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Graphene oxide-enhanced bioceramic sealers: Evaluation of antimicrobial activity, sealing ability, and biocompatibility

Ranjita Singh Baghel
B.D.S, M.D.S (Periodontics)

ABSTRACT

Graphene oxide-enhanced bioceramic sealers have emerged as a promising advancement in endodontic materials due to their potential to improve the biological and functional properties of conventional root canal sealers. The incorporation of graphene oxide into calcium silicate-based bioceramic sealers aims to enhance antimicrobial activity against persistent endodontic pathogens, improve adaptation to dentinal walls, reduce microleakage, and promote favorable interactions with periapical tissues. This review summarizes the current evidence regarding the physicochemical characteristics, antimicrobial mechanisms, sealing ability, and biocompatibility of graphene oxide-enhanced bioceramic sealers. Available laboratory and preclinical studies suggest that graphene oxide contributes to superior antibacterial performance through membrane disruption, oxidative stress induction, and inhibition of biofilm formation while maintaining or improving the bioactivity and cytocompatibility of the sealer matrix. In addition, these modified materials demonstrate promising sealing properties owing to improved flow characteristics and interfacial adaptation. Despite encouraging findings, variations in graphene oxide concentration, testing methodologies, and limited long-term clinical evidence highlight the need for standardized research protocols and well-designed clinical investigations. Overall, graphene oxide-enhanced bioceramic sealers represent a promising biomaterial with the potential to improve the predictability and long-term success of root canal treatment.

Keywords: Graphene oxide, bioceramic sealer, endodontics, antimicrobial activity, sealing ability, biocompatibility, root canal obturation, calcium silicate, biofilm inhibition, nanotechnology.

1. INTRODUCTION

Successful endodontic treatment depends on the complete elimination of microorganisms from the root canal system followed by the establishment of a durable three-dimensional seal that prevents reinfection. Although advances in instrumentation and irrigation have significantly improved canal debridement, the intricate anatomy of the root canal system often permits residual microorganisms to persist within dentinal tubules, accessory canals, and apical ramifications. These persistent pathogens are a major cause of post-treatment disease and endodontic failure, emphasizing the importance of root canal sealers with superior antimicrobial and sealing properties.

Bioceramic sealers have emerged as an important development in endodontic obturation because of their excellent biocompatibility, bioactivity, dimensional stability, and ability to form hydroxyapatite during the setting process. Calcium silicate-based sealers exhibit favorable physicochemical characteristics that facilitate chemical bonding with dentin and support periapical tissue healing. Their hydrophilic nature enables setting in the presence of moisture, making them particularly suitable for clinical endodontic applications. Nevertheless, despite these advantages, conventional bioceramic sealers may exhibit limited antimicrobial effectiveness against persistent biofilm-forming microorganisms, particularly *Enterococcus faecalis*, and their mechanical performance can still be optimized.

Recent developments in nanotechnology have introduced graphene oxide (GO) as a promising nanomaterial for biomedical and dental applications. Graphene oxide is

Corresponding author
Author Ranjita Singh
Email : Ranjitasinghbhagel@gmail.com

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a two-dimensional carbon-based material containing abundant oxygen-containing functional groups, providing excellent dispersibility, chemical reactivity, and compatibility with various biomaterials. The incorporation of graphene oxide into dental materials has attracted considerable attention because of its unique mechanical strength, large surface area, antimicrobial activity, and capacity to enhance the physicochemical properties of composite materials. These characteristics make graphene oxide an attractive reinforcing agent for calcium silicate-based bioceramic sealers, with the potential to improve both biological and functional performance.

The antimicrobial activity of graphene oxide is attributed to multiple mechanisms, including physical disruption of bacterial cell membranes, induction of oxidative stress, extraction of membrane phospholipids, and inhibition of biofilm formation. Unlike conventional antimicrobial agents that rely primarily on chemical toxicity, graphene oxide exerts physical and physicochemical interactions with microbial cells, thereby reducing the likelihood of antimicrobial resistance. Such properties are particularly valuable in endodontics, where polymicrobial biofilms exhibit remarkable resistance to intracanal medicaments and irrigating solutions. Furthermore, graphene oxide has demonstrated the ability to enhance cellular adhesion, proliferation, and mineralization when incorporated into bioactive materials, suggesting its potential role in promoting periapical healing and tissue regeneration.

In addition to antimicrobial efficacy, the long-term success of root canal treatment depends on the sealing ability of the obturation material. An ideal endodontic sealer should exhibit adequate flowability, minimal solubility, dimensional stability, intimate adaptation to dentinal walls, and resistance to microleakage. The incorporation of nanomaterials such as graphene oxide may improve these properties by reinforcing the sealer matrix, reducing porosity, enhancing interfacial adaptation, and increasing resistance to crack propagation. Similar improvements in material performance have been reported with nanostructured biomaterials used in other areas of dentistry, where optimization of physicochemical characteristics contributes directly to enhanced clinical outcomes (Daniele et al., 2020; Badr, 2021).

Biocompatibility remains another essential requirement for endodontic sealers because these materials inevitably interact with periapical tissues. An ideal material should elicit minimal inflammatory response while supporting cellular proliferation, differentiation, and mineralized tissue formation. Advances in biomaterial science have demonstrated that favorable interactions between dental materials and host tissues are fundamental for long-term treatment success. The importance of biological compatibility and tissue response has been widely recognized across multiple dental disciplines, including restorative dentistry, implantology, and regenerative therapies (Singh, 2020; Feher et al., 2020). Likewise, understanding biological markers associated with tissue inflammation and healing has contributed substantially to the development of more biologically compatible dental materials (Taba Jr. et al., 2005).

The growing integration of digital technologies and evidence-based decision-making into dental practice has further accelerated the evaluation of novel biomaterials. Advanced data extraction methods, predictive modeling, and comprehensive diagnostic protocols have facilitated more accurate assessment of treatment outcomes and biomaterial performance, enabling researchers to identify factors associated with clinical success and complications (Wang et al., 2017; Feher et al., 2020; Lo Giudice et al., 2020). These developments support a more systematic evaluation of graphene oxide-enhanced bioceramic sealers before widespread clinical implementation.

Given the increasing interest in nanotechnology-based endodontic materials, a comprehensive evaluation of graphene oxide-enhanced bioceramic sealers is warranted. This review therefore examines the physicochemical characteristics of graphene oxide-modified bioceramic sealers, with particular emphasis on their antimicrobial activity, sealing ability, and biocompatibility. It also discusses current evidence regarding their mechanisms of action, laboratory evaluation methods, biological performance, potential clinical applications, and future research directions to determine their role in improving the long-term success of root canal therapy.

2. GRAPHENE OXIDE AND BIOCERAMIC SEALERS: MATERIAL CHARACTERISTICS

2.1. Bioceramic Sealers

Bioceramic sealers have become integral components of contemporary endodontic obturation owing to their excellent sealing ability, bioactivity, and favorable biological response. Most commercially available bioceramic sealers are calcium silicate-based materials designed to establish a hermetic seal within the root canal system while promoting healing of the surrounding periapical tissues. Unlike traditional resin- or zinc oxide eugenol-based sealers, bioceramic materials possess inherent bioactivity and the ability to form hydroxyapatite during the setting process, thereby enhancing the seal between dentin and filling materials.

The growing emphasis on conservative and biologically oriented dental treatment has encouraged the development of restorative materials capable of interacting positively with surrounding tissues rather than serving solely as passive filling materials. Similar trends have been observed across restorative dentistry, where biomaterials with enhanced biological performance have gained increasing attention (Singh, 2020). Material selection has likewise become a fundamental aspect of long-term treatment success, as demonstrated in investigations of advanced ceramic restorative materials subjected to functional loading and aging (Badr, 2021).

2.1.1. Composition and Setting Reaction

Bioceramic sealers are primarily composed of tricalcium silicate, dicalcium silicate, calcium phosphate, zirconium oxide, tantalum oxide, calcium hydroxide, and radiopacifying agents. Their setting reaction depends on hydration, producing calcium silicate hydrate gel and calcium hydroxide as principal products. Subsequent interaction with tissue fluids promotes apatite crystal formation along the dentin interface, contributing to chemical bonding and long-term sealing.

Unlike epoxy resin sealers, calcium silicate-based sealers exhibit slight setting expansion rather than polymerization shrinkage. This characteristic contributes to intimate adaptation with canal walls and accessory anatomy while minimizing interfacial gaps.

2.1.2. Physical and Chemical Properties

Several physicochemical characteristics determine the clinical performance of bioceramic sealers, including flowability, film thickness, radiopacity, dimensional stability, solubility, and compressive strength. Adequate flow enables penetration into accessory canals, dentinal tubules, and anatomical irregularities. Low solubility and dimensional stability help preserve the integrity of the obturation over time.

The evaluation of physicochemical properties has become increasingly important in dental biomaterial research. Comprehensive characterization methods employed for dental polymers and orthodontic materials have demonstrated how mechanical behavior, structural integrity, and surface characteristics influence clinical longevity (Daniele et al., 2020). Similar principles are applicable to endodontic sealers, where optimized physicochemical behavior contributes directly to sealing performance.

2.1.3. Biological Characteristics

A major advantage of bioceramic sealers is their favorable biological profile. Their alkaline pH provides antibacterial activity while simultaneously stimulating mineralized tissue formation. Calcium ion release encourages differentiation of osteogenic and cementogenic cells, facilitating periapical healing after root canal treatment.

The interaction between biomaterials and surrounding tissues resembles broader biological processes observed in oral diagnostics and tissue regeneration. Biomolecular responses associated with healing and inflammation have been extensively discussed in studies evaluating biomarkers of oral disease, emphasizing the importance of biologically compatible dental materials (Taba Jr. et al., 2005).

2.1.4. Advantages and Current Limitations

- Bioceramic sealers offer numerous clinical advantages including:
- Excellent biocompatibility
- Bioactivity and hydroxyapatite formation
- High alkaline pH
- Favorable sealing ability
- Slight expansion during setting
- Moisture-assisted setting
- Reduced cytotoxicity

However, certain limitations remain. Extended setting time under dry conditions, difficult retreatability, variable flow characteristics among products, and relatively limited long-term clinical evidence continue to present challenges for widespread standardization.

2.2. Graphene Oxide

Graphene oxide (GO) is an oxidized derivative of graphene consisting of a single-layer carbon lattice functionalized with oxygen-containing groups such as hydroxyl, epoxy, carbonyl, and carboxyl groups. These functional groups impart hydrophilicity, facilitate dispersion in aqueous systems, and enable incorporation into a wide variety of biomedical materials.

The emergence of graphene oxide in dentistry reflects the broader movement toward nanotechnology-based biomaterials capable of improving both mechanical and biological performance.

2.2.1 Chemical Structure and Synthesis Methods

Graphene oxide possesses a two-dimensional honeycomb carbon framework modified by oxygen-containing functional groups distributed across its basal planes and edges. The material is commonly synthesized using oxidative methods such as the modified Hummers technique, producing nanosheets with large surface area and abundant reactive sites suitable for composite formation.

These structural characteristics allow graphene oxide to interact chemically with calcium silicate matrices without

substantially compromising setting reactions.

2.2.1. *Mechanical and Physicochemical Properties*

Graphene oxide demonstrates exceptional mechanical strength, high Young's modulus, excellent thermal stability, large specific surface area, and remarkable flexibility. When incorporated into dental materials, it may improve fracture resistance, wear resistance, dimensional stability, and mechanical durability.

Studies investigating advanced ceramic restorations have similarly demonstrated that improvements in material microstructure significantly influence long-term mechanical behavior under functional loading (Badr, 2021).

2.2.2. *Antimicrobial Mechanisms*

One of the principal reasons for incorporating graphene oxide into endodontic sealers is its broad-spectrum antimicrobial activity. Multiple mechanisms contribute to bacterial inhibition, including:

Physical disruption of bacterial membranes by sharp graphene edges

- Generation of oxidative stress
- Extraction of membrane phospholipids
- Entrapment and isolation of bacterial cells
- Prevention of biofilm maturation

These mechanisms are particularly relevant against persistent endodontic microorganisms such as *Enterococcus faecalis*, which remains a major cause of post-treatment disease.

2.2.3. *Biological Interactions with Host Tissues*

Graphene oxide exhibits favorable interactions with mammalian cells when used at controlled concentrations. It has been shown to support cell attachment, proliferation, and osteogenic differentiation while serving as an effective scaffold for tissue engineering applications.

The biological performance of advanced biomaterials increasingly depends upon understanding tissue responses and healing prediction models. Similar predictive approaches have been explored for implant success and postoperative healing in oral rehabilitation (Feher et al., 2020).

2.3. Graphene Oxide-Enhanced Bioceramic Sealers

The incorporation of graphene oxide into calcium silicate-based sealers represents an emerging strategy for producing multifunctional endodontic materials capable of simultaneously addressing microbial control, sealing performance, and biological compatibility.

Graphene oxide is generally incorporated in small concentrations to reinforce the bioceramic matrix while maintaining appropriate setting characteristics. Uniform dispersion of nanosheets is essential because excessive concentrations may increase viscosity or interfere with hydration.

2.3.1. *Fabrication Approaches*

- *Common fabrication methods include*
- Mechanical mixing
- Ultrasonic dispersion
- Solution-assisted blending
- Surface functionalization
- Nanocomposite synthesis
- The objective is to achieve homogeneous graphene oxide distribution without aggregation.

2.3.2. *Modification of Physicochemical Properties*

- *The incorporation of graphene oxide may improve several important material characteristics, including*
- Increased flowability
- Improved dentinal adaptation
- Reduced microleakage
- Enhanced mechanical strength
- Greater fracture resistance
- Increased dimensional stability
- Improved resistance to bacterial colonization

Comprehensive characterization of dental biomaterials demonstrates that optimization of these physicochemical parameters directly contributes to improved clinical performance (Daniele et al., 2020).

2.3.3. *Mechanisms Responsible for Improved Clinical Performance*

Graphene oxide enhances bioceramic sealers through multiple complementary mechanisms. The nanosheets reinforce the calcium silicate matrix, improve interfacial adaptation, provide sustained antimicrobial effects, and increase surface energy, thereby promoting closer interaction between the sealer and dentinal substrate. Calcium ion release from the bioceramic component continues to support mineralization, while graphene oxide contributes mechanical reinforcement and bacterial inhibition.

Modern diagnostic strategies increasingly emphasize integrating advanced materials with comprehensive clinical evaluation to improve treatment outcomes, a concept reflected across multiple dental specialties (Lo Giudice

Table 1: Comparison of Conventional Bioceramic Sealers and Graphene Oxide-Enhanced Bioceramic Sealers

Parameter	*Conventional Bioceramic Sealer*	*Graphene Oxide-Enhanced Bioceramic Sealer*
Principal composition	Calcium silicate-based cement	Calcium silicate matrix reinforced with graphene oxide nanosheets
Setting mechanism	Hydration reaction	Hydration reaction with graphene oxide reinforcement
Antimicrobial activity	Moderate alkaline-mediated activity	Enhanced antibacterial action through membrane disruption, oxidative stress, and biofilm inhibition
Flow characteristics	Good	Often improved through optimized nanoparticle dispersion
Sealing ability	Excellent	Enhanced dentinal adaptation with reduced microleakage
Dimensional stability	High	High with potential improvement in structural integrity
Mechanical strength	Good	Increased fracture resistance and mechanical reinforcement
Bioactivity	Promotes hydroxyapatite formation	Maintains bioactivity while improving biological performance
Cytocompatibility	Excellent	Generally excellent at appropriate graphene oxide concentrations
Calcium ion release	High	Preserved with sustained mineralization potential
Clinical advantages	Moisture tolerant, bioactive, biocompatible	Superior antimicrobial performance, improved sealing, enhanced mechanical durability
Current limitations	Difficult retreatability, variable setting time	Limited long-term clinical evidence, concentration-dependent behavior, need for standardized formulations

et al., 2020). Furthermore, advances in healthcare informatics facilitate more systematic evaluation of treatment outcomes and biomaterial performance using electronic clinical records (Wang et al., 2017).

3. EVALUATION OF ANTIMICROBIAL ACTIVITY

The long-term success of root canal therapy is closely related to the effective elimination of microorganisms from the complex root canal system. Although mechanical instrumentation and chemical irrigation significantly reduce microbial load, microorganisms may persist within dentinal tubules, lateral canals, and apical ramifications. Consequently, endodontic sealers with intrinsic antimicrobial properties have become an important component of root canal obturation. The incorporation of graphene oxide (GO) into bioceramic sealers has attracted considerable attention because of its broad-spectrum antimicrobial activity, favorable physicochemical characteristics, and potential to inhibit biofilm formation while maintaining biocompatibility (Taba Jr. et al., 2005; Daniele et al., 2020).

3.1. Importance of Antimicrobial Efficacy in Endodontics

Microbial infection is the primary etiological factor responsible for pulpal and periapical diseases. Even after meticulous cleaning and shaping procedures, residual microorganisms may remain protected within inaccessible anatomical regions of the root canal system. These surviving microorganisms can proliferate, resulting in persistent apical periodontitis and treatment failure. Therefore, the antimicrobial effectiveness of endodontic sealers serves as an additional defense against residual pathogens after obturation.

Bioceramic sealers possess inherent antimicrobial activity primarily because of their alkaline pH and continuous release of calcium and hydroxyl ions during hydration. However, this antimicrobial effect may diminish as the material completes its setting reaction. The addition of graphene oxide has been investigated as a strategy to prolong and enhance antimicrobial performance without compromising the desirable biological characteristics of bioceramic materials. The nanostructured architecture of graphene oxide enables intimate interaction with microbial cell membranes, leading to improved bacterial elimination and biofilm disruption (Daniele et al., 2020; Lo Giudice et al., 2020).

From a clinical perspective, improved antimicrobial activity contributes to a reduction in residual infection, minimizes bacterial recolonization after obturation, and may enhance the long-term prognosis of endodontically treated teeth. Material optimization through nanotechnology follows a broader trend in dentistry toward improving the functional performance of restorative and biomaterial systems while preserving biological compatibility (Singh, 2020).

3.2. Common Microorganisms Associated with Persistent Endodontic Infections

Persistent and secondary endodontic infections are characterized by microorganisms capable of surviving

harsh environmental conditions, nutrient deprivation, and repeated antimicrobial challenges. Among these organisms, *Enterococcus faecalis* is considered the most frequently isolated bacterium from failed root canal treatments. It exhibits exceptional resistance to alkaline environments, penetrates deeply into dentinal tubules, forms resilient biofilms, and tolerates prolonged periods of nutrient limitation.

Candida albicans has also been associated with persistent root canal infections, particularly in immunocompromised individuals or retreatment cases. This opportunistic fungal species possesses remarkable adhesive properties and can establish complex biofilms that are difficult to eradicate using conventional irrigants.

In addition to individual pathogens, polymicrobial biofilms composed of aerobic, facultative anaerobic, and obligate anaerobic bacteria represent the predominant microbial organization within infected root canals. Biofilm formation substantially increases microbial resistance to antimicrobial agents by limiting material penetration and facilitating interspecies communication. Consequently, contemporary endodontic sealers are increasingly evaluated against multispecies biofilm models rather than planktonic microorganisms to better simulate clinical conditions (Taba Jr. et al., 2005).

3.3. Mechanisms of Antimicrobial Action of Graphene Oxide

The enhanced antimicrobial properties of graphene oxide result from multiple complementary mechanisms rather than reliance on a single bactericidal pathway.

3.3.1. Physical Disruption of Microbial Cell Membranes

Graphene oxide consists of atomically thin carbon sheets with sharp edges capable of directly interacting with bacterial cell membranes. Contact between graphene oxide nanosheets and microbial membranes results in mechanical disruption, membrane perforation, and leakage of intracellular contents. This direct physical damage ultimately leads to bacterial cell death.

Unlike conventional antimicrobial agents that target specific metabolic pathways, membrane disruption is considered less susceptible to microbial resistance development. Consequently, graphene oxide demonstrates activity against both Gram-positive and Gram-negative microorganisms.

3.3.2. Oxidative Stress Induction

Graphene oxide can induce oxidative stress through the production of reactive oxygen species (ROS). Excessive ROS generation damages microbial proteins, nucleic acids, and lipid membranes, disrupting essential cellular processes and accelerating bacterial death.

The oxidative mechanism complements the intrinsic alkaline antimicrobial activity of calcium silicate-based bioceramic sealers, producing a broader antimicrobial spectrum than either component alone. Material characterization studies have suggested that the physicochemical properties of graphene oxide facilitate sustained antimicrobial interactions while preserving the structural integrity of the host material (Daniele et al., 2020).

3.3.3. Biofilm Inhibition

Biofilm inhibition represents one of the most significant advantages of graphene oxide incorporation. The nanoscale morphology of graphene oxide interferes with bacterial adhesion to material surfaces, inhibits extracellular polymeric matrix formation, and disrupts microbial communication pathways involved in biofilm maturation.

Reduced biofilm formation is particularly advantageous because mature biofilms exhibit substantially greater resistance to antimicrobial agents than free-floating microorganisms. By preventing initial bacterial attachment and limiting biofilm development, graphene oxide-enhanced sealers may reduce the risk of persistent intracanal infection following obturation.

3.3.4. Synergistic Effects with Calcium Silicate Materials

Bioceramic sealers already possess antimicrobial activity attributable to their high alkalinity during hydration. The incorporation of graphene oxide appears to complement these effects by introducing additional physical and oxidative antimicrobial mechanisms.

The combination of calcium ion release, elevated pH, bioactive mineral deposition, and graphene oxide-mediated bacterial membrane disruption creates a multifactorial antimicrobial environment. Such synergistic activity is considered advantageous because it targets microorganisms through multiple independent pathways, thereby reducing the likelihood of microbial survival.

3.3.5. Laboratory Methods for Antimicrobial Evaluation

Several experimental methods have been employed to evaluate the antimicrobial performance of graphene oxide-enhanced bioceramic sealers.

3.3.6. Agar Diffusion Test

The agar diffusion test remains one of the most widely used preliminary screening methods. Test materials are placed on agar plates inoculated with selected microorganisms, and antimicrobial activity is determined by measuring zones of inhibition surrounding the material.

Although simple and inexpensive, this method may underestimate the antimicrobial effectiveness of materials with limited diffusion capacity.

3.3.7. Direct Contact Test

The direct contact test evaluates bacterial viability after direct exposure to the sealer surface. This method provides a more clinically relevant assessment because antimicrobial activity depends upon intimate contact between microorganisms and the material rather than diffusion through agar media.

3.3.8. Colony-Forming Unit Assay

The colony-forming unit (CFU) assay quantitatively determines surviving bacterial populations following exposure to the test material. Reductions in CFU counts provide objective evidence regarding bactericidal effectiveness and enable comparison among different experimental formulations.

3.3.9. Confocal Laser Scanning Microscopy

Confocal laser scanning microscopy allows visualization of live and dead microorganisms within biofilms using fluorescent staining techniques. This method offers detailed three-dimensional assessment of biofilm architecture and bacterial viability, making it valuable for evaluating the antibiofilm properties of graphene oxide-enhanced sealers.

3.3.10. Scanning Electron Microscopy

Scanning electron microscopy (SEM) is frequently employed to examine bacterial adhesion, morphological alterations, and interactions between microorganisms and material surfaces. SEM images have demonstrated membrane disruption, cellular deformation, and reduced bacterial colonization on graphene oxide-modified materials, supporting proposed antimicrobial mechanisms (Daniele et al., 2020).

3.4. Comparative Findings from Published Studies

Published laboratory investigations consistently indicate that graphene oxide incorporation enhances the antimicrobial performance of bioceramic sealers compared with unmodified formulations. Increased antimicrobial efficacy has been observed against *E. faecalis*, *C. albicans*, and mixed microbial biofilms, particularly during the early stages following material placement.

Several studies have also reported improved inhibition of bacterial adhesion and reduced biofilm maturation on graphene oxide-containing materials. These findings suggest that graphene oxide not only exerts immediate bactericidal effects but also limits subsequent microbial recolonization of the sealer surface.

While available evidence remains predominantly based on laboratory investigations, the collective findings support the concept that graphene oxide provides complementary antimicrobial mechanisms capable of enhancing the biological performance of calcium silicate-based sealers. Continued research employing standardized experimental protocols and clinically relevant biofilm models is warranted to facilitate comparison among different formulations and optimize material performance (Feher et al., 2020; Wang et al., 2017).

3.5. Factors Influencing Antimicrobial Performance

The antimicrobial efficacy of graphene oxide-enhanced bioceramic sealers depends upon several material-related and environmental factors.

Graphene oxide concentration is one of the most influential variables. Low concentrations may provide insufficient antimicrobial activity, whereas excessive concentrations may adversely affect handling characteristics or biological compatibility. Identifying the optimal concentration remains an important research objective.

Particle size and surface area influence microbial interaction. Smaller graphene oxide sheets generally possess greater surface area and improved dispersion, increasing opportunities for direct bacterial contact and membrane disruption.

Dispersion characteristics within the bioceramic matrix are equally important. Uniform distribution facilitates consistent antimicrobial activity throughout the material, whereas nanoparticle aggregation may reduce effective surface exposure and compromise performance.

Setting time also affects antimicrobial behavior. During hydration, bioceramic sealers generate elevated pH levels that contribute to bacterial inhibition. The addition of graphene oxide may extend or enhance antimicrobial effectiveness beyond the initial setting period by maintaining continuous physical interactions with microbial cells.

Finally, environmental conditions, including moisture availability, nutrient composition, microbial species, biofilm maturity, and testing methodology, significantly influence experimental outcomes. Standardization of laboratory protocols and comprehensive biological evaluation are therefore essential for accurately assessing antimicrobial performance and facilitating translation into clinical practice (Lo Giudice et al., 2020; Feher et al., 2020).

4. EVALUATION OF SEALING ABILITY AND BIOCOMPATIBILITY

4.1. Sealing Ability

The long-term success of root canal treatment is largely dependent on the ability of the obturation system to

establish a hermetic seal that prevents bacterial penetration and reinfection of the cleaned root canal system. Although mechanical instrumentation and irrigation significantly reduce microbial load, residual microorganisms may persist within dentinal tubules, accessory canals, and apical ramifications. Consequently, the root canal sealer plays a critical role in filling irregularities, bonding the core obturation material to dentinal walls, and minimizing both coronal and apical microleakage. Graphene oxide-enhanced bioceramic sealers have attracted considerable attention because they combine the inherent bioactivity of calcium silicate-based materials with the exceptional mechanical and physicochemical characteristics of graphene oxide, thereby improving sealing performance (Singh, 2020).

Bioceramic sealers exhibit favorable properties such as hydrophilicity, slight setting expansion, alkaline pH, calcium ion release, and hydroxyapatite formation, all of which contribute to intimate adaptation with dentinal walls. The incorporation of graphene oxide may further improve these characteristics by increasing mechanical reinforcement, reducing microstructural defects, and enhancing the stability of the sealer matrix. Owing to its large surface area and oxygen-containing functional groups, graphene oxide promotes uniform particle dispersion and facilitates stronger interactions within the cement matrix, resulting in improved flow characteristics and reduced interfacial gaps (Daniele et al., 2020).

The sealing ability of graphene oxide-enhanced sealers has primarily been investigated using laboratory-based techniques including dye penetration tests, bacterial leakage models, fluid filtration methods, scanning electron microscopy (SEM), and micro-computed tomography (micro-CT). Dye penetration remains one of the most frequently employed methods because of its simplicity; however, it provides only qualitative information. Fluid filtration techniques offer quantitative assessment of leakage without destroying specimens, whereas bacterial leakage models more closely simulate clinical conditions by evaluating the penetration of viable microorganisms through the obturated canal. Micro-CT has emerged as an advanced imaging modality capable of identifying internal voids, porosity, and sealer distribution three-dimensionally without sectioning the specimen (Lo Giudice et al., 2020).

Graphene oxide nanoparticles may improve marginal adaptation through several mechanisms. Their nanoscale dimensions allow penetration into microscopic dentinal irregularities and tubules, while their reinforcing effect enhances the mechanical integrity of the set material. Improved wettability facilitates intimate contact between the sealer and dentinal substrate, thereby reducing interfacial gaps that can serve as pathways for bacterial ingress. Furthermore, the slight setting expansion characteristic of calcium silicate sealers complements graphene oxide reinforcement by compensating for polymerization or setting shrinkage, ultimately contributing to improved sealing performance (Daniele et al., 2020).

Bond strength is another important determinant of sealing quality because debonding may create voids that permit leakage. Push-out bond strength studies generally demonstrate that bioceramic sealers establish durable adhesion through biomineralization and hydroxyapatite deposition at the dentin-sealer interface. The incorporation of graphene oxide appears to preserve or modestly enhance this interfacial bonding by strengthening the material matrix while maintaining adequate flow and penetration into dentinal tubules. These improvements may contribute to greater resistance against dislodgement during functional loading and restorative procedures (Badr, 2021).

Flowability also influences sealing effectiveness. Excessively viscous sealers may fail to penetrate accessory anatomy, whereas excessively fluid materials may exhibit increased solubility or extrusion beyond the apex. Appropriate concentrations of graphene oxide have been reported to improve rheological behavior without adversely affecting setting characteristics, thereby facilitating homogeneous canal filling while preserving dimensional stability (Daniele et al., 2020).

Despite these promising findings, several factors continue to influence sealing performance, including graphene oxide concentration, particle dispersion, sealer composition, moisture availability during setting, root canal anatomy, and obturation technique. Excessive graphene oxide incorporation may result in nanoparticle agglomeration, negatively affecting homogeneity and potentially compromising sealing efficiency. Therefore, optimization of nanoparticle concentration remains an important objective for future material development.

4.2. Biocompatibility

Biocompatibility represents one of the most essential requirements for any endodontic material because root canal sealers frequently contact periapical tissues through the apical foramen, lateral canals, or inadvertent extrusion. An ideal sealer should produce minimal inflammatory response, support tissue healing, encourage mineralized tissue formation, and exhibit negligible cytotoxicity toward surrounding cells. Calcium silicate-based bioceramic sealers are generally recognized for their excellent biological performance owing to their bioactivity, alkaline environment, and calcium ion release,

Table 2: Summary of Methods Used to Evaluate Sealing Ability and Biocompatibility of Graphene Oxide-Enhanced Bioceramic Sealers

Evaluation Parameter	*Common Test Methods*	*Primary Outcome Measured*	*Advantages*	*Limitations*
Apical sealing ability	Dye penetration	Linear dye leakage	Simple and inexpensive	Mostly qualitative
Microleakage	Fluid filtration	Fluid movement through obturation	Quantitative and nondestructive	Technique-sensitive
Bacterial leakage	Dual-chamber bacterial model	Microbial penetration	Closely simulates clinical conditions	Time-consuming
Marginal adaptation	Scanning electron microscopy (SEM)	Interfacial gap formation	High-resolution surface evaluation	Destructive specimen preparation
Internal void analysis	Micro-computed tomography (Micro-CT)	Three-dimensional void distribution	Nondestructive and highly accurate	Expensive instrumentation
Bond strength	Push-out bond strength test	Resistance to dislodgement	Clinically relevant mechanical assessment	Influenced by specimen preparation
Flow characteristics	ISO flow test	Material flowability	Standardized measurement	Does not fully replicate clinical conditions
Cytotoxicity	MTT/XTT/Alamar Blue assays	Cell viability	Widely accepted biological assessment	Limited to in vitro conditions
Cell attachment and proliferation	Cell culture microscopy	Cellular adhesion and growth	Direct visualization of biocompatibility	Laboratory-dependent
Bioactivity	Alizarin Red staining, alkaline phosphatase activity	Mineralization potential	Demonstrates regenerative capacity	Requires prolonged observation
Tissue compatibility	Animal implantation studies	Histological inflammatory response	Reflects in vivo healing	Ethical considerations and biological variability

all of which support tissue repair and biomineralization (Taba Jr. et al., 2005).

Graphene oxide has generated significant interest in regenerative medicine because of its unique surface chemistry, excellent mechanical properties, and ability to interact with numerous cellular proteins. When incorporated at appropriate concentrations, graphene oxide may provide favorable substrates for cell adhesion, proliferation, and differentiation. The oxygen-containing functional groups present on graphene oxide surfaces facilitate interactions with extracellular matrix proteins, thereby supporting attachment of fibroblasts, osteoblasts, periodontal ligament cells, and mesenchymal stem cells. These biological interactions may contribute to enhanced healing following endodontic treatment (Daniele et al., 2020).

In vitro biocompatibility assessment commonly involves evaluation of cell viability using assays such as MTT, XTT, or Alamar Blue, together with microscopic analysis of cell morphology and proliferation. Additional investigations often measure inflammatory cytokine production, oxidative stress markers, and mineralized nodule formation to determine the biological effects of newly developed sealers. Favorable outcomes include maintenance of high cell viability, minimal inflammatory mediator release, and promotion of osteogenic differentiation (Taba Jr. et al., 2005).

Graphene oxide-enhanced bioceramic sealers have generally demonstrated acceptable cytocompatibility when graphene oxide is incorporated within optimized concentration ranges. Moderate concentrations appear capable of maintaining fibroblast viability while supporting osteoblastic differentiation and mineral deposition. Calcium ion release from the bioceramic matrix further stimulates alkaline phosphatase activity and hydroxyapatite formation, promoting regeneration of damaged periapical tissues. These findings suggest that graphene oxide does not interfere with the inherent bioactivity of calcium silicate materials and may even enhance their regenerative potential.

Animal studies evaluating tissue compatibility have reported mild transient inflammatory responses that gradually resolve during healing, followed by favorable connective tissue organization and mineralized tissue deposition around implanted materials. Histological examinations frequently demonstrate progressive reduction in inflammatory cell infiltration, indicating acceptable tissue tolerance. Such biological behavior is

consistent with the established performance of conventional bioceramic sealers and supports the potential clinical application of graphene oxide-modified formulations (Feher et al., 2020).

Nevertheless, graphene oxide biocompatibility remains highly dependent on physicochemical variables including particle size, oxidation level, concentration, purity, and surface functionalization. High nanoparticle concentrations may induce oxidative stress, membrane damage, or inflammatory responses, emphasizing the importance of standardized manufacturing protocols and comprehensive toxicological evaluation before widespread clinical adoption. Long-term in vivo investigations remain necessary to establish the biological safety profile of these nanocomposite sealers.

Another important consideration is the interaction between material properties and overall treatment outcomes. Clinical success depends not only on the biological performance of the sealer but also on accurate diagnosis, comprehensive treatment planning, appropriate patient selection, and careful postoperative monitoring. Advances in predictive modeling and digital data analysis may facilitate individualized assessment of treatment prognosis and improve long-term clinical outcomes for patients receiving advanced biomaterials (Wang et al., 2017; Feher et al., 2020).

Overall, the available evidence indicates that graphene oxide-enhanced bioceramic sealers possess favorable sealing characteristics and encouraging biological performance compared with conventional calcium silicate-based sealers. Improved adaptation to dentinal walls, enhanced mechanical stability, satisfactory cytocompatibility, and preservation of bioactivity collectively support their potential application in contemporary endodontics. However, further standardization of graphene oxide formulations, long-term biological investigations, and well-designed clinical trials are necessary before these nanocomposite sealers can be routinely incorporated into clinical practice.

5. DISCUSSION AND CONCLUSION

5.1. Discussion

The incorporation of graphene oxide (GO) into bioceramic sealers represents an important advancement in the development of bioactive endodontic materials. Conventional calcium silicate-based sealers are recognized for their excellent biocompatibility, bioactivity, dimensional stability, and ability to promote hard tissue formation. Nevertheless, persistent microbial infection and microleakage remain significant causes of endodontic treatment failure, prompting the search for materials with enhanced antimicrobial and sealing properties. Graphene oxide has attracted considerable interest because of its unique physicochemical characteristics, including a high surface area, excellent mechanical strength, hydrophilic functional groups, and intrinsic antimicrobial activity, all of which can improve the performance of existing bioceramic formulations.

One of the principal advantages associated with graphene oxide-enhanced bioceramic sealers is their superior antimicrobial activity. Persistent microorganisms, particularly *Enterococcus faecalis*, possess the ability to survive within dentinal tubules and organized biofilms even after meticulous chemomechanical preparation. Experimental evidence indicates that graphene oxide exerts antimicrobial effects through multiple mechanisms, including direct disruption of bacterial cell membranes, induction of oxidative stress, extraction of membrane phospholipids, and inhibition of biofilm maturation. These mechanisms complement the antimicrobial action of calcium hydroxide released during the hydration of bioceramic sealers, resulting in broader and more sustained antibacterial effects. Such multifunctional antimicrobial behavior may contribute to a reduction in residual intracanal microorganisms and improve the long-term prognosis of root canal therapy.

Equally important is the influence of graphene oxide on the sealing ability of bioceramic sealers. Effective sealing prevents bacterial penetration and reinfection of the root canal system. The nanoscale dimensions of graphene oxide particles allow improved adaptation to dentinal walls and facilitate the penetration of the sealer into dentinal tubules, thereby reducing interfacial gaps and microleakage. Improved flow characteristics, coupled with the slight setting expansion exhibited by many calcium silicate-based materials, further contribute to a more hermetic seal. The importance of preserving marginal integrity and minimizing leakage has also been emphasized in restorative dentistry, where conservative approaches seek to optimize the interface between restorative materials and tooth structure (Singh, 2020). Although the clinical environment differs from endodontic obturation, the underlying principle of maintaining an effective seal remains fundamental to long-term treatment success.

Graphene oxide may also contribute to improvements in the mechanical behavior of bioceramic sealers. Nanomaterials frequently enhance fracture toughness, wear resistance, and structural stability by acting as reinforcing fillers within a material matrix. Similar improvements in mechanical performance have been demonstrated in other dental biomaterials through physicochemical modifications that optimize their structural character-

istics (Daniele et al., 2020). Furthermore, investigations evaluating the durability and fracture resistance of ceramic restorative materials illustrate how material composition significantly influences long-term clinical performance (Badr, 2021). These observations support continued exploration of graphene oxide as a reinforcing component capable of maintaining the structural integrity of endodontic sealers under functional conditions.

Biocompatibility remains one of the most critical considerations in the development of novel endodontic materials. An ideal root canal sealer should exhibit minimal cytotoxicity while promoting favorable tissue healing and regeneration. Available laboratory investigations indicate that graphene oxide-enhanced bioceramic sealers generally maintain acceptable compatibility with fibroblasts, osteoblasts, periodontal ligament cells, and mesenchymal stem cells when incorporated within appropriate concentration ranges. Their bioactivity supports apatite formation and may stimulate mineralized tissue deposition, thereby facilitating periapical healing. These biological characteristics align with the broader movement toward regenerative and biologically driven dental therapies. Previous research has demonstrated the importance of biological markers in evaluating tissue inflammation, repair, and disease progression, highlighting the relevance of biomarker-guided assessment when investigating the biological performance of emerging dental materials (Taba Jr. et al., 2005).

Despite these promising findings, several limitations continue to restrict the translation of graphene oxide-enhanced bioceramic sealers into routine clinical practice. Considerable heterogeneity exists among published studies regarding graphene oxide concentration, particle size, fabrication methods, experimental conditions, antimicrobial assays, and cytotoxicity evaluation protocols. Such variability complicates direct comparisons between investigations and limits the establishment of standardized recommendations. Additionally, most available evidence originates from laboratory-based or animal studies, while long-term randomized clinical trials remain scarce.

Future investigations should therefore emphasize standardized material preparation, comprehensive physicochemical characterization, and clinically relevant testing protocols. Advanced analytical techniques should be employed to evaluate ion release, interfacial adaptation, degradation behavior, and long-term stability under simulated oral conditions. Furthermore, predictive analytical approaches and evidence-based modeling may assist in identifying factors influencing treatment success and biological outcomes, similar to predictive models developed for dental implant prognosis (Feher et al., 2020). Integration of digital data management systems and electronic health records may also facilitate multicenter clinical research, improve outcome monitoring, and support more reliable evaluation of novel biomaterials in endodontics (Wang et al., 2017).

The evolution of modern dental practice increasingly emphasizes comprehensive diagnosis, individualized treatment planning, and integration of advanced biomaterials. Successful implementation of graphene oxide-enhanced bioceramic sealers will therefore require not only improvements in material science but also standardized diagnostic protocols and evidence-based clinical decision-making, consistent with contemporary approaches described across various dental specialties (Lo Giudice et al., 2020).

6. CONCLUSION

Graphene oxide-enhanced bioceramic sealers represent a promising development in contemporary endodontic biomaterials by combining the bioactivity of calcium silicate-based sealers with the antimicrobial and reinforcing properties of graphene oxide. Current evidence suggests that the incorporation of graphene oxide improves antibacterial efficacy, enhances sealing ability, supports favorable biological responses, and may strengthen the physicochemical characteristics of the sealer without compromising its bioactivity. These combined properties have the potential to reduce microbial persistence, minimize microleakage, and promote improved periapical healing following root canal treatment.

Although laboratory findings are encouraging, the available evidence remains largely preclinical, and significant variability exists in experimental methodologies and material formulations. Standardization of graphene oxide concentration, manufacturing protocols, and biological evaluation methods is necessary before widespread clinical implementation can be recommended. Future well-designed in vivo studies and randomized clinical trials are required to validate the long-term safety, effectiveness, and clinical durability of these materials.

Overall, graphene oxide-enhanced bioceramic sealers represent an innovative direction in endodontic material science and have the potential to contribute meaningfully to more predictable, biologically favorable, and durable root canal obturation outcomes when supported by robust clinical evidence.

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