Does the Incorporation of Graphene Oxide into PMMA Influence its Antimicrobial Activity?

Keywords

PMMA
Graphene Oxide
Antimicrobial
Coatings
Incorporation

Authors

Izabela Ferreira *
(DDS, MSc)

Beatriz D. Sahm *
(DDS, MSc)

Oswaldo L. Alves §
(DDS, MSc, PhD. In Memorian)

José A. M. Agnelli ‡
(DDS, MSc, PhD)

Andrea C. dos Reis *
(DDS, MSc, PhD)

Address for Correspondence

Andrea C. dos Reis *

Email: andreare73@yahoo.com.br

* *Department of Dental Materials and Prosthesis, Ribeirão Preto School of Dentistry, University of São Paulo (USP), Ribeirão Preto, Brazil*

§ *Department of Inorganic Chemistry, Institute of Chemistry, State University of Campinas (UNICAMP), Campinas, Brazil*

‡ *Materials Engineering Department (DEMa), Federal University of São Carlos (UFSCar), Brazil*

Received: 23.11.2023
Accepted: 12.02.2024

doi: 10.1922/EJPRD_2651Ferreira07

ABSTRACT

Introduction: The increase in infectious diseases mediated by oral bacteria has been one of the most potent threats to human health, specifically to the elderly. Researchers seek to incorporate antimicrobial nanomaterials into PMMA to control the colonization of microorganisms and reduce deadly diseases. Objective: To conduct a systematic review to answer the question: "Does graphene oxide incorporated into polymethylmethacrylate influence its antimicrobial activity?" Methods: The electronic search was performed in PubMed, Embase, Web of Science, Scopus, and Google Scholar databases, and articles published until October 2021 were selected. The search terms were: ("acrylic resin" OR PMMA) AND (graphene OR "oxide graphene") AND (antimicrobial OR antibacterial OR "anti-infective agent"). The risk of bias analysis of the articles was performed using the quasi-experimental study assessment tool adapted from the JBI. Results: Two hundred and fifty-nine articles were found in the databases. After removing the duplicates, 245 were analyzed by title and abstract. Of these, 06 were selected for full reading, of which 03 met the eligibility criteria and were included in this systematic review. Conclusion: This systematic review's findings can infer that the incorporation of graphene in the form of graphene-based coatings to PMMA showed antimicrobial effectiveness.

INTRODUCTION

The rapid increase in infectious diseases mediated by oral bacteria is one of the most potent threats to human health, specifically to the elderly, due to the large use of dentures and their hygiene difficulties, which can favor the environment for microorganisms to adhere.[1-3] The colonization of microorganisms on the surfaces of dental materials composes the oral biofilm[4-6] and this biofilm is the main cause of diseases related to the oral cavity.[7]

The initial approach to this challenge is to incorporate antimicrobial agents into dental materials, such as acrylic resin, endodontic cement, soft liners, and dental porcelain as they present an increase in their antimicrobial efficacy,[4-6,8-10] and reduce failures of dental treatment caused by microorganisms.[8] However, the development of materials modified to express antimicrobial activity may present changes in properties, making it difficult to balance satisfactory mechanical and antimicrobial properties concurrently in the same material.[11]

Polymethylmethacrylate (PMMA) is commonly used in the manufacture of dental prostheses, due to its satisfactory aesthetics, low density, ease of manipulation, precision of adjustment, good cost-benefit ratio, physico-mechanical properties compatible with the oral environment to resist dynamic

masticatory forces, parafunctional habits and good durability.[12-16] As a disadvantage, the material has high porosity, which can contribute to the process of colonization of microorganisms on the surface of PMMA.[10] However, the innovation of PMMA through the incorporation of an antimicrobial that can reduce the growth of microorganisms in dental prostheses, can configure materials that will effectively protect human health from deadly infectious diseases.[17,18]

One possibility to improve the antimicrobial performance of dental materials is the use of nanotechnology.[4,19] Nanomaterials have excellent antimicrobial properties due to their reduced size, which gives them a greater contact surface with microorganisms, and for metallic nanomaterials, there is a greater release of ions,[20-23] which improves the performance of dental materials.[8-24]

Graphene, based on graphite, applied as graphene oxide, and reduced graphene oxide, has microbiological properties due to the ability to disrupt bacterial cell membrane integrity and produce reactive oxygen species.[19,25,26] In view of the nanoparticles, graphene has a greater contact surface area that allows a better performance against microorganisms, is one of the most promising nanomaterials, and was discovered in 2004.[18,26,27] Despite being biocompatible,[27,29-31] graphene is cytotoxic when used in high concentrations, which harms human cells by damaging the cell membrane through mechanical stress or the removal of phospholipids.[27,30,31] However, given its success as a coating for implants in osseointegration and its antimicrobial action, its use is considered promising in dental materials.[19,31-33]

The limitations of graphene are related to the agglomeration of the material and the processing difficulty,[34] as much as silver particles.[4,17,35] Therefore, chemical modification of graphene is necessary to produce the derivatives that are more versatile and applicable, such as graphene oxide (GO) and reduced graphene oxide (rGO)which draws attention to its use in engineering, electronics, environmental engineering and biomedical engineering.[18,36,37]

In view of the articles found in the literature referring exclusively to the role of graphene and the role in PMMA to attribute antimicrobial properties, and the importance of the longevity of rehabilitative treatments that use PMMA as a material and the reduction of the adhesion of microorganisms and infectious diseases in patients with dental prostheses, we are facing an innovative possibility. Therefore, this study aimed to carry out a systematic search in the literature regarding the antimicrobial effectiveness of the polymethylmethacrylate material incorporated with the graphene oxide nanomaterial.

MATERIAL AND METHODS

PROTOCOL

This systematic review (SR) was structured according to the Preferred reporting items for systematic reviews and meta-analyses (PRISMA), and its protocol was registered in the Open Science Framework (osf.io/4wc8t). The question of this review was formulated based on PICO: Population = acrylic resins or materials used to manufacture them; Intervention = incorporation of graphene oxide; Comparison = control group; Outcome = assessment of antimicrobial activity. To answer the question "Does graphene oxide incorporated into polymethylmethacrylate influence its antimicrobial activity?".

ELIGIBILITY CRITERIA

Inclusion criteria were *in vitro* experimental studies that evaluated the incorporation of graphene oxide into the acrylic resin composition and its antimicrobial effectiveness. Studies that a) incorporated graphene oxide together with another antimicrobial in the acrylic resin were excluded; b) clinical studies, systematic reviews, book chapters, conference abstracts, short communications, personal opinions, and case reports.

SEARCH STRATEGY

The electronic search of articles was performed in PubMed, Embase, Scopus, and Science Direct databases, and articles published until December 2021 were selected. The search terms were: ("acrylic resin" OR PMMA) AND (graphene OR "oxide graphene") AND (antimicrobial OR antibacterial OR "anti-infective agent"). In addition, complementary research was carried out, where the reference lists and citations of the included articles were analyzed, to find possible additional inclusions.

SELECTION PROCESS

The search for articles was performed by two authors (I.F and B.D.S) and attached to the Rayyan software. After reading the titles and abstracts, the initial selection of studies. Studies were only excluded when not meet the inclusion criteria for an SR. In the second stage were fully evaluated the preselected studies.

ANALYSIS OF THE RISK OF BIAS

The quasi-experimental studies (non-randomized experimental studies) from the JoannaBriggs Institute (JBI) have been adapted to assess the risk of bias. For the classification of the methodological quality of the studies, each question was scored with "yes", "no" and "uncertain". The analysis was performed using the RevMan 5.3 software.

RESULTS

SELECTION PROCESS

Two hundred and fifty-nine articles were found in the databases. After removing the duplicates, 245 were analyzed by title and abstract. Of these, 06 were selected for full reading, of which 03 met the eligibility criteria and were included in this systematic review. Figure 1 describes the study workflow.

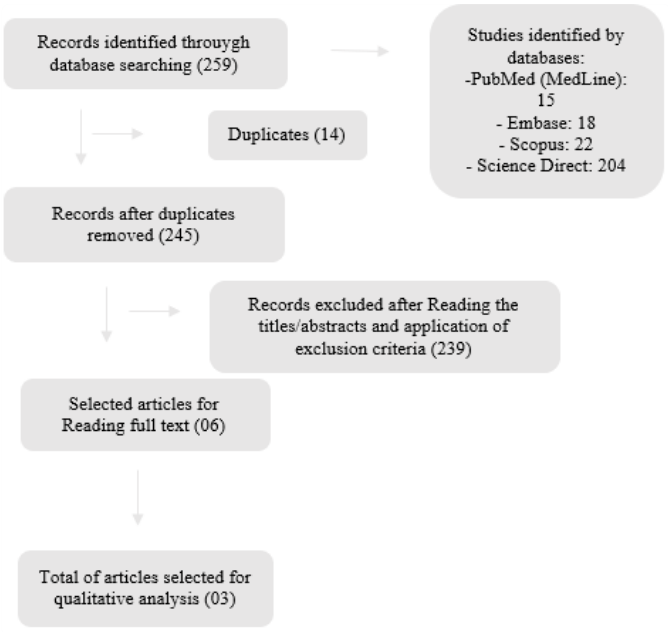

Figure 1: Flowchart of the article selection process.

RISK OF BIAS

The results obtained after evaluating the quality of the studies are shown in Figures 2 and 3. The exception was regarding the criteria "Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest?" which presented a high risk of bias[37] because the study presented other causes in the characterization of the material, which may change its final result and the criterion "Was appropriate statistical analysis used?" who presented a high risk of bias[36,37] for not presenting statistical analysis.

STUDY CHARACTERISTICS

Table 1 provides information regarding the included studies, which were published until December 2021.

The studies included in this systematic review evaluated the polymethylmethacrylate material with the incorporation of graphene oxide coating, such as graphene oxide nanosheets, in percentages of incorporation 0.25, 0.5, 1.0 and 2.0wt%[19] and 2.0, 4.0 and 8.0wt%[37] and graphene oxide nanoplatelets, in percentages. 2.0, 4.0, and 8.0wt%.[36]

To assess the antimicrobial activity of the modified material, the studies used colony forming units (CFU) counts[36,37] and the anti-adhesive mechanism was investigated by a non-thermal oxygen plasma treatment[19] on pathogenic E.coli species,[19,36,37] *C. albicans, S. aureus, S. mutans*[19] and *P. aeruginosa*.[36]

DISCUSSION

The growth of bacterial-mediated infectious diseases is one of the most potent threats to human health. In dentistry, the elderly is the most affected due to the use of extensive prostheses made with PMMA.[2,3] However, the development of antibacterial materials can effectively protect the health of society against deadly infectious diseases.[24]

Materials incorporated with antibacterials are generally evaluated against groups of pathogenic bacteria such as *Listeria monocytogenes, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium, Streptococcus mutans, Escherichia coli, Staphylococcus epidermidis, Vibrio harveyi* and *Enterococcus faecalis*, which are the main cause of many infections.[38,39]

Alternatively, nanomaterials are used by researchers[4,5,6,8] to improve the quality and functioning of dental materials, mainly because they have the ability to reduce the adhesion and biofilm formation of pathogenic microorganisms and consequently reduce oral diseases.[7,40,41] Among them, graphene-based materials have surprising characteristics to fight bacterial infections.[24]

Graphene is a two-dimensional (2D) nanostructure composed of sp2 carbons. It appears as a block with allotropes of carbon, in a hexagonal honeycomb structure.[18] Graphene emerged as a promising nanomaterial in 2004, due to its crystalline form with various oxygen-containing functional groups (hydroxyl, carboxyl, etc.), it confers antimicrobial, physical and mechanical properties such as high compressive strength, high tensile strength, high elasticity, high impermeability, large surface area superior to other nanomaterials such as carbon nanotubes,[26,42,43] as biocompatibility with the oral cavity, high fracture toughness module, excellent electrical conductivity, and high optical transparency.[44,45] In addition to these characteristics, its use is renewable, low cost, and easy to obtain.[46,47]

Graphene can damage the cell membrane wall through antimicrobial mechanisms, such as oxidative stress, mechanical damage to the membrane and cell wall, involvement, and electron transfer.[45,48] However, oxidative stress is the main cause of graphene toxicity as it causes the bacteria to no longer proliferate after deactivating its proteins and lipids.[49] In addition, graphene may also present antibacterial activity by electron transfer, which consists of an exchange of electrons between extracellular graphene that captures electrons from the bacteria's respiratory metabolism and leads to membrane rupture with consequent microbial death and infection control.[24,45]

Although graphene coatings exhibit antibacterial properties, the particles tend to aggregate and thus limit the material's antimicrobial action[18,50] and its processing difficulty.[34] It then becomes necessary to chemically modify it into graphene oxide (GO) or reduced graphene oxide (rGO), with the main differences between the coatings being their carbon ratios / oxygen and their degree of hybridization.[18] In 2010, Hu *et al* reported for the first time the antibacterial efficacy of two graphene-based materials, through graphene oxide (GO) and reduced graphene oxide (rGO) nanosheets that significantly inhibited the growth of *E. coli* bacteria.

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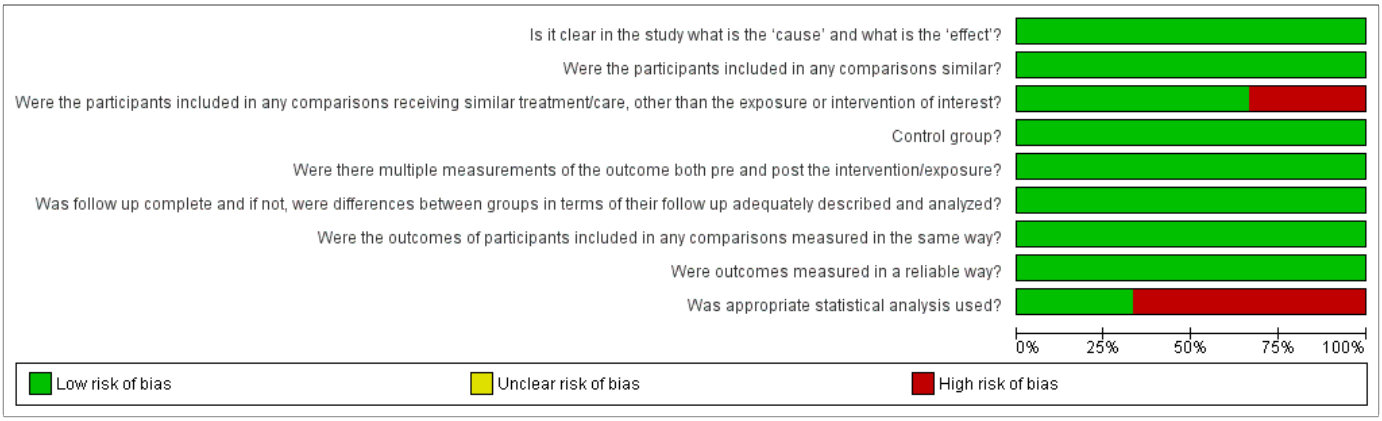

Figure 2: Qualitative analysis with adapted the quasi-experimental studies appraisal tool by the Joanna Briggs Institute.

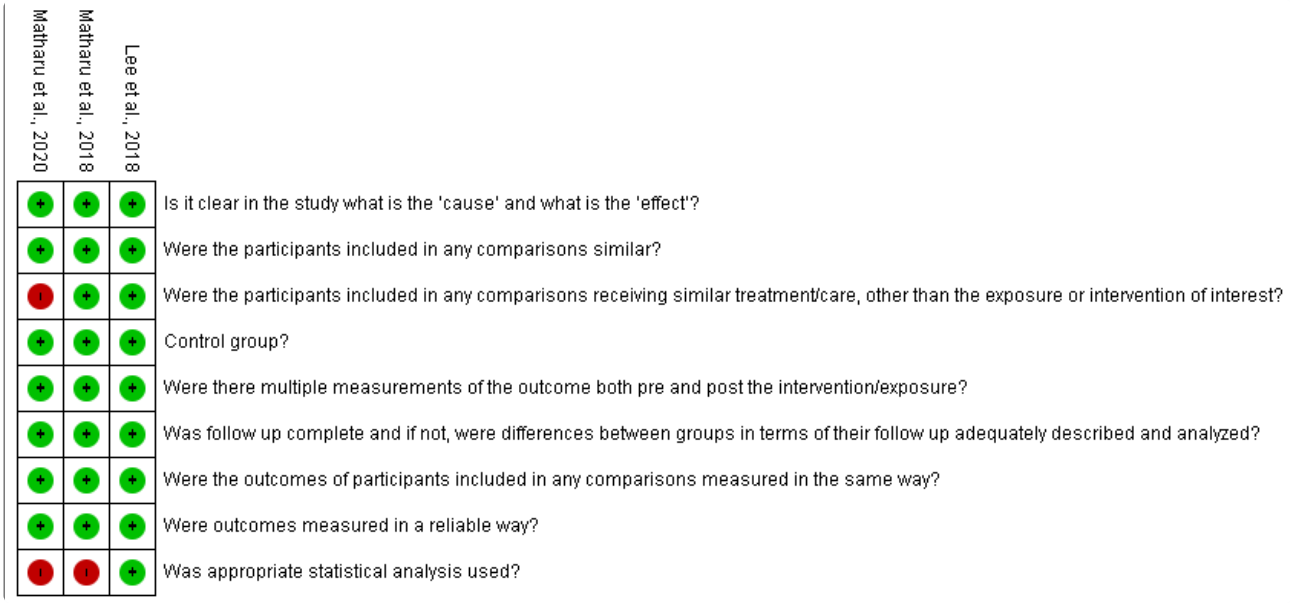

Figure 3: Qualitative analysis of each study according to adaptation of the Joanna Briggs Institute's quasi-experimental studies evaluation tool.

Table 1. Summary of included studies.

Study	Graphene	wt%	Antimicrobial evaluation	Pathogenic species	Conclusion
Lee et al., 2018	Nano-sheets	0, 0.25, 0.5, 1.0, or 2.0% wt%	anti-adhesive mechanism was investigated by a non-thermal oxygen plasma treatment	*E. coli, C. albicans, S. aureus, S. mutans*	2% showed antimicrobial effect
Matharu et al., 2018	Nanoplates	0, 2, 4 and 8 wt%	CFU	*E. coli, P. aeruginosa*	8% showed antimicrobial effect
Matharu et al., 2020	Nano-sheets	0, 2, 4 and 8 wt%	CFU	*E. coli*	8% showed greater antimicrobial effect

These limitations can also be overcome by incorporating the nanomaterial into the polymer matrix to avoid the aggregation of the nanoparticles, through a stable dispersion of graphene-polymer.[51] Polymers can be easily biodegraded by being attacked by different microorganisms, by breaking chemical bonds in the main chain of the polymer, using the elements of the molecule as a food source.

However, polymer-based composites have tunable properties to develop high mechanical-performance antimicrobial biomaterials for clinical applications.[52] There are three strategies to develop graphene-polymer nanocomposites, such as the physical mixing of GO with the polymer, the covalent bonding of the polymer matrix by GO functional groups, and the bonding of GO with the aromatic group of the polymer. through non-covalent interactions.[24]

Recently, polymer-graphene nanocomposites have been developed to enhance antibacterial activity using polymeric matrices, as in a study by Matharu *et al*, which incorporated GO nanosheets into PMMA, to assess the antimicrobial efficacy of the material, resulting in inhibition against *E. coli*. Lee *et al* demonstrated that GO nanosheets improve not only antimicrobial properties against dental pathogens but also the mechanical properties of PMMA acrylic resin, such as flexural strength.

Due to this, an important issue to be observed when adding graphene to PMMA is the consideration of unwanted changes that interfere with the functionality of the material due to its alteration so that it has antimicrobial properties. For example, the roughness pattern can be controlled by standardizing the surface topography of the material.[18] Initially, the effect of roughness may or may not be desirable, the wrinkled graphene surfaces consisting of peaks and valleys are means of suitability for colonization of microorganisms.[53] However, Zou, *et al* and Seifi, Kamali, demonstrated that the sharp edge of the peak on the surface of the nanomaterial inhibited bacterial adhesion.

Yu *et al* and Zou *et al*, showed in the literature that the antimicrobial, physicochemical, and mechanical properties of graphene coatings also depend on the size and concentration of its flakes in the material.[54,55] The incorporation of up to 2% by weight of GO nanosheets increased the hydrophilicity of PMMA and its antimicrobial effects with minimal toxicity to human cells.[19] As for Matharu *et al*, the incorporation of GO of 8% by weight showed better antimicrobial activity when compared to lower percentages of the nanomaterial.

Although promising, there are still difficulties and challenges associated with graphene-based PMMA, due to the material's innovation and research. How to understand the interaction between bacteria and the surface of the material, changes in physicochemical and mechanical properties, and level of cellular toxicity for clinical applicability of the antibacterial material, in addition to studies that evaluate the effectiveness of the biomaterial in several pathogenic species, since the most studied was E. coli.

Therefore, a summary of recent literary advances in the incorporation of graphene-based coatings in the polymethylmethacrylate material was concluded in this review, presenting the inherent antimicrobial efficacy of graphene. In addition, graphene has been used in functionalization for several nanomaterials, specifically, PMMA, being able to improve the antibacterial activity of the material and thus reduce worldwide infectious diseases predisposed in the elderly, caused by bacteria present in the oral environment.

CONCLUSION

It can be inferred through this systematic review that the incorporation of graphene-based nanomaterial coatings in the polymethylmethacrylate material has antimicrobial activity and the study of its applicability in materials aimed at dentistry should grow to provide more information since the material proved to be viable as an antimicrobial. Researchers should propose the addition of nanomaterial to reduce the risk of infections while maintaining the mechanical and physicochemical properties of PMMA for longevity and success in clinical practice. The limitation of this study is related to the innovation of the material. Thus, few study its advantages, disadvantages, and characteristics that tend to be amplified by the great potential presented by the antimicrobial.

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