activity of Graphene Oxide Tests for Selected Microorganisms. *Materials*, 16(11), 4199.^[11]

Olczak and collaborators investigated the bactericidal activity of graphene oxide against selected microorganisms. Their work provides insights into the specificity of graphene oxide's antibacterial effects, contributing to a better understanding of its potential applications in microbial control.

7. Mousavi et al. (2021): Bioinorganic synthesis of polyrhodanine stabilized Fe₃O₄/Graphene oxide in microbial supernatant media for anticancer and antibacterial applications. *Bioinorganic Chemistry and Applications*.^[12]

Mousavi and co-authors explored the bioinorganic synthesis of polyrhodanine stabilized Fe₃O₄/Graphene oxide for both anticancer and antibacterial applications. This study offers a multifaceted perspective on the potential of graphene-based materials, highlighting their versatility in addressing both cancer and bacterial challenges.

8. Gholibegloo et al. (2018): Carnosine-graphene oxide conjugates decorated with hydroxyapatite as promising nanocarrier for ICG loading with enhanced antibacterial effects in photodynamic therapy against *Streptococcus mutans*. *Journal of Photochemistry and Photobiology B: Biology*, 181, 14-22.^[13]

Gholibegloo and collaborators investigated the potential of carnosine-graphene oxide conjugates decorated with hydroxyapatite as nanocarriers for indocyanine green (ICG) in photodynamic therapy. Their work explores the synergistic antibacterial effects of this nanocomposite, offering a promising avenue for enhanced bacterial control, particularly against *Streptococcus mutans*.

9. Kim and Min (2023): Combined effect of apigenin and reduced graphene oxide against *Enterococcus faecalis* biofilms. *Journal of Oral Science*.^[14]

Kim and Min explored the combined effect of apigenin and reduced graphene oxide against *Enterococcus faecalis* biofilms. This study investigates a synergistic approach to biofilm management, providing valuable insights into the potential of combined therapies in addressing persistent bacterial infections in endodontics.

5. REVIEW OF LITERATURE

1. Liu et al. (2011): Antibacterial activity of graphite, graphite oxide, graphene oxide, and reduced graphene oxide: membrane and oxidative stress. ACS Nano, 5(9), 6971-80.

Sl. No.	Particulars	Description
1.	Year	2011
2.	Authors	S. Liu, T.H. Zeng, M. Hofmann, E. Burcombe, J. Wei, R. Jiang, J. Kong, and Y. Chen.
3.	Place	Boston,USA
4.	Design	RCT
5.	Duration	4 WEEKS
6.	Study setup	The study involved the preparation and characterization of four types of graphene-based materials: graphite (Gt), graphite oxide (GtO), graphene oxide (GO), and reduced graphene oxide (rGO). Antibacterial activity against Escherichia coli was assessed for these materials.
7.	Intervention	The primary intervention involved exposing Escherichia coli cells to dispersions of graphene-based materials (Gt, GtO, GO, and rGO) in isotonic saline solutions at 37 °C with 250 rpm shaking speed for 2 hours. The study assessed the loss of viability of E. coli cells as a result of this exposure.
8.	Outcome measuring tool	cell viability loss of viability
9.	Type of treatment	exposure of Escherichia coli cells to dispersions of graphene-based materials (Gt, GtO, GO, and rGO) at different concentrations in isotonic saline solutions.
10.	Type of treatment in control group	isotonic saline solution without graphene-based materials.
11.	Funding	National Research Foundation, Singapore (NRF-CRP2-2007-02 and NRF2010-POC001-021) and Center for Excitonics at Massachusetts Institute of Technology, an Energy Frontier Research Center funded by the U.S. Department of Energy,Office of Science,Office of Basic Energy Sciences under Award Number DE-SC0001088.
12.	Conflict of interest	none

2. Correa et al. (2017): Antimicrobial activity from polymeric composites-based polydimethylsiloxane/TiO₂/GO: evaluation of filler synthesis and surface morphology. Polymer Bulletin, 74, 2379-90.

Sl. No.	Particulars	Description
1.	Year	2017
2.	Authors	Camila F. Correa,Luiza R. Santana,Ricardo M. Silva,Bruno S. Noremberg,Rafael G. Lund,Juliana S. Ribeiro,Fabiana V. Motta,Mauricio R. D. Bomio3,Rubens M. Nascimento3

3.	Place	Brazil
4.	Design	RCT
5.	Study setup	Not mentioned
6.	Intervention	The intervention in the study involved the preparation and incorporation of graphene oxide (GO) and TiO ₂ (titanium dioxide) nanoparticles into a polydimethylsiloxane (PDMS) matrix.
7.	Outcome measuring tool	X-Ray Analysis (XRD) Field-Emission Scanning Electron Microscope (FESEM) Brunauer-Emmett-Teller (BET) Surface Area Analysis Atomic Force Microscope (AFM) Analysis Water Contact Angle Measurements Antibacterial Activity Evaluation (Modified Direct Contact Test - mDCT)
8.	Type of preparation	Preparation of PDMS Matrix: PDMS (Sylgard 184, Dow Corning) was prepared in a 10:1 ratio of basic mixture to curing agent. The PDMS mixture was poured onto Petri dishes and left at 24 °C to remove oxygen bubbles. TiO₂ Preparation via Microwave-assisted Hydrothermal Method: Titanium glycolate was dispersed in absolute ethanol, subjected to ultrasound, heated, and then cooled to obtain TiO₂. Preparation of Solutions and Samples: Solutions containing different percentages of GO and TiO₂ were prepared in absolute ethanol. Samples were then prepared by either dripping solutions on the surface of the PDMS film or through manual mixing in uncured PDMS.
9.	Type of treatment in control group	No control group
10.	Funding	No funding
11.	Conflict of interest	none

3. Martini et al. (2020): Antimicrobial and antibiofilm properties of graphene oxide on *Enterococcus faecalis*. Antibiotics, 9(10), 692.

Sl. No.	Particulars	Description
1.	Year	2020
2.	Authors	Cecilia Martini, Francesca Longo, Ra_aellaCastagnola , Luca Marigo Nicola Maria Grande, Massimo Cordaro, Margherita Cacaci, MassimilianoPapi, Valentina Palmieri, Francesca Bugli and Maurizio Sanguinetti
3.	Place	Italy
4.	Design	RCT

5.	Study setup	integrating microbiological assays, material characterization techniques, and analysis of dentin disc
6.	Intervention	The intervention in this study involves the application of Graphene Oxide (GO) to investigate its impact on Enterococcus faecalis and biofilm formation on dentin discs.
7.	Outcome measuring tool	1.Viability Testing 2.Zeta Potential Characterization 3.AFM (Atomic Force Microscopy) 4. Biofilm Formation Assay 5.SEM Analysis (Scanning Electron Microscopy)
8.	Type of preparation	1. Graphene oxide preparation 2. Bacterial growth media preparation 3. Dentin disc sample preparation 4. Biofilm formation assay
9.	Type of treatment in control group	The control group involves dentin discs that do not receive the Graphene Oxide (GO) treatment.
10.	Funding	none
11.	Conflict of interest	none

4. Perreault et al. (2015): Antimicrobial properties of graphene oxide nanosheets: why size matters. ACS Nano, 9(7), 7226-36.

Sl. No.	Particulars	Description
1.	Year	2015
2.	Authors	Franc-oisPerreault, Andreia Fonseca de Faria, SiamakNejati, and Menachem Elimelech
3.	Place	USA
4.	Design	Experimental
5.	Duration	
6.	Study setup	The study setup involves the synthesis and characterization of graphene oxide (GO) and its subsequent application to assess antimicrobial activity.
7.	Intervention	The intervention in this study is the application of graphene oxide (GO). Specifically, GO is synthesized using a modified Hummers' method
8.	Outcome measuring tool	1. CFU 2. Fluorescent staining assays 3. Spectrophotometry 4. Microscopy
9.	Type of preparation	1. Graphene oxide synthesis 2. Graphene oxide coating 3. Graphene oxide in suspension

		4. Glutathione oxidation experiment
10.	Type of treatment in control group	none
11.	Funding	none
12.	Conflict of interest	none

5. Nasim et al. (2022): Antioxidant and anti-inflammatory activity of a nanoparticle-based intracanal drug. Bioinformation, 18(5), 450.

Sl. No.	Particulars	Description
1.	Year	2022
2.	Authors	Iffat Nasim1*, Marimuthu Shamly1, KrishnaKanth Jaju1, Veeraraghavan Vishnupriya2 & Zohra Jabin
3.	Place	India
4.	Design	Experimental
5.	Study setup	The study setup involves the preparation of silver and graphene oxide nanoparticles using plant extracts, followed by the assessment of their antioxidant and anti-inflammatory activities.
6.	Intervention	The intervention involves the preparation of silver nanoparticles and graphene oxide nanoparticles using plant extracts, specifically from Andrographis paniculata and Ocimum sanctum Linn leaves.
7.	Outcome measuring tool	1. UV Vis Spectrophotometer 2. DPPH Free Radical Scavenging Activity 3. Nitric Oxide Radical Scavenging Activity 4. Protein Denaturation Assay 5. Xanthine Oxidase Inhibitory Activity
8.	Type of preparation	silver nanoparticles and graphene oxide nanoparticles using plant extracts
9.	Type of treatment in control group	None
10.	Funding	None
11.	Conflict of interest	None

6. Olczak et al. (2023): Bactericidal Activity of Graphene Oxide Tests for Selected Microorganisms. Materials, 16(11), 4199.

Sl. No.	Particulars	Description
1.	Year	2023
2.	Authors	Katarzyna Olczak 1,* , Witold Jakubowski 2 and Witold Szymański
3.	Place	Poland
4.	Design	Experimental

5.	Study setup	In Vitro
6.	Intervention	The intervention in the study involves exposing bacterial strains to graphene oxide (GO) at different concentrations and time intervals.
7.	Outcome measuring tool	The outcome measuring tool in this study is an Accuri C6 flow cytofluorimeter (BD Biosciences), along with CSampler software. Additionally, a viability/cytotoxicity assay for live and dead bacteria cells (Biotium) was used to analyze the results.
8.	Type of preparation	Bacterial Culture Graphene Oxide (GO) Cytotoxicity Assay Live/Dead Staining
9.	Type of treatment in control group	none
10.	Funding	None
11.	Conflict of interest	None

7. Mousavi et al. (2021): Bioinorganic synthesis of polyrhodanine stabilized Fe₃O₄/Graphene oxide in microbial supernatant media for anticancer and antibacterial applications. Bioinorganic Chemistry and Applications.

Sl. No.	Particulars	Description
1.	Year	2021
2.	Authors	Seyyed Mojtaba Mousavi,¹ Seyyed Alireza Hashemi,² Ahmad Gholami,³Navid Omidifar,⁴ Maryam Zarei,⁵ Sonia Bahrani,⁵ Khadije Yousefi,⁵Wei-Hung Chiang,¹ and Aziz Babapoor⁶
3.	Place	Canada
4.	Design	Experimental
5.	Study setup	The study setup involves the synthesis of Polyrhodanine/GO/Fe ₃ O ₄ and its variations, along with the characterization of the synthetic compounds. Additionally, antimicrobial assays (MIC, MBC, MFC) and in vitro cell toxicity assays on the Hep-G2 cell line were conducted.
6.	Intervention	The main interventions in the study include the synthesis of Polyrhodanine/GO/Fe ₃ O ₄ and its variations, as well as the preparation of Graphene Oxide-Coated Fe ₃ O ₄ Nanoparticles (GO/Fe ₃ O ₄).
7.	Outcome measuring tool	Tensor __ FT-IR Spectroscopy (Bruker, Germany) EDX Spectroscopy and Morphology (MRA __ TESCAN) Magnetization Characterization (MDKB VSM, Mdk, Iran) Antimicrobial Assays (MIC, MBC, MFC) In Vitro Cell Toxicity Assay (MTT Colorimetric Assay)
8.	Type of preparation	- Polyrhodanine (PR) Synthesis - Graphene Oxide-Coated Fe₃O₄ Nanoparticles (GO/Fe₃O₄) Preparation

		3. Polyrhodanine/GO/Fe₃O₄ Preparation 4. Polyrhodanine/GO/Fe₃O₄ Based on Kombucha Solvent (PR/GO/Fe₃O₄/Ko) Preparation 5. Antimicrobial Assay Preparation 6. In Vitro Cell Toxicity Assay Preparation
9.	Type of treatment in control group	None
10.	Funding	None
11.	Conflict of interest	None

8. Gholibegloo et al. (2018): Carnosine-graphene oxide conjugates decorated with hydroxyapatite as promising nanocarrier for ICG loading with enhanced antibacterial effects in photodynamic therapy against *Streptococcus mutans*. *Journal of Photochemistry and Photobiology B: Biology*, 181, 14-22.

Sl. No.	Particulars	Description
1.	Year	2018
2.	Authors	ElhamGholibeglooa,b†, AshkanKarbasib†, Maryam Pourhajibagherc, NasimChiniforushd, Ali Ramazania, TayebbehAkbarie, Abbas Bahadorf*, Mehdi Khoobib,g*
3.	Place	Iran
4.	Design	Experimental
5.	Study setup	The study is set up as a laboratory-based experimental investigation involving the synthesis and characterization of graphene-based nanocomposites. Graphene oxide (GO) is conjugated with carnosine (Car) and combined with hydroxyapatite (HAp), followed by the loading of indocyanine green (ICG) onto these nanocomposites. Various characterization techniques, such as FT-IR, XRD, UV-Visible spectroscopy, and microscopy, are employed to analyze the synthesized nanocomposites.
6.	Intervention	The intervention in this study involves the use of graphene-based nanocomposites for antimicrobial purposes, particularly against <i>S. mutans</i> . The synthesized nanocomposites include graphene oxide (GO) conjugated with carnosine (Car) and combined with hydroxyapatite (HAp). Additionally, indocyanine green (ICG) is loaded onto these nanocomposites. The antimicrobial intervention is implemented through a technique known as antimicrobial photodynamic therapy (aPDT), where the nanocomposites are applied to both planktonic and biofilm forms of <i>S. mutans</i> , and subsequently exposed to diode laser illumination.
7.	Outcome measuring tool	Colony-Forming Units (CFU/mL) Biofilm Formation Assessment Quantitative Real-Time PCR (qRT-PCR) UV-Visible Spectrophotometer
8.	Type of preparation	Graphene Oxide (GO) Nanosheets Graphene Oxide-Carnosine (GO-Car) Conjugation

		Hydroxyapatite (HAp) Synthesis Indocyanine Green (ICG) Loading Aqueous Stability Testing
9.	Type of treatment in control group	none
10.	Funding	None
11.	Conflict of interest	None

9. Kim and Min (2023): Combined effect of apigenin and reduced graphene oxide against *Enterococcus faecalis* biofilms. *Journal of Oral Science*.

Sl. No.	Particulars	Description
1.	Year	2023
2.	Authors	Mi-Ah Kim and Kyung-San Min
3.	Place	Korea
4.	Design	Experimental
5.	Study setup	The study setup involves the formation of <i>Enterococcus faecalis</i> (E. faecalis) biofilms on hydroxyapatite (HA) discs. The bacteria are cultured in brain heart infusion medium overnight and then transferred to a 24-well plate containing HA discs and fresh media. Biofilms are allowed to form over a period of 7 days at 37°C without agitation, with daily media changes.
6.	Intervention	Apigenin Treatment Combined Apigenin and RGO Treatment
7.	Outcome measuring tool	Colony Counting Crystal Violet Staining Confocal Laser Scanning Microscopy (CLSM) Scanning Electron Microscopy (SEM)
8.	Type of preparation	Bacterial Inoculation Biofilm Formation Treatment Preparations
9.	Type of treatment in control group	None
10.	Funding	None
11.	Conflict of interest	None

6. RESULT

The investigation into carnosine-graphene oxide (GO) conjugates revealed promising attributes for dental applications. Fourier-transform infrared (FT-IR) analysis confirmed successful conjugation, marked by a significant decrease in carbonyl groups, indicating the linkage between GO and carnosine (Car). Introduction of hydroxyapatite (HAp) enhanced the stability of nanocarriers, as evident in FT-IR and X-ray diffraction (XRD) analyses. The conjugates displayed a robust capacity for

loading Indocyanine Green (ICG), essential for photodynamic therapy (aPDT). Surprisingly, carnosine not only facilitated conjugation but also exhibited an unexpected enhancement of antibacterial effects when combined with GO. This unforeseen synergy opens avenues for exploring the interplay between graphene-based materials and bioactive compounds, potentially revolutionizing biofilm-targeted therapies.

The studies demonstrated the efficient loading of ICG onto carnosine-GO conjugates, a critical aspect of aPDT. The highest ICG loading capacity was observed for carnosine-GO/HAp nanocarriers, comparable to reported carriers. More notably, the nanocarriers significantly improved ICG stability over time, with carnosine-GO/HAp showing remarkable stability after one week. This stability enhancement, attributed to the mesoporous structure of hydroxyapatite, indicates the potential of these nanocarriers to protect ICG from degradation. The findings suggest that not only do these nanocarriers increase the amount of ICG loading, but they also significantly improve its stability, crucial for sustained therapeutic efficacy.

The functionalized nanocarriers exhibited considerable antibacterial effects against *Streptococcus mutans*. The application of aPDT using carnosine-GO/ICG and carnosine-GO/HAp/ICG significantly decreased bacterial survival and effectively reduced the count of *S. mutans* strains. Moreover, the nanocarriers demonstrated the ability to inhibit biofilm formation, with carnosine-GO/ICG showing a pronounced effect. The expression profile of biofilm-associated genes of *S. mutans* revealed a substantial reduction, emphasizing the potential of these nanocarriers in controlling biofilm formation. These antibacterial and biofilm inhibition properties highlight the practical applicability of carnosine-GO conjugates for combating oral pathogens.

The investigation into the combined effect of apigenin and reduced graphene oxide (RGO) against *Enterococcus faecalis* biofilms demonstrated a synergistic antibacterial impact. Apigenin exhibited a dose-dependent decrease in *E. faecalis* viability, with minimal impact on biomass. In contrast, RGO displayed a significant dose-dependent reduction in biomass, suggesting its potential in disrupting biofilm formation. Confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) visualizations validated these quantitative findings. The combination of apigenin and RGO showcased a considerable reduction in live bacteria and morphological changes in *E. faecalis* biofilms, underscoring the promising potential of nanomaterial combinations in combating biofilm-related infections.

In summary, the systematic review reveals promising outcomes for dental therapeutics employing nanomaterials against biofilms. The carnosine-GO conjugates exhibited unexpected synergies, enhancing antibacterial effects and stability for effective aPDT against *Streptococcus mutans*. Simultaneously, the combination of apigenin and RGO showcased a synergistic antibacterial impact against *Enterococcus faecalis* biofilms, emphasizing the potential of nanomaterial combinations for improved therapeutic outcomes in dental care. These findings contribute valuable insights to the growing field of nanotechnology in dentistry, providing novel strategies for addressing challenges posed by oral biofilms. Further research is warranted to elucidate underlying mechanisms, optimize formulations, and assess long-term safety for the eventual clinical translation of these innovative approaches.

7. DISCUSSION

The systematic review amalgamates findings from distinct studies investigating the efficacy of nanomaterials against oral pathogens. The first study focused on carnosine-graphene oxide (GO) conjugates and their unexpected synergistic effects in combating *Streptococcus mutans* biofilms. The second study explored the combined impact of apigenin and reduced graphene oxide (RGO) against *Enterococcus faecalis* biofilms. These studies provide valuable insights into the potential of nanomaterials for biofilm-targeted therapies in dentistry.

The carnosine-GO conjugates showcased a dual advantage, exhibiting enhanced antibacterial effects and improved stability of loaded Indocyanine Green (ICG). The unexpected augmentation of antibacterial properties upon combining carnosine with GO opens new avenues for research in graphene-based materials and bioactive compounds. The stability of ICG, crucial for sustained therapeutic efficacy, was notably improved in the presence of hydroxyapatite (HAp), a characteristic feature of carnosine-GO/HAp nanocarriers. The mesoporous structure of HAp played a pivotal role in protecting ICG from degradation, indicating the potential longevity of these nanocarriers in clinical applications.

The application of carnosine-GO conjugates in aPDT effectively reduced bacterial survival and inhibited biofilm formation in *Streptococcus mutans*. The unexpected enhancement of antibacterial effects in the presence of carnosine suggests a novel mechanism that warrants further investigation. Additionally, the modulation of biofilm-associated gene expression, particularly the significant reduction in the *gtfB* gene expression, highlights the potential of carnosine-GO conjugates in controlling the molecular mechanisms underlying biofilm formation. These findings not only emphasize the practical applicability of these nanocarriers but also open avenues for targeted gene expression modulation as a strategy against biofilm-related infections.

The studies exploring the combined effect of apigenin and RGO against *Enterococcus faecalis* biofilms, revealed a synergistic antibacterial impact. The dose-dependent decrease in *E. faecalis* viability in the presence of apigenin, coupled with the significant reduction in biomass induced by RGO, underscores the potential of combining natural compounds with nanomaterials for enhanced therapeutic outcomes. Visualizations through CLSM and SEM provided compelling evidence of

the impact on biofilm architecture, showcasing the potential of nanomaterial combinations in disrupting established biofilms. These studies contribute valuable insights to the evolving landscape of nanotechnology in dentistry. The unexpected synergies observed in both studies highlight the need for further exploration of nanomaterial combinations for targeted and effective biofilm control. The translational potential of these findings to clinical applications underscores the promise of nanotechnology in addressing challenges posed by oral biofilms. Future research should delve into elucidating the underlying mechanisms of synergies observed, optimizing formulations for clinical use, and conducting thorough safety assessments to ensure the viability of these innovative approaches in dental care.

The unexpected synergistic effects observed in the combination of carnosine-graphene oxide (GO) conjugates and their enhanced antibacterial properties against *Streptococcus mutans* biofilms suggest a promising avenue for further research in graphene-based materials and bioactive compounds^[15,16]. The improved stability of Indocyanine Green (ICG) in carnosine-GO conjugates, attributed to the presence of hydroxyapatite (HAp) in the nanocarriers, highlights the potential longevity of these formulations for sustained therapeutic efficacy^[17,18]. Application of carnosine-GO conjugates in antimicrobial photodynamic therapy (aPDT) demonstrated significant reductions in bacterial survival and inhibition of biofilm formation in *Streptococcus mutans*, suggesting a novel mechanism for biofilm control^[19,20]. Furthermore, the modulation of biofilm-associated gene expression, particularly the significant reduction in *gtfB* gene expression, underscores the potential of carnosine-GO conjugates in targeting molecular mechanisms underlying biofilm formation^[21]. Similarly, the synergistic antibacterial impact observed in the combination of apigenin and reduced graphene oxide (RGO) against *Enterococcus faecalis* biofilms suggests a promising strategy for enhanced therapeutic outcomes in dentistry^[22,23]. Visualizations through confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) provided compelling evidence of the disruptive effects of nanomaterial combinations on biofilm architecture, further emphasizing their potential for clinical applications^[24,25]. These findings contribute valuable insights to the growing field of nanotechnology in dentistry, highlighting the need for continued exploration of nanomaterial combinations for effective biofilm control^[26]. Future research endeavors should focus on elucidating the underlying mechanisms of synergies observed, optimizing formulations for clinical use, and conducting comprehensive safety assessments to ensure the translational potential of these innovative approaches in dental care^[27,28].

In conclusion, the systematic review consolidates evidence supporting the potential of nanomaterials, particularly carnosine-GO conjugates and the combination of apigenin with RGO, in combating oral biofilms. These findings pave the way for continued exploration and innovation in the integration of nanotechnology for enhanced therapeutic strategies in dentistry.

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