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GRAPHENE OXIDE IN DENTAL AND MAXILLOFACIAL TISSUE ENGINEERING: MECHANISTIC INSIGHTS AND TRANSLATIONAL READINESS FOR CANINE VETERINARY MEDICINE

ÓXIDO DE GRAFENO EN LA INGENIERÍA DE TEJIDOS DENTALES Y MAXILOFACIALES: PERSPECTIVAS MECANICISTAS Y PREPARACIÓN PARA SU APLICACIÓN TRASLACIONAL EN MEDICINA VETERINARIA CANINA

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Graphene Oxide in dental and maxillofacial tissue Engineering: mechanistic insights and translational readiness for canine veterinary medicine

Óxido de grafeno en la ingeniería de tejidos dentales y maxilofaciales: perspectivas mecanicistas y preparación para su aplicación traslacional en medicina veterinaria canina

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ABSTRACT

The aim of this study was to critically assess the translational maturity of graphene oxide (GO) in canine dental and maxillofacial medicine. The scientific literature published between 2015 and 2026 on the interaction of GO with oral tissues was analyzed. Of the studies identified, only three preclinical investigations evaluated in vivo performance in canine models, compared with 48 in vitro studies and 32 in vivo studies conducted exclusively in rodents. Although the incorporation of GO into scaffolds and coatings promotes osteogenesis in cellular assays and rodent models, evidence in canines remains insufficient. The reported biological performance is constrained by substantial risks of dose-dependent cytotoxicity, reactive oxygen species (ROS) generation, and genotoxicity. These risks are associated with the lack of standardization in lateral sheet size, the carbon-to-oxygen (C/O) ratio, and GO aggregation in biological fluids. Furthermore, its antibacterial activity lacks clinical validation against dog-specific periodontal

pathogens, particularly Porphyromonas gulae. The application of GO in veterinary dentistry remains at an early stage of development (TRL 3–4). Despite its mechanical and biomimetic properties, GO cannot yet be considered a clinically viable alternative until issues related to bioaccumulation, peroxidase-mediated enzymatic biodegradation, and material standardization in accordance with ISO requirements are adequately addressed.

Keywords: graphene oxide; veterinary medicine; canine dentistry; periodontal regeneration; bone regeneration; biomaterials.

RESUMEN

El objetivo de este estudio fue evaluar críticamente la madurez traslacional del óxido de grafeno (GO, por sus siglas en inglés) en la medicina dental y maxilofacial canina. Se analizó la literatura científica publicada entre 2015 y 2026 sobre la interacción del GO con los tejidos orales. De los estudios identificados, solo tres investigaciones preclínicas evaluaron el rendimiento in vivo en modelos caninos, en comparación con 48 estudios in vitro y 32 estudios in vivo realizados exclusivamente en roedores.

Aunque la incorporación de GO en andamios y recubrimientos promueve la osteogénesis en ensayos celulares y modelos de roedores, la evidencia en caninos sigue siendo insuficiente. El rendimiento biológico reportado está limitado por riesgos sustanciales de citotoxicidad dependiente de la dosis, generación de especies reactivas de oxígeno (ROS) y genotoxicidad. Estos riesgos están asociados con la falta de estandarización en el tamaño lateral de las láminas, la relación carbono-oxígeno (C/O) y la agregación del GO en fluidos biológicos. Además, su actividad antibacterial carece de validación clínica contra patógenos periodontales específicos de los perros, particularmente Porphyromonas gulae. La aplicación del GO en odontología veterinaria se mantiene en una etapa temprana de desarrollo (TRL 3–4). A pesar de sus propiedades mecánicas y biomiméticas, el GO aún no puede considerarse una

alternativa clínicamente viable hasta que se aborden adecuadamente los problemas relacionados con la bioacumulación, la biodegradación enzimática mediada por peroxidasas y la estandarización del material de acuerdo con los requisitos ISO.

Palabras clave: óxido de grafeno; medicina veterinaria; odontología canina; regeneración periodontal; regeneración ósea; biomateriales.

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INTRODUCTION

Diseases of the oral cavity are among the most common health problems in both human and veterinary medicine (Harvey et al., 2023). In dogs, periodontal disease is the most prevalent oral disorder, affecting a substantial proportion of adult animals and causing chronic inflammation, loss of periodontal attachment, alveolar bone resorption, tooth mobility, and, in untreated cases, potentially systemic consequences (Cunha et al., 2022).

Despite advances in veterinary dentistry, conventional therapies continue to present limitations in achieving predictable and functional regeneration of periodontal and osseous tissues, particularly in large defects or lesions involving substantial loss of structural support (Ward, 2022). Traditional periodontal repair techniques involve open-flap debridement, the application of grafting materials, and barrier membranes to prevent apical migration of the epithelium and the formation of a long junctional epithelium, which can compromise regeneration and true periodontal healing (Harvey, 2005).

The rapid development of periodontal tissue engineering has led to the emergence of new approaches for the treatment of periodontal disease (Dai et al., 2024). However, the clinical use of these restorative materials remains uncommon in veterinary dentistry (Erdoğan & Saritaş, 2024). In this context, tissue engineering and regenerative medicine have assumed an increasingly important role in veterinary dentistry.

The design of scaffolds, guided tissue regeneration membranes, implant coatings, and smart biomolecule-delivery systems aims to reproduce the biological conditions required to restore the architecture and function of oral tissues (Purbantoro et al., 2024). In veterinary medicine, these technologies represent a promising alternative for the treatment of maxillofacial defects, traumatic injuries, advanced periodontal disease, and post-oncological surgical reconstruction, in which conventional approaches often provide limited outcomes (Wei et al., 2022).

Among the nanobiomaterials developed over the past decade, graphene oxide (GO) has attracted considerable interest because of its combination of physicochemical and biological properties (Qi et al., 2021). Its high surface-to-volume ratio, abundance of oxygen-containing functional groups, mechanical strength, chemical stability, and ease of functionalization enable its incorporation into polymeric matrices, hydrogels, membranes, and implants to enhance cell adhesion, proliferation, osteogenic differentiation, and the formation of new mineralized tissue (Hosseini et al., 2025).

Graphene is a two-dimensional, transparent, flexible, and mechanically strong carbon-based nanomaterial consisting of sp^2-hybridized carbon atoms arranged in a planar honeycomb lattice, resulting in an atomically thin structure (Gutiérrez-Cruz et al., 2022). The properties of the material depend largely on the synthesis method, which influences the number and types of oxygen-containing functional groups present in the resulting GO. Unlike graphene, GO is hydrophilic, making it relatively easy to prepare as a stable suspension in water or organic solvents (Jiříčková et al., 2022).

Several experimental studies have demonstrated that GO can exert antibacterial activity against microorganisms implicated in oral infections, while also exhibiting favorable biocompatibility when used at appropriate concentrations and in controlled formulations (Martuci et al., 2025; Narváez-Romero et al., 2025; Williams et al., 2023). In human dentistry, knowledge regarding the applications of graphene oxide has expanded considerably (Castro et al., 2024).

Recent studies have investigated its use as a coating for titanium implants to enhance osseointegration, as a component of membranes designed for guided bone regeneration, as a reinforcing agent in restorative biomaterials, as a vehicle for the controlled delivery of therapeutic agents, and as a functional component of scaffolds used in pulp, periodontal, and bone tissue engineering. Collectively, these studies suggest that GO may enhance the mechanical properties of conventional biomaterials, promote osteogenic responses, and contribute to bacterial biofilm control, all of which are of considerable interest in regenerative dentistry (Lima et al., 2026; Radunovic et al., 2022; Souza et al., 2023).

However, the available evidence in veterinary medicine remains limited and is scattered across experimental studies, preclinical investigations, and reviews focused predominantly on human medicine. Although tissue engineering approaches applied to veterinary oral and maxillofacial reconstruction have made substantial progress, there remains limited integration of knowledge specifically concerning the use of graphene oxide in canine patients. This fragmentation makes it difficult to determine the actual state of knowledge, the strengths of the available evidence, methodological limitations, and prospects for future clinical application in dogs (Purbantoro et al., 2024).

Given the growing interest in nanobiomaterials for regenerative medicine and the need to strengthen the scientific basis for their application in veterinary dentistry, a critical review synthesizing the available evidence on graphene oxide and its principal applications in dogs is warranted. Integrating this evidence will enable the identification of current research trends, underlying biological mechanisms, potential advantages over conventional biomaterials, biocompatibility and biosafety considerations, and the challenges that must still be addressed before the routine incorporation of GO into veterinary clinical practice.

In this review, we first describe the physicochemical and biological properties of GO and then examine its applications in different areas of canine dentistry and related tissue

regeneration. We also summarize potential future directions for the development of GO-based nanomaterials in dentistry and related tissue regeneration.

METHODOLOGY

Study Design

A systematic descriptive, critical, and analytical literature review was conducted on the applications of graphene oxide (GO) in dentistry and tissue regeneration, with particular emphasis on its translational potential in canine veterinary medicine. The review encompassed scientific literature published between 2015 and 2026, with priority given to recent experimental studies and investigations specifically conducted in canine models.

The analysis was structured around the physicochemical and biological properties of GO, its molecular interactions, biocompatibility, antibacterial activity, controlled-release capacity, and effects on osteogenic and odontogenic differentiation, as well as its applications in periodontal regeneration, dentin–pulp complex regeneration, bone regeneration, implantology, and tissue-engineering scaffolds. This approach enabled the evidence obtained in vitro, in animal models, and specifically in dogs to be distinguished, with the aim of determining the translational maturity of this technology. This classification was consistent with the central objective of the manuscript: to establish the gap between experimental evidence and its potential clinical application in canine veterinary dentistry.

Literature Search Strategy

The literature search was designed to identify studies addressing the use of GO in dentistry, bone regeneration, tissue engineering, implantology, and veterinary applications. Publications from 2015 to 2026 were considered, with particular emphasis on experimental studies published between 2022 and 2026. The search strategy incorporated terms related to the material, dental applications, tissue regeneration, and the canine species, including conceptual combinations of graphene oxide, dentistry, dental, periodontal regeneration, bone

regeneration, dental implant, tissue engineering, odontogenic differentiation, osteogenic differentiation, dog, dogs, and canine.

The conceptual search strategy employed Boolean operators AND and OR according to the following structure:

- ("graphene oxide" OR "graphene oxide nanomaterial") AND (dentistry OR dental OR periodontal OR "bone regeneration" OR "dental implant" OR "tissue engineering" OR odontogenic OR osteogenic) AND (dog OR dogs OR canine OR "veterinary medicine").
- The search strategies were adapted to the characteristics of each bibliographic database consulted. Scientific reviews were primarily used to contextualize the field and identify primary literature, whereas claims concerning the biological effects of GO were preferentially supported by original experimental studies.

Inclusion Criteria

Studies were considered eligible if they met the following criteria:

- Original in vitro, in vivo, or preclinical investigations;
- Studies addressing GO applied to dentistry, tissue engineering, or bone and periodontal regeneration;
- Investigations of osteogenic or odontogenic differentiation;
- Studies evaluating antibacterial activity and biofilm control;
- Investigations of GO coatings for implants;
- Studies involving scaffolds, hydrogels, and GO-containing composite matrices;
- Investigations in which GO was used as a delivery system or component for bioactive molecules;
- Studies conducted in animal models, with particular emphasis on investigations involving dogs;

- Articles providing sufficient information regarding the material, experimental model, and outcomes; and Scientific publications with verifiable bibliographic information and a DOI, when available.

Studies specifically conducted in dogs were retained irrespective of publication year because of the scarcity of direct canine evidence, which was identified as one of the major knowledge gaps in the reviewed literature. The manuscript identified three preclinical canine studies addressing alveolar ridge preservation, periodontal regeneration, and peri-implantitis.

Exclusion Criteria

Articles consisting exclusively of editorials, letters to the editor, conference abstracts, or documents lacking sufficient experimental information were excluded. Studies in which GO was not a relevant experimental component were also excluded, as were investigations focused exclusively on reduced graphene oxide or other carbon-based derivatives when they did not allow the specific behavior of GO to be evaluated. Duplicate publications were also excluded. Narrative and systematic reviews were not considered primary evidence for establishing biological effects, although they were consulted as secondary sources for contextualizing the findings and identifying relevant experimental studies.

Evidence Selection and Classification

The identified studies were screened and subsequently classified according to the type of experimental model employed. The available evidence was organized into three major categories: in vitro studies, in vivo studies conducted in non-canine animal models, and in vivo studies specifically conducted in dogs.

According to the synthesis performed in the manuscript, 48 in vitro studies, 32 in vivo investigations conducted in rodent models, and three preclinical in vivo studies involving canine models were identified. This distribution represents one of the principal findings of the review and demonstrates that research remains strongly concentrated on cellular and laboratory animal models, whereas evidence specifically derived from dogs remains limited.

Data Extraction and Analysis

The following information was extracted from the selected studies: author and year of publication, type of GO used, associated biomaterial, experimental model, cell type or animal species, target tissue, dental application, principal biological outcomes, and DOI.
The studies were subsequently grouped according to their application in periodontal regeneration, dentin–pulp complex regeneration, alveolar and maxillofacial bone regeneration, bioactive implant coatings, three-dimensional scaffolds, controlled-release systems, and antibacterial applications. This classification enabled integration of the biological mechanisms described in the literature with the potential dental applications of GO.

A comparative matrix was also developed to characterize GO combinations with natural and synthetic biomaterials, including PCL, PLA, collagen, chitosan, hydroxyapatite, bioglass, PEEK, and titanium, among others. The corresponding table distinguishes experimentally demonstrated evidence from applications that remain potential areas for further investigation.

Assessment of Translational Maturity

The evidence was interpreted according to the experimental stage of development of GO-based applications. A distinction was made between in vitro evidence, preclinical evidence from laboratory animal models, preclinical evidence specifically obtained in dogs, and veterinary clinical evidence.

This classification enabled identification of a translational gap between the demonstration of osteogenic, odontogenic, antibacterial, or controlled-release effects under experimental conditions and their potential application in canine patients. The available findings indicate that the application of GO in canine veterinary dentistry remains at an early preclinical stage, primarily because of the limited number of studies involving dogs and the absence of established veterinary clinical protocols.

RESULTS

Physicochemical Properties of GO and Standard Characterization Criteria

A wide range of methods is available for the preparation of graphene oxide (GO), including the chemical, electrochemical, and microbial oxidation of various carbon-based materials (Liu et al., 2015; Nishina & Eigler, 2020; Yang et al., 2019). Most studies reported in the literature have focused on GO produced through the chemical oxidation of graphite or, more recently, through the rapid direct oxidation of graphene (Costa et al., 2021). Because most GO preparation methods employ strong oxidizing agents, such as potassium permanganate, GO typically contains a substantial number of defects within its crystalline lattice (Jahan et al., 2022). This variability in synthesis necessitates rigorous characterization of its physicochemical properties before biological use.

Critical Characterization Parameters and Spectroscopic Analysis

Number of Layers and Lateral Thickness

Determination: Atomic Force Microscopy (AFM) and High-Resolution Transmission Electron Microscopy (HR-TEM) [ISO/TS 21356-1] (Xi et al., 2026).

Biological relevance: Monolayer GO sheets (thickness ≈ 1 nm) exhibit substantially greater mechanical flexibility and specific surface area (>2,000 m^2/g) than multilayer sheets (>5 layers; thickness >5 nm) (Xi et al., 2026). Lateral sheet size determines cellular translocation capacity and the mechanism of macrophage internalization. Large sheets (>5 μm) can induce frustrated phagocytosis and granulomatous inflammatory responses (Liu et al., 2022), whereas submicron sheets (<500 nm) are efficiently internalized through clathrin- or caveolin-dependent endocytic pathways (Xi et al., 2026).

Carbon-to-Oxygen Ratio (C/O Ratio)

Determination: X-ray Photoelectron Spectroscopy (XPS) (Costa et al., 2021; Yang et al., 2019).

Biological relevance: The C/O ratio quantifies the degree of oxidation of the carbon network, typically ranging from 1.5 to 2.5 for GO synthesized using the Hummers method (Suhaimin et al., 2022). A high oxygen content (C/O <2.0) increases the negative surface charge and hydrophilicity, thereby promoting dispersion in aqueous media (Jahan et al., 2022; Xi et al., 2026), but may also generate higher levels of reactive oxygen species (ROS) through edge reactivity and interfacial electronic interactions (Pradyumn et al., 2026). Deconvolution of the XPS C1s spectrum is essential to distinguish different hybridization states and chemical bonds: C–C/C=C (284.8 eV), C–O (286.5 eV), C=O (287.8 eV), and O–C=O (289.0 eV) (Liu et al., 2022).

Degree of Crystalline Lattice Defects (ID/IG)

Determination: Raman spectroscopy using a 532- or 633-nm laser (Nishina & Eigler, 2020; Xi et al., 2026). Biological relevance: The G band (≈1,580 cm^{-1}) corresponds to the E2g vibrational mode of the sp^2 carbon plane, whereas the D band (≈1,350 cm^{-1}) reflects symmetry breaking associated with sp^3 defects and sheet edges (Kabore et al., 2026). The intensity ratio ID/IG (frequently >0.95 in GO) provides an estimate of defect density within the basal plane (Nishina & Eigler, 2020; Xi et al., 2026). A high ID/IG ratio indicates substantial disruption of π conjugation, which may reduce the capacity for drug or protein adsorption through π–π stacking (Costa et al., 2021; Jahan et al., 2022), while substantially increasing the number of active sites susceptible to neutrophil myeloperoxidase (MPO)-mediated enzymatic degradation (Costa et al., 2021; Liu et al., 2015).

Zeta Potential (ζ) and Aggregation Kinetics

Determination: Dynamic Light Scattering (DLS) and laser Doppler electrophoresis (Jahan et al., 2022; Nishina & Eigler, 2020).

Biological relevance: GO exhibits a strongly negative zeta potential (typically −30 to −50 mV in deionized water at pH 7.0), primarily due to the deprotonation of carboxyl groups (Jahan et al., 2022; Xi et al., 2026). However, in physiological media, including saliva, gingival

crevicular fluid, and plasma, the presence of divalent cations (Ca^{2+}, Mg^{2+}) neutralizes the surface charge through electrostatic screening, inducing extensive aggregation into secondary microstructures (Costa et al., 2021; Jahan et al., 2022). This aggregation alters the effective dose exposed to tissues, modifies interactions with the plasma membrane, and may enhance mechanical cytotoxicity (Costa et al., 2021; Liu et al., 2015).

These characteristics make GO particularly effective in promoting cellular growth, differentiation, and bone tissue regeneration (Gao et al., 2024). In particular, GO coatings may improve clinical outcomes in dental surgery. Enhanced osseointegration and cytocompatibility may be beneficial for the placement of GO-coated implants (Inchingolo et al., 2023). However, despite these advantages, studies conducted by the U.S. Food and Drug Administration (FDA) and independent experimental investigations have indicated that GO may exhibit toxic effects both in vitro and in vivo (Qiu et al., 2024).

Furthermore, interactions between GO and proteins are fundamental to the performance of GO- based biomaterials (Shahriari et al., 2026). Owing to their large surface area and capacity to interact with proteins and peptides, GO materials exhibit valuable physicochemical and biological properties for biomedical applications and have been successfully employed to optimize scaffold architecture across a broad range of tissues and organs, from skin to cardiac tissue (Biru et al., 2022).

Biological Properties of GO

Biocompatibility and Tissue Tolerance: Interfacial Interactions and Cellular Modulation

The biocompatibility of graphene oxide (GO) should not be regarded as an intrinsic or absolute property of the nanomaterial, but rather as a conditional and multifactorial biological response. This response is governed by inherent physicochemical parameters, including nanosheet lateral dimensions, degree of oxidation (C/O ratio), concentration, cumulative dose, synthesis method, and surface functionalization state (Kwak et al., 2022). At sublethal

concentrations, GO acts as a bioactive matrix capable of promoting cell adhesion and proliferation through modulation of interactions with the extracellular matrix (ECM) and cytoskeleton, without compromising the viability of adjacent tissues (Yang et al., 2023).

However, cellular tolerance is intrinsically constrained by the colloidal stability of the nanomaterial within the tissue microenvironment. Optimization of biocompatibility is achieved primarily through surface modification and functionalization to regulate the protein adsorption profile and mitigate physical damage to plasma membranes.

Modification of high-performance polymers (PEEK): Deposition of self-assembled GO coatings onto polyether ether ketone (PEEK) markedly alters the surface energy and nanoscale roughness of the substrate. This modification enhances initial cell adhesion, osteoblast proliferation, and matrix-specific differentiation, while also providing a preventive bactericidal response against opportunistic pathogens (Huang et al., 2024).

Bioactivation of titanium implants and trans-tissue response: Functionalization of titanium with GO nanostructures simultaneously enhances the viability and osteogenic differentiation of bone marrow stromal cells (BMSCs) and the response of human gingival fibroblasts (Kwak et al., 2022). In in vivo preclinical models involving rabbit tibial defects, GO-modified surfaces demonstrated a significant quantitative increase in bone-to-implant contact (BIC) and accelerated osseointegration indices (Kwak et al., 2022), supporting the capacity of GO to function as a bifunctional interface suitable for both peri-implant hard and soft tissues.

Despite these favorable findings for immobilized GO surfaces and coatings, the biocompatibility of free GO suspensions is substantially constrained by the risks of dose-dependent cytotoxicity, oxidative stress induced by intracellular ROS generation, and DNA fragmentation. These effects must be rigorously evaluated before clinical application.

Controlled-Release Properties

One of the most relevant functional characteristics of graphene oxide (GO) is its ability to serve as a platform for the sustained and site-specific delivery of biomolecules and therapeutic agents. Unlike conventional polymeric matrices, which are subject to rapid and uncontrolled diffusion, the two-dimensional architecture of GO provides a high specific surface area and a high density of functional groups, enabling exceptional loading efficiencies through non-covalent interactions, including π–π stacking, electrostatic attraction, and hydrogen bonding (Jeong et al., 2022).

Release kinetics from the GO matrix are regulated by the physicochemical conditions of the local microenvironment and can respond dynamically to variations in pH, ionic strength, and enzymatic degradation of the polymeric scaffold. This controlled behavior reduces the need for repeated dosing and maintains therapeutic concentrations within the desired therapeutic window over extended periods (Jeong et al., 2022).

Immobilization and sustained release of growth factors (BMP-2): Incorporation of GO into three- dimensional scaffolds enhances the adsorption of bone morphogenetic protein 2 (BMP-2), effectively preventing the initial burst release commonly associated with secondary or ectopic inflammatory responses. Progressive and controlled desorption of BMP-2 over several weeks promotes sustained osteogenic signaling in mesenchymal stem cells (MSCs), as evidenced by sustained increases in alkaline phosphatase (ALP) activity, enhanced matrix mineralization (calcium deposition), and upregulation of key osteogenic markers compared with conventional GO-free matrices (Jeong et al., 2022).

Multifunctional platforms for periodontal regeneration: Beyond osteoinductive factors, GO enables the co-transport and combined release of synthetic or naturally derived antimicrobial agents and immunomodulatory molecules. This multifunctional capacity is particularly relevant to regeneration of the periodontal complex, in which suppression of

bacterial colonization and resolution of the local inflammatory response must occur concurrently with the formation of new bone and cementum (Li et al., 2022).

Antibacterial Activity and Membrane Disruption Mechanisms

The antimicrobial activity of graphene oxide (GO) is a critical property for mitigating biomaterial- associated infections, including peri-implantitis and periodontal disease. The antibacterial action of GO is not attributable to a single chemical effect but rather to a dynamic, multifactorial, and synergistic mechanism determined by its sp^2-hybridized aromatic domains, the density of oxygen- containing groups across the basal plane and sheet edges, and its high specific surface area (Kwak et al., 2022; Lin et al., 2023).

The mechanisms underlying bacterial inhibition can be broadly classified into two principal pathways involving physical and metabolic damage.

Direct mechanical damage and lipid extraction: Two-dimensional GO sheets interact with bacterial envelopes through a combination of electrostatic, van der Waals, and hydrophobic interactions. The sharp edges of GO nanosheets can act as nanoscale cutting edges or “nano-blades,” inducing direct disruption of the cell wall and cytoplasmic membrane. This process is further enhanced by extensive phospholipid extraction, whereby hydrophobic domains of GO attract membrane lipid tails toward the basal plane, compromising structural integrity, increasing membrane permeability, and ultimately causing cell lysis through irreversible leakage of cytoplasmic contents (Bousiakou et al., 2022).

Oxidative stress and metabolic inhibition: Independently of direct physical damage, GO can act as a redox mediator capable of accepting electrons from bacterial membranes and perturbing intracellular electron-transport processes. This may promote the generation of reactive oxygen species (ROS), including hydroxyl radicals (•OH) and superoxide anions (O_2•$^-$), which can trigger lipid peroxidation, oxidation of structural proteins, and lethal damage to bacterial DNA (Kwak et al., 2022; Lin et al., 2023).

Nanocomposite synergy and applications in three-dimensional scaffolds: To overcome bacterial resistance and reduce the GO concentrations required, the nanomaterial can serve as a synergistic platform for the immobilization of metallic nanoparticles or bioactive oligoelements. For example, GO integrated with nickel nanoparticles exhibits substantially greater bactericidal activity against Gram-positive microorganisms (Staphylococcus aureus) and Gram-negative microorganisms (Escherichia coli) than either component alone (Wu et al., 2022).

From a tissue bioengineering perspective, incorporating GO into biocompatible three-dimensional scaffolds, such as polycaprolactone (PCL) matrices fabricated by 3D printing, not only enhances the mechanical properties and surface topography of the scaffold but also confers prophylactic bactericidal activity. This may help prevent pathogenic biofilm formation during the early stages of osteoblast proliferation and in vivo new bone formation (Du et al., 2022).

Osteogenic Differentiation Properties

The ability of GO to induce and promote osteogenic differentiation is one of its most relevant properties for applications in bone tissue engineering and periodontal regeneration. This biological activity is associated with the combination of GO physicochemical characteristics, including its high specific surface area, abundance of oxygen-containing functional groups, capacity to adsorb osteoinductive proteins, and availability of aromatic domains capable of interacting with extracellular matrix components (Jeong et al., 2022).

At the cellular level, GO can modulate signaling pathways associated with osteogenesis, particularly the Wnt/β-catenin, BMP/Smad, and MAPK pathways, thereby promoting the expression of genes involved in osteoblastic differentiation, including RUNX2, ALP, COL1A1, OCN, and OPN. In addition, its two-dimensional structure facilitates the local concentration of calcium and phosphate, thereby promoting extracellular matrix mineralization (Jeong et al., 2022).

The influence of GO on osteogenic differentiation is also associated with its ability to enhance cell–biomaterial surface interactions. The presence of carboxyl and hydroxyl groups facilitates the adsorption of cell-adhesion proteins, such as fibronectin and vitronectin, thereby promoting integrin-mediated adhesion and activation of intracellular signaling pathways associated with bone formation (Kwak et al., 2022).

In addition to directly stimulating osteoblastic activity, GO may promote bone regeneration through immunomodulatory mechanisms. The initial inflammatory response following tissue injury is a major determinant of regenerative success, and biomaterials capable of regulating macrophage polarization toward a reparative phenotype may substantially enhance new bone formation (Jeong et al., 2022).

Induction of Osteogenic Differentiation and Signal Transduction Mechanisms

The ability of graphene oxide (GO) to promote lineage commitment and accelerate the osteogenic differentiation of progenitor cells, such as mesenchymal stem cells (MSCs) and bone marrow stromal cells (BMSCs), represents one of the strongest rationales for its integration into bone and periodontal regeneration matrices. This osteoinductive effect is not merely contact-induced but rather results from a complex physicochemical and biomechanical interplay driven by its high specific surface area, nanoscale topography, and functional domains (Lin et al., 2023).

The biological and molecular mechanisms through which GO modulates the cellular microenvironment to promote osteogenesis can be organized into the following principal pathways.

Activation of signal-transduction pathways and gene expression: Interfacial interactions with GO induce the phosphorylation and activation of key signaling cascades involved in osteoblastic maturation, predominantly the Wnt/β-catenin, BMP/Smad, and MAPK/ERK pathways. This mechanotransduction culminates in the coordinated upregulation of master transcription factors, including RUNX2 (Runt-related transcription factor 2) and OSTERIX, as

well as matrix-associated genes encoding alkaline phosphatase (ALP), type I collagen (COL1A1), osteocalcin (OCN), and osteopontin (OPN) (Lin et al., 2023). In addition, the electronegative basal plane of GO attracts and nucleates divalent ions, acting as a physical template that accelerates hydroxyapatite precipitation and extracellular matrix (ECM) mineralization (Lin et al., 2023).

Selective adsorption of adhesion proteins: The carboxyl and hydroxyl groups of GO, together with its sp^2-hybridized aromatic domains, facilitate the selective adsorption of extracellular matrix proteins, such as fibronectin and vitronectin, while preserving conformations conducive to biological activity (Ahn et al., 2020). This protein corona exposes specific Arg-Gly-Asp (RGD) tripeptide sequences, promoting their interaction with cell-surface integrins. The resulting actin cytoskeletal rearrangement generates cellular tension forces that activate osteogenic signaling through direct mechanotransduction (Ahn et al., 2020).

Osteogenic immunomodulation and macrophage polarization: Beyond its direct effects on the osteoblastic lineage, GO plays an active role in the peri-implant immune microenvironment. Following implantation, GO can modulate the acute inflammatory response by promoting the phenotypic transition of macrophages from a pro-inflammatory M1 state toward a pro-regenerative M2 phenotype (Lin et al., 2023). This immunomodulatory polarization promotes the secretion of pro- osteogenic cytokines and pro-angiogenic factors, such as VEGF and TGF-β1, thereby establishing an immune microenvironment conducive to sustained tissue repair and the formation of highly mineralized new bone (Lin et al., 2023).

Applications of Graphene Oxide in Veterinary Dentistry

Advanced Periodontal Regeneration: Immunomodulation and Reconstruction of the Root- Associated Complex Periodontal regeneration represents one of the major challenges in dental and veterinary tissue engineering because of the complex tripartite architecture of the periodontal attachment apparatus. Successful regeneration requires the coordinated restoration of root cementum, the periodontal ligament (PDL), and alveolar bone, together with the

functional reinsertion of Sharpey's fibers (Mashhadi et al., 2024; Sánchez-Cepeda et al., 2024; Xie et al., 2024).

Within this context, periodontal ligament stem cells (PDLSCs) represent a major progenitor cell population involved in periodontal tissue repair. Graphene oxide (GO) derivatives can act as instructive modulators of the PDLSC cellular microenvironment through advanced biological.

Modulation of mitochondrial dynamics and GO quantum dots (GQDs): Incorporation of graphene oxide quantum dots (GQDs) into gelatin methacrylate (GelMA) hydrogel matrices promotes the osteogenic differentiation of PDLSCs by regulating cellular metabolic homeostasis. Specifically, GQDs promote mitochondrial fusion and attenuate excessive fission, thereby preserving mitochondrial membrane potential and accelerating the repair of periodontal bone defects in vivo (An et al., 2024).

Three-dimensional PCL/GO scaffolds: Surface functionalization of three-dimensional polycaprolactone (PCL) matrices with GO coatings optimizes nanoscale topography and surface free energy. This results in enhanced initial adhesion and proliferation of PDLSCs and promotes rapid extracellular matrix (ECM) mineralization (Park et al., 2021).

Preclinical evidence in canine models: Application of GO-modified three-dimensional collagen scaffolds to Class II furcation periodontal defects in canines demonstrated superior regenerative capacity after four weeks of implantation. Histological analyses revealed not only new alveolar bone formation and tissues exhibiting cementum- and periodontal ligament-like morphology, but also active perpendicular insertion of Sharpey's fibers into the newly formed root surface, supporting restoration of the biomechanical integrity of the periodontal attachment apparatus (Kawamoto et al., 2018).

Immunomodulation in compromised microenvironments: Under complex pathophysiological conditions, such as diabetic microenvironments or chronic periodontitis, the regenerative potential of GO extends beyond direct osteoinduction. Alginate/gelatin hybrid

matrices functionalized with polydopamine (PDA)-modified GO and hydroxyapatite nanoparticles (nHA) exhibit substantial reactive oxygen species (ROS)-scavenging capacity and the ability to activate Ca^{2+} signaling pathways, thereby modulating the inflammatory macrophage phenotype and promoting osteogenesis in tissues characterized by elevated oxidative stress (Li et al., 2022).

Critical Perspective and Veterinary Clinical Translation

Despite the advances reported to date—including in vivo proof-of-concept studies in canine models (Kawamoto et al., 2018)—the application of graphene oxide (GO) in clinical veterinary dentistry, particularly for the treatment of canine and feline periodontal disease, remains at an early experimental stage.

Most findings reported to date have originated from in vitro studies using human cells or in vivo experiments conducted in rodent models. Before systematic clinical translation into veterinary medicine can be considered, several critical knowledge gaps must be addressed. Physicochemical standardization: The effective dose, optimal lateral size range of GO nanosheets, and density of oxygen-containing functional groups must be established to minimize the risk of systemic cytotoxicity.

Biodegradation kinetics and long-term safety: Biological clearance, myeloperoxidase-mediated degradation, and the potential for undesirable tissue deposition following defect resolution must be systematically evaluated.

Controlled clinical trials in canine patients: Prospective randomized studies should be conducted in real-world veterinary patients with naturally occurring periodontitis, with long-term assessment of clinical periodontal parameters, including probing depth, clinical attachment level, and tomographic bone density.

Regeneration of the Dentin–Pulp Complex: Odontogenic Induction and Tissue Reconstruction Preservation and regeneration of the dentin–pulp complex represent primary

objectives in modern regenerative dentistry, as loss of pulp vitality interrupts tertiary dentinogenesis, compromises proprioception, and leaves the tooth highly susceptible to fracture and root resorption. Unlike conventional endodontic treatment, which focuses on inert obturation of the root canal, tissue engineering aims to restore a highly vascularized and innervated tissue characterized by a palisaded layer of functional odontoblasts capable of synthesizing reparative tubular dentin (Huang et al., 2024; Qin et al., 2022).

In this process, dental pulp stem cells (DPSCs) represent the most relevant progenitor cell population. Graphene oxide (GO) nanostructures act on DPSCs as potent biological and physicochemical inducers, modulating odontogenesis through the following principal mechanisms.

Table 1.

Graphene oxide combinations with natural and synthetic biomaterials investigated experimentally

Material combination	Experimental model	Study objective	Main findings
GO+PCL	Rabbit bone defect	To evaluate 3D-printed PCL scaffolds enriched with GO for bone regeneration	GO-containing scaffolds showed greater new bone formation; GO concentrations of 1% and 3% were
GO+PLA+ hardystonite	Characterization and experimental evaluation of 3D scaffolds	To develop a nanocomposite scaffold using 3D printing	GO acted as a component/coupling agent within the PLA–hardystonite system, improving scaffold-related properties for bone regeneration
GO+collagen +curcumin	Biological and structural evaluation of a scaffold	To develop a collagen scaffold incorporating GO and loaded with curcumin	The system exhibited favorable structural and biological characteristics for bone regeneration and antibacterial

GO+PLA	Cell culture and 3D scaffold	To evaluate the effect of a GO coating on 3D-printed PLA scaffolds	GO surface treatment increased wettability and resulted in an increase of up to 220% in cell viability under dynamic culture
GO+PLA+ β-TCP	3D-printed scaffolds; physicochemical, mechanical, and biological evaluation	To develop a PLA scaffold reinforced with GO and β-tricalcium phosphate	Structure, mechanical properties, and bioactivity of the system were characterized
GO+PLGA	Rats with mandibular fractures	To compare absorbable GO–PLGA nanofibrous plates with titanium plates	Bone stability, viability, and healing of mandibular fractures were evaluated
GO+PEEK	Experimental surface evaluation	To develop a self-assembled GO coating on PEEK	The coating improved the bactericidal and osteogenic properties of PEEK
GO+collagen+ Attapulgite	3D scaffold; experimental bone regeneration	To fabricate a biocompatible scaffold using 3D printing	The combination was designed to provide structural support and promote bone regeneration, with potential for patient-specific fabrication
GO+bioglass	Scaffold evaluation for bone regeneration	To develop a composite scaffold comprising GO and nanobioglass	A composite material with favorable characteristics for bone tissue engineering was obtained
GO+chitosan +alginate + Se	Rabbit with bone defect	To evaluate a biopolymeric scaffold containing GO and selenium	GO and Se enhanced the physical properties of the scaffold and resulted in significantly greater bone regeneration than the control
GO + PLLA/PGA+ polydopamine+Sr	Scaffold for bone tissue engineering; mechanical and osteogenic evaluation	To incorporate strontium-functionalized GO into a PLLA/PGA scaffold	The system demonstrated improved mechanical properties and osteogenic induction
GO + titanium + antimicrobial peptide Nal-P-113	Titanium surface; antimicrobial and cytocompatibility	To develop a GO coating loaded with an antimicrobial peptide	The coating was evaluated for antibacterial activity and cytocompatibility

GO+titanium	Dental implant surface; antimicrobial evaluation	To evaluate GO nanoparticles as a surface treatment for titanium implants	The synthesis, characterization, and potential antimicrobial activity of the coating were
GO+titanium + minocycline	Titanium implants; experimental evaluation	To develop a GO/multilayer coating incorporating minocycline	Coating characteristics, cellular response, and properties related to bacterial control were evaluated
GO+bioglass + PEEK	Experimental dental implant	To develop a bioglass/GO composite coating for PEEK	System was designed to overcome the low bioactivity and limited antimicrobial activity of PEEK

Modulation of cellular metabolism and AMPK/mTOR signaling by GQDs: Exposure of DPSCs to optimized concentrations of graphene oxide quantum dots (GQDs) significantly stimulates cell proliferation, alkaline phosphatase (ALP) activity, and mineralized nodule deposition. At the molecular level, this odontogenic stimulus is mediated by regulation of the AMPK/mTOR metabolic pathway, which promotes the upregulation of key dentin-matrix proteins, including dentin sialophosphoprotein (DSPP), dentin matrix protein 1 (DMP-1), and the transcription factor RUNX2 (Lin et al., 2023).

Synergy in three-dimensional mesoporous bioactive glass/GO (MBG/GO) nanocomposites: Incorporation of GO into mesoporous bioactive glass (MBG) matrices generates an ionic-exchange and topographical microenvironment conducive to human DPSC activity. This hybrid platform not only induces coordinated upregulation of advanced odontogenic markers, including DSPP, DMP- 1, BMP-2, MEPE, RUNX2, and ALP, but also accelerates biomineralization kinetics through the epitaxial nucleation of apatite crystals on the surface network (Ahn et al., 2020).

Multifunctional growth-factor retention platforms: The abundance of carboxyl and hydroxyl groups at the edges and basal plane of GO enables the non-covalent adsorption of bioactive biomolecules, particularly members of the transforming growth factor-β (TGF-β) superfamily, such as BMP-2 and BMP-7. Incorporation of GO into injectable hydrogels or porous scaffolds enables the development of sustained-release systems that mimic the native

odontogenic niche, protecting growth factors from proteolytic degradation and maintaining their local bioavailability to guide DPSC differentiation (Ahn et al., 2020).

Critical Perspective and Clinical Challenges in Canine Veterinary Dentistry

Despite promising in vitro and in vivo findings from basic preclinical models, the current scientific literature presents conceptual and methodological limitations that must be addressed before clinical translation into veterinary dentistry can be contemplated:

Partial evidence versus comprehensive regeneration: Most available evidence evaluates indirect markers or partial endpoints, such as in vitro differentiation, formation of mineralized nodules, or gene expression (Huang et al., 2024; Qin et al., 2022). However, there is still no conclusive evidence that GO alone can orchestrate the complete regeneration of a vascularized and innervated dental pulp within complex pulp chambers.

Translational gap to the canine species: Direct extrapolation of findings obtained from human DPSCs to canine veterinary clinical practice is neither biomechanically nor immunologically valid. The canine oral cavity presents specific challenges, including:

Severe masticatory forces and microleakage: Substantial occlusal loads in dogs impose more stringent mechanical-resistance requirements on the regenerated dentin–pulp complex

Species-specific microbial burden: The canine endodontic microbiota differs in composition and pathogenic potential from that of humans, necessitating biomaterials with enhanced bactericidal capacity against dog-specific pathogens.

Need for species-specific in vivo trials: GO should currently be regarded as a promising experimental biomaterial platform. Progress toward clinical applications in direct pulp capping or regenerative pulpotomy in dogs requires the design of orthotopic in vivo studies in canine models, with quantitative assessment of tertiary dentin formation, pulp revascularization, and long-term pulp biocompatibility.

Alveolar Bone Regeneration and Maxillofacial Reconstruction: Hybrid Nanocomposites and Osteoangiogenic Systems.

The reconstruction of alveolar and maxillofacial bone defects represents a major biomechanical and physiological challenge because it requires simultaneous restoration of structural rigidity, alveolar ridge stability, and bone volume lost following severe periodontitis, dental avulsion, high- energy trauma, or oncological surgical resection. Many of these defects exceed the physiological capacity for spontaneous regeneration and therefore constitute critical-sized defects, requiring three-dimensional matrices that integrate osteoconductive, osteoinductive, and angiogenic properties (Kumari et al., 2022; Yang et al., 2024).

The physicochemical properties of graphene oxide (GO) enable its use as both a mechanical reinforcing agent and a bioactive component in bone tissue engineering, giving rise to several families of hybrid nanocomposites.

Hydroxyapatite/GO hybrid systems and bone immunomodulation: Combining GO with hydroxyapatite (HA) mimics the native inorganic phase of bone while enhancing the fracture toughness of the inorganic matrix. Multifunctional matrices composed of polydopamine (PDA)-modified GO and integrated hydroxyapatite nanoparticles (nHA) within alginate/gelatin hydrogels have been shown to orchestrate regeneration in inflammatory microenvironments. This nanocomposite scavenges reactive oxygen species (ROS) and promotes macrophage polarization from the pro-inflammatory M1 phenotype toward the reparative M2 phenotype, thereby increasing the density of mineralized matrix formation in vivo (Li et al., 2022).

Mesoporous bioactive glass/GO composites (MBG/GO): Incorporation of GO into bioactive glasses modifies surface microtopography and ion-exchange kinetics. These composites enhance protein adsorption and stimulate epitaxial biomineralization, promoting progenitor-cell differentiation through accelerated formation of a biologically active carbonate-substituted apatite layer (Li et al., 2022).

Dual loading and sustained release of osteoangiogenic factors (BMP-2 and VEGF): Reconstruction of critical-sized defects requires rapid vascularization coordinated with osteogenesis. GO acts as a highly efficient molecular reservoir through non-covalent interactions, including π–π stacking, hydrogen bonding, and electrostatic attraction. Incorporation of GO into PCL/PVP nanofibrous scaffolds suppresses the initial burst release of bone morphogenetic protein 2 (BMP-2), maintaining sustained desorption over prolonged periods. This modulated release kinetics results in enhanced osteoblastic maturation and calcium phosphate deposition (Jeong et al., 2022). Similarly, incorporation of vascular endothelial growth factor (VEGF) into GO-based matrices enables coupling of neovascularization with bone mineralization.

Tissue integration and in vivo immuno-osseointegration: Preclinical studies demonstrate that titanium surfaces modified with GO nanolayers quantitatively increase bone-to-implant contact (BIC) and accelerate osseointegration in rabbit tibial models (Yang et al., 2024), supporting the interfacial biocompatibility of the nanomaterial with both cortical and trabecular bone in vivo (Kwak et al., 2022).

Critical Perspective and Translation to Canine Veterinary Dentistry

The development of GO-based biomaterials for alveolar and maxillofacial reconstruction in dogs has considerable potential given the high prevalence of advanced periodontal disease and extensive bone loss encountered in small-animal clinical practice. However, the clinical translation gap requires several technical and biological limitations to be addressed.

Species-specific biomechanical requirements: The canine maxillofacial complex is subjected to substantially greater masticatory occlusal forces than those experienced in rodents and rabbits.

It is therefore essential to characterize the compressive, shear, and fatigue properties of GO-based hybrid scaffolds under physiologically relevant cyclic loading conditions.

Dependence on vascularization in extensive defects: Although VEGF adsorption onto GO- based matrices has demonstrated biological plausibility, the in vivo degradation kinetics of the scaffold must be precisely synchronized with the rate of angiogenesis and capillary ingrowth from adjacent cortical bone to prevent central necrosis in large-volume defects.

Optimization of Synthesis Parameters and Longitudinal Safety

Standardization of the threshold dose, degree of oxidation (C/O ratio), and lateral dimensions of GO sheets is required in orthotopic canine maxillofacial models. In addition, long-term histomorphometric and tomographic assessments are imperative to rule out chronic foreign-body inflammation or undesirable tissue migration of free GO nanosheets.

Bioactive Coatings on Titanium Dental Implants: Surface Modification and Interfacial Performance.

Commercially pure titanium (cp-Ti) and its alloys (e.g., Ti-6Al-4V) constitute the gold standard in dental and maxillofacial implantology because of their high yield strength, corrosion resistance, and passive biocompatibility conferred by the titanium dioxide (TiO_2) surface oxide layer. However, the relative biological inertness of titanium may result in prolonged osseointegration times or premature failure associated with bacterial peri-implantitis or inadequate primary and secondary stability (Kwak et al., 2022).

Topographical and functional modification of titanium surfaces using graphene oxide (GO) nanocoatings transforms a passive interface into a bioactive instructive microenvironment, modulating osteogenesis and providing antimicrobial protection without altering the structural mechanical properties of the metallic core.

Surface anchoring and integration techniques (micro-arc oxidation—MAO): Deposition of GO onto titanium by micro-arc oxidation (MAO) generates a porous TiO_2 layer that functions as a rough mechanical anchor at the micro- and submicrometre scales. Subsequent immobilization of GO nanosheets within the pores of the MAO layer optimizes surface free energy and

wettability, generating a nanotopography with high affinity for cellular attachment (Kwak et al., 2022).

Mechanotransduction and in vitro and in vivo osteogenic response: Ti/MAO-GO surfaces induce a significant increase in the expression of alkaline phosphatase (ALP), the transcription factor RUNX2, osteopontin (OPN), and osteocalcin (OCN) in bone progenitor cells (Kwak et al., 2022). In in vivo animal models (rabbit tibia), implants modified with GO coatings demonstrated a marked quantitative increase in bone-to-implant contact (BIC) and peri-implant trabecular bone volume compared with conventional surfaces (Kwak et al., 2022), thereby accelerating bone consolidation at the implant– bone interface.

Multifunctional multilayer coatings: The ability of GO to function as an immobilization matrix enables the development of multifunctional coatings on titanium.
Biomimetics and mineralization: Co-deposition of GO with hydroxyapatite (HA) accelerates the formation of a biologically active apatite layer.

Dual antimicrobial effect: The presence of GO prevents the initial colonization of oral pathogens in the peri-implant sulcus through mechanical membrane damage and ROS generation.

Sustained growth-factor release: GO enables the physical adsorption and kinetically regulated release of BMP-2 or immunomodulatory agents into the peri-implant surgical site (Kwak et al., 2022).

Critical Perspective and Challenges for Translation to Canine Veterinary Implantology
The use of GO-functionalized titanium implants in canine patients represents a highly attractive alternative for oral rehabilitation, fixation of maxillofacial prostheses, and stabilization of complex bone defects in veterinary medicine. However, safe clinical translation requires the resolution of specific methodological and biomechanical challenges.

Coating adhesion and shear resistance: During dental implant insertion into the bone bed under mechanical torque, the GO coating is exposed to substantial frictional and shear

forces. It is therefore essential to quantify delamination resistance and mechanical stability of the coating to prevent unintended release of free nanosheets into the bone bed, which could trigger foreign-body responses or frustrated phagocytosis by macrophages.

Canine oral environment and peri-implant biofilm: Canine saliva differs from standard laboratory models in pH, viscosity, and bacterial burden, including the presence of species such as Porphyromonas gulae. It is therefore necessary to determine whether the bioactivity and antibacterial effects of GO coatings remain stable in the presence of enzymatic degradation and canine dental plaque formation in vivo.

Standardization and veterinary clinical trials: Prospective canine studies evaluating primary stability, implant stability quotient (ISQ) values using resonance frequency analysis, and long-term implant success rates under physiological occlusal loading are currently lacking. Consequently, Ti–GO remains an experimental biomaterial system with substantial translational potential.

Three-Dimensional Scaffolds: Hybrid Nanocomposite Architectures and Biofabrication Three-dimensional scaffolds constitute a fundamental component of dental and maxillofacial tissue engineering by functioning as synthetic extracellular matrix mimetics that provide temporary mechanical support, guide cellular behavior, and immobilize osteoinductive cues. Incorporation of graphene oxide (GO) into polymeric, protein-based, or hydrogel matrices can overcome the inherent limitations of pristine materials, such as low toughness, rapid degradation, or limited bioactivity, through the formation of multifunctional hybrid networks.

The interfacial integration of GO into the different families of three-dimensional matrices can be organized according to the following biomaterial strategies:

Bioprintable Hydrogels (GelMA and Bioactive Peptides)

Gelatin methacryloyl (GelMA) hydrogels mimic the viscoelasticity of soft tissues and facilitate cellular encapsulation. Incorporation of GO optimizes the hydrogel storage modulus, increases shape fidelity during the 3D bioprinting process, and promotes mesenchymal stem

cell adhesion and osteogenic differentiation, as evidenced by the upregulation of RUNX2, osteopontin, and osteocalcin. In addition, GelMA/GO hydrogels functionalized with bone-forming peptide-1 (BFP- 1) act as delivery platforms for osteogenic cues, accelerating alkaline phosphatase (ALP) activity and mineralized matrix deposition.

Biodegradable Synthetic Polymers and the Formulation Window Effect (PCL/GO)

Poly(ε-caprolactone) (PCL) offers high processability for scaffold fabrication by fused deposition modeling (FDM). Incorporation of GO reduces the hydrophobicity of PCL and increases nanoscale surface roughness. In vivo experiments in rabbit bone defects demonstrated a narrow formulation window: the addition of 1 wt% GO significantly increased new bone formation. However, higher concentrations induced nanosheet aggregation, undesirable acceleration of biodegradation due to structural mismatch, and local cytotoxicity, demonstrating that the biological response does not exhibit a direct linear relationship with nanophase loading.

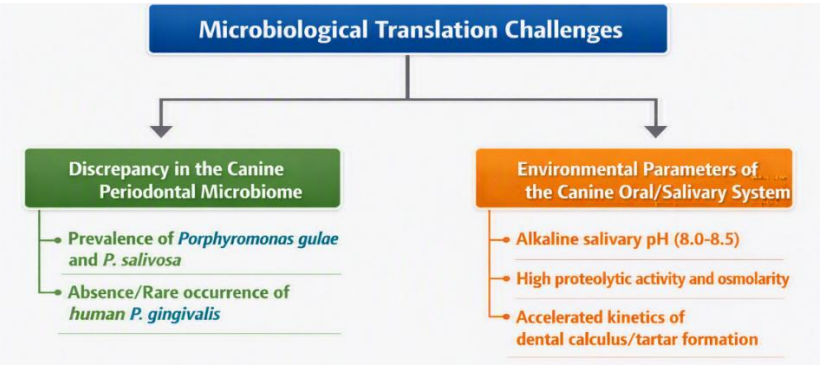
Natural Protein Matrices and Antimicrobial Biopolymers (Collagen and Chitosan)

Collagen/GO systems: The combination of GO with collagen restores the protein component of the extracellular matrix (ECM), while the nanosheets enhance thermal stability and resistance to enzymatic degradation by collagenases. The incorporation of minerals (attapulgite) or phytochemicals (curcumin) into three-dimensional collagen/GO matrices generates multifunctional scaffolds that combine structural support with antibacterial and antioxidant activity.

Chitosan/GO composites: Electrostatic interactions between the positively charged amino groups of chitosan and the electronegative carboxyl groups of GO reinforce the three-dimensional network. In critical-sized defects, chitosan/GO scaffolds increase new bone formation and the expression of osteogenic markers (BMP, RUNX2, OPN, and OCN) compared with the biopolymer alone, while also serving as controlled-release matrices for bactericidal agents.

Figure 1

Critical Parameters of GO/Scaffold Systems



The systematic balance among these variables determines whether the scaffold will induce a regenerative tissue response or a foreign-body reaction, thereby establishing the framework for the development of three-dimensional matrices for clinical veterinary and biomedical applications.

Incorporation of Bioceramics and Multicomponent Hybrid Systems

Coupling GO with osteoconductive bioceramics, including hydroxyapatite, β-tricalcium phosphate (β-TCP), and bioactive glasses, generates ternary or quaternary nanocomposites (e.g., chitosan/gelatin/bioactive nanoglass/GO or β-TCP/polymer/GO). In these systems, the bioceramic component provides inorganic nucleation sites, whereas GO acts as a network-forming cross- linker and mechanical reinforcement against bending and compressive stresses.

Critical Perspective on Scaffold Synthesis and Design

The accumulated evidence indicates that GO should be conceptualized as an interfacial dopant and instructive agent rather than merely as a passive reinforcing nanofiller. To maximize the regenerative performance of three-dimensional scaffolds and ensure an appropriate cellular response, biomaterial design should consider a multidimensional matrix of variables:

Nanofunctional Platforms for Controlled Release and Local Delivery of Bioactive Agents

The ability of graphene oxide (GO) to function as a sustained and site-specific delivery platform (targeted delivery system) stems from its interfacial physicochemical properties. Its

high specific surface area, combined with the basal plane of sp^2-hybridized carbon atoms and peripheral functional groups (carboxyl, hydroxyl, and epoxy groups), enables stable yet reversible non- covalent interactions, including π–π stacking, hydrogen bonding, hydrophobic interactions, and electrostatic forces.

In dental and maxillofacial tissue engineering, localized release kinetics can minimize rapid dissipation or washout through blood or salivary flow, suppressing the initial burst release and maintaining the bioactive dose within the effective therapeutic window for prolonged periods compatible with bone and periodontal histogenesis.

The major advances in the delivery of bioactive and therapeutic agents using GO-based systems can be organized into the following main areas

Delivery of Osteoinductive Factors (BMP-2) Using Macro- and Nanoscale Matrices

Macro-/microstructured scaffold systems (PCL/GO–PVP): The incorporation of GO/polyvinylpyrrolidone (PVP) hydrogels into electrospun nanofibrous poly(ε-caprolactone) (PCL) scaffolds modulates the desorption of bone morphogenetic protein-2 (BMP-2). Non-covalent interactions between GO and BMP-2 prolong the bioavailability of the osteoinductive protein, continuously promoting mineral deposition and the upregulation of osteogenic markers in critical-sized defects.

Nanoscale delivery platforms (GO–PLL/albumin): Beyond rigid three-dimensional scaffolds, functionalization of bovine serum albumin nanoparticles with poly-L-lysine and GO (GO–PLL) enables the formation of colloidal nanocarriers for BMP-2. This GO–PLL/BMP-2 nanoscale complex attenuates the initial diffusion rate, enhances cellular uptake, and stimulates the differentiation of bone marrow-derived mesenchymal stem cells (BMSCs), providing a potential route for injectable therapies targeting alveolar defects with irregular geometries.

Stimuli-Responsive Systems for Antimicrobial Agents

pH-dependent antibiotic release (vancomycin): Incorporation of GO into polymeric hydrogel matrices composed of poly (vinylsulfonic acid)–Sterculia gum enables modulation of vancomycin diffusion kinetics through pH responsiveness. Changes in the protonation state of GO carboxyl groups under acidic or neutral conditions alter electrostatic interactions with the charged drug, thereby regulating its release. Although this model was initially developed for gastrointestinal drug delivery, it establishes the biophysical principle underlying GO-based hydrogels responsive to microenvironmental changes, such as the acidification observed in periodontal disease or active peri-implant infection.

Carriers for Low-Molecular-Weight Phytotherapeutic Bioactive Molecules

PLLA/GO biomimetic scaffolds (salvianolic acid B): The incorporation of GO into porous poly (L-lactic acid) (PLLA) matrices alters polymer-fiber topography and enables sustained release of small molecules such as salvianolic acid B. π–π stacking interactions between the aromatic ring of the drug and the sp^2 domains of GO regulate the desorption profile, coupling the antioxidant and vasodilatory effects of the bioactive molecule with structural regeneration of the matrix.

Critical Perspective and Veterinary Clinical Translation: The integration of four independent functions—structural support, osteoinduction/odontogenesis, bacterial control, and kinetically regulated bioactive delivery—within a single biomaterial platform positions GO as a highly versatile material.

However, before translation into canine dental practice, particularly for the regeneration of periodontal and osseous defects associated with previous infection, the following scientific gaps must be addressed

Species-specific in vivo evidence in dogs: Although the release kinetics of factors such as BMP- 2 and antibiotics such as vancomycin have been demonstrated in vitro or in non-dental preclinical models, empirical evidence validating the efficacy of GO–vancomycin or GO–BMP-2 systems within the canine periodontal microenvironment is still lacking.

Kinetic–metabolic synchronization: Growth-factor release kinetics must be synchronized with scaffold degradation and the rate of bone remodeling in dogs. Decoupled release may result in ectopic bone formation if release is excessively rapid, or fibrous encapsulation if the bioactive agent becomes irreversibly entrapped within the GO network.

Validation of stability and immunological safety: Local pharmacokinetics, long-term cytotoxicity profiles, stability of the nanocarrier–drug complex in the presence of canine salivary enzymes, and the immune response to repeated or prolonged administration of GO-based delivery vectors must be determined.

Surface Antimicrobial Strategies and Control of Multispecies Oral Biofilms

Pathogenic colonization and invasion mediated by oral biofilms on biomaterials constitute a primary etiological factor in the pathogenesis of dental caries, periodontal disease, and peri- implantitis. The limited efficacy of conventional therapies in eliminating the extracellular polymeric substance (EPS) matrix of established biofilms has driven the development of bioactive and instructive GO-based surfaces that act through direct contact (physical action/"nano-knives"), oxidative stress generation, or function as nanovectors for pharmacological synergy.

The antibacterial applications of GO in dental and regenerative biomaterials can be organized into three principal platforms:

Functionalization of Implant and Titanium/Zirconia Device Surfaces

Layer-by-layer (LbL) nanocoatings and antimicrobial peptides: Immobilization of the antimicrobial peptide Nal-P-113 onto titanium using GO as a supporting matrix enables continuous and controlled release of the peptide agent. This hybrid coating significantly suppresses the growth of Streptococcus mutans and Porphyromonas gingivalis without inducing cytotoxicity in human gingival fibroblasts (hGFs).

GO/ε-poly-L-lysine multilayers: The nanostructured assembly of 20 alternating layers of GO and ε-poly-L-lysine on titanium generates a highly effective biocidal coating against the primary periodontal pathogen P. gingivalis, while maintaining adequate cellular biocompatibility.

Plasma-mediated surface modification of zirconia: Plasma treatment for deposition of graphene/GO nanocoatings onto zirconium oxide substrates (zirconia, ZrO_2) markedly reduces initial bacterial adhesion and the hydration energy required for attachment of S. mutans and P. gingivalis, demonstrating that graphene-based surface modification can be extended to ceramic substrates used in restorative and prosthodontic applications.

Direct and Indirect Restorative Materials (Resin Composites and Doped Hydroxyapatite)

Hybrid resin cements (GO/HA-Ag): The formulation of experimental dental resins and cements enriched with GO and silver-doped hydroxyapatite (HA-Ag) inhibits the proliferation of cariogenic pathogens (S. mutans) and periodontopathogens (P. gingivalis). Incorporation of trace concentrations of GO nanophases enhances the fracture toughness of the polymer composite and acts as a bactericidal synergist, potentially preventing secondary caries at restoration margins.

Inhibition of primary colonizers and bridging species (historical model): Two-dimensional GO nanosheets exert a direct bactericidal effect against the reference pathogenic bacterial triad: S. mutans (matrix initiator), Fusobacterium nucleatum (bridging/adhesive microorganism), and P. gingivalis (strict periodontal pathogen).

Targeted Antimicrobial Photodynamic Therapies (aPDT)

Aptamer–NGO systems: Conjugation of DNA aptamers specifically targeting membrane receptors of P. gingivalis onto nano-sized graphene oxide (NGO) enables targeted delivery of photosensitizing agents. Upon irradiation, antimicrobial photodynamic therapy (aPDT) generates a localized burst of reactive oxygen species (ROS), selectively eliminating P. gingivalis biofilms while preserving the commensal microbial balance of the oral tissue microenvironment.

Critical Perspective and Translational Gap in Canine Veterinary Medicine

Although in vitro and in vivo evidence demonstrates high bactericidal efficacy against human- derived strains (S. mutans, P. gingivalis, and F. nucleatum), translation of GO-based antibacterial biomaterials to canine dentistry faces critical biological limitations that must be addressed.

Divergence of the periodontal microbiome: The canine periodontal disease-associated microbial complex is predominantly characterized by Porphyromonas gulae, Porphyromonas salivosa, and Peptostreptococcus canis, rather than human P. gingivalis. Therefore, findings demonstrated against human periodontal pathogens should be regarded as evidence of translational potential rather than as proof of in vivo efficacy in dogs.

Canine biofilm resilience in alkaline microenvironments: Canine saliva exhibits a substantially more alkaline pH (pH 8.0–8.5) and a higher rate of mineral precipitation (accelerated tartar/calculus formation) than human saliva. This necessitates evaluation of whether GO surface bioactivity and the release of antimicrobial peptides or ions remain stable in the face of rapid mineralization and alkalinization of the canine oral environment.

Research perspective: Experimental evaluation of GO coatings against wild-type strains isolated from canine P. gulae is required, together with the development of orthotopic studies to validate biocompatibility and control of periodontal dysbiosis in dogs with naturally occurring periodontitis.

Current State of Research and Translational Gaps in Canine Veterinary Medicine

The development of graphene oxide (GO)-based biomaterials for dental applications and tissue regeneration has grown exponentially over the past decade. Nevertheless, a recent systematic review (2013–2023) of GO applications in oral surgery identified 293 initial records, of which only 19 publications met the inclusion criteria for specific applications involving implant surfaces, prosthetic abutments, guided bone regeneration (GBR) membranes, and bone-regeneration scaffolds (Inchingolo et al., 2023). This quantitative analysis confirms that,

although interest in GO for oral tissue engineering is rapidly expanding, the available evidence remains predominantly preclinical (Inchingolo et al., 2023).

When the literature is examined specifically for canine subjects, the available evidence is reduced to a limited number of isolated preclinical studies published between 2016 and 2020: Post-extraction alveolar ridge preservation: Implantation of GO-coated collagen scaffolds into dental extraction sockets in dogs resulted in an approximately fivefold increase in new bone formation compared with non-functionalized controls, accompanied by substantial cellular infiltration and stromal vascularization (Miyaji et al., 2016).

Periodontal regeneration in furcation defects: The use of three-dimensional collagen/GO matrices in Class II furcation defects in dogs promoted histological reconstruction of the periodontal attachment apparatus, with newly formed alveolar bone, cementum-like tissue, and functional periodontal ligament being observed (Kawamoto et al., 2018).

Management of experimental peri-implantitis: In beagle dog models with experimentally induced peri-implantitis, the use of minocycline-functionalized GO films on implant abutments attenuated marginal bone resorption and inhibited the progression of inflammatory infiltration (Qian et al., 2020).

Despite these pioneering findings spanning the three major areas of regenerative dentistry— alveolar preservation, periodontal reconstruction, and peri-implant therapy— evidence in dogs has not yet developed into a continuous or consolidated research programme. Notably, there has been no proportional increase in in vivo canine studies after 2022; instead, recent research has shifted towards in vitro cultures of stem cells (MSCs, PDLSCs, and DPSCs) and rodent/rabbit models.

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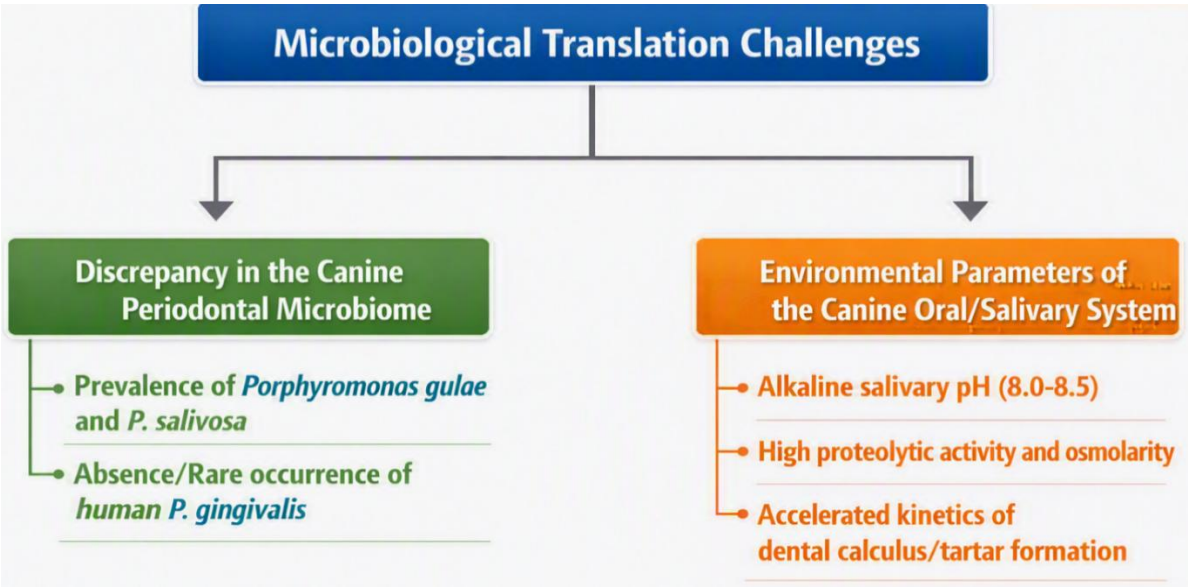
Figure 2

Evidence flow of research on GO in dentistry and canine veterinary medicine.



Methodological Limitations and Barriers to Clinical Translation

The safe translation of GO-based biomaterials from laboratory research to clinical veterinary practice requires overcoming three fundamental methodological biases and barriers: Methodological bias arising from the predominance of in vitro studies and model incompatibility: The abundance of monolayer cell assays, in which upregulation of ALP, RUNX2, DSPP, or DMP-1 is interpreted as evidence of biological potential, does not capture the haemodynamic complexity, occlusal biomechanics, or immunological and microbiological microenvironment of a living dog.

Likewise, rodent and rabbit models differ substantially from domestic carnivores in terms of bone-remodelling rates and maxillofacial mechanical loading. Physicochemical heterogeneity of GO: The designation "graphene oxide" encompasses materials exhibiting substantial variation in nanosheet lateral dimensions, degree of oxidation (C/O ratio), number of layers, purity, and synthesis method. These variables can markedly alter cytotoxicity thresholds, enzymatic degradation rates, and tissue reactivity, making direct comparisons between studies

impossible unless the physicochemical profile of the starting material is standardized. Figure 2.

Translational gap of graphene oxide in canine veterinary dentistry.

Note. The figure synthesizes the distribution of the available evidence, ranging from in vitro studies and experimental animal models to studies conducted specifically in dogs and their potential eventual application in veterinary clinical practice. The identified canine evidence is concentrated on alveolar regeneration, periodontal regeneration, and peri-implantitis, whereas a marked lack of veterinary clinical studies persists.

Scarcity of long-term immunosafety and clearance assessments: The lack of longitudinal studies prevents adequate characterization of myeloperoxidase-mediated biodegradation kinetics, interactions with macrophages, and potential accumulation or tissue migration of nanosheets following degradation of the host matrix (PCL, GelMA, or collagen). In veterinary patients, short- term acute biocompatibility cannot substitute for evidence of long-term safety against foreign- body reactions or delayed chronic peri-implant inflammation.

Future Research Opportunities in Veterinary Dentistry

Because the dog is a biologically relevant translational model for periodontology and implantology owing to its anatomical and pathophysiological similarities to higher mammals, there is a key opportunity to reinvigorate targeted preclinical research. Rigorous in vivo studies are needed to evaluate contemporary multifunctional formulations—including GO composites with GelMA, hydroxyapatite, bioactive glass, bioceramics, and osteoangiogenic factors—under orthotopic conditions that reproduce the actual pathogenesis of canine periodontal disease. Such studies should incorporate histomorphometric analyses, micro-computed tomography (micro-CT), pathogen-specific microbiological assessment against Porphyromonas gulae, and longitudinal monitoring of biocompatibility.

Accordingly, the available evidence currently supports classifying the application of GO in canine veterinary dentistry as a promising preclinical technology that remains insufficiently validated. The available literature provides proof-of-concept evidence in dogs and a substantial

experimental basis derived from human and laboratory animal models; however, there is still insufficient evidence to establish veterinary clinical protocols, optimal therapeutic concentrations, or standardized formulations. This gap between biomaterial development and clinical validation represents one of the major challenges for the future application of GO in canine dentistry and, at the same time, constitutes one of the areas with the greatest potential for translational research.

Future Perspectives and Translational Research The gap between nanostructured material design and application in canine patients defines the priorities for future research in biomaterials and veterinary dentistry: Standardization and Physicochemical Characterization: Establish strict formulation ranges (the “therapeutic window”) that optimize bioactivity without triggering cytotoxicity or foreign- body responses. Species-Specific Orthotopic Models: Develop longitudinal preclinical studies in dogs to evaluate contemporary multifunctional scaffolds (GO-doped GelMA, PCL, collagen, chitosan, or bioceramics) under conditions that reproduce naturally occurring periodontal disease and peri- implantitis, integrating histomorphometric analyses, micro-CT, and pathogen-specific microbiological assessment. Long-Term Tissue Safety: Evaluate the shear strength of coatings on titanium implants, the kinetics of biodegradation mediated by local enzymes, and extended-term systemic biocompatibility.

DISCUSSION

The evidence analyzed indicates that graphene oxide (GO) is a nanobiomaterial with physicochemical and biological characteristics of considerable interest for regenerative dentistry. The presence of oxygen-containing functional groups, its high specific surface area, and its ability to interact with proteins, polymers, and bioactive molecules allow its incorporation into scaffolds, coatings, and controlled-release systems. However, the properties of GO depend on variables such as the degree of oxidation, lateral sheet size, concentration, and surface functionalization; therefore, it cannot be regarded as a material with uniform biological behavior.

Jiříčková et al. (2022) and Kabore et al. (2026) specifically highlighted the influence of the structural and physicochemical characteristics of GO on its biological behavior, an aspect that is essential for the appropriate interpretation of experimental findings.

Osteogenic differentiation represents one of the applications with the strongest experimental support. Park et al. (2021) demonstrated that a poly(ε-caprolactone) scaffold coated with GO enhanced the adhesion, proliferation, and mineralization of periodontal ligament stem cells, whereas An et al. (2024) observed that GO quantum dots promoted the osteogenic differentiation of periodontal ligament stem cells through mechanisms associated with mitochondrial dynamics. These findings support the potential use of GO as a bioactive component of matrices designed for periodontal and bone regeneration. Complementarily, Qin et al. (2022) and Alazab et al. (2023) demonstrated the potential of GO-based collagen and PCL composite scaffolds, respectively, for bone-regeneration applications.

In dentin–pulp regeneration, Qiu et al. (2024) demonstrated that a GO/poly-L-lactic acid scaffold could favorably modulate the biological properties of human dental pulp stem cells. These findings suggest that GO may contribute to the development of microenvironments conducive to processes associated with odontogenesis and mineralization. Nevertheless, the expression of odontogenic markers or the formation of mineralized deposits must be distinguished from complete functional regeneration of the dentin–pulp complex. Therefore, further in vivo studies are required to evaluate tissue organization, vascularization, and maintenance of pulp vitality.

Another relevant application involves controlled-release systems. Jeong et al. (2022) developed a GO/polyvinylpyrrolidone-based system incorporated into a PCL scaffold, which enabled BMP-2 release and stimulated mineralization processes. This strategy is particularly attractive for bone regeneration because it combines structural support with localized delivery of bioactive factors. However, demonstration of controlled release in vitro does not, by itself,

guarantee therapeutic efficacy in vivo. Parameters such as release kinetics, stability, biodistribution, and formulation safety must therefore be determined.

The ability of GO to combine regenerative and antibacterial properties represents a potential advantage for dentistry. Radunovic et al. (2022) demonstrated antibiofilm activity on GO-functionalized titanium discs and collagen membranes, whereas Huang et al. (2024) developed GO coatings on PEEK with enhanced bactericidal and osteogenic properties. Similarly, Al-Noaman and Rawlinson (2023) evaluated a bioactive glass/GO coating for dental implants. These findings suggest that GO may serve as a multifunctional platform capable of controlling bacterial colonization while simultaneously promoting biomaterial–tissue interactions. The evidence becomes particularly relevant when specifically considering canine veterinary medicine. Miyaji et al. (2016) demonstrated that a GO-modified collagen scaffold promoted bone formation in post-extraction dental sockets in dogs. Subsequently, Kawamoto et al. (2018) evaluated a GO-based scaffold in periodontal furcation defects in dogs and observed the formation of alveolar bone and tissues compatible with cementum and periodontal ligament. In turn, Qian et al. (2020) demonstrated that minocycline-loaded GO films could reduce marginal bone loss and the inflammatory response in a canine model of peri-implantitis. These three studies constitute particularly relevant evidence because they demonstrate that effects observed in cellular models can, at least partially, translate to the oral tissues of dogs.

However, these findings also highlight the principal translational gap identified in this review. Research on GO in dentistry is dominated by in vitro studies and laboratory animal models, whereas canine-specific evidence remains limited. The studies by Miyaji et al. (2016), Kawamoto et al. (2018), and Qian et al. (2020) represent important advances but are insufficient to establish veterinary clinical protocols. Furthermore, the heterogeneity of GO formulations makes comparisons across studies difficult and hinders the identification of optimal concentrations and physicochemical characteristics.

Consequently, GO can currently be considered a promising but still experimental biomaterial for canine veterinary dentistry. Its greatest potential appears to lie in multifunctional systems integrating structural support, antibacterial activity, osteogenic or odontogenic stimulation, and localized delivery of therapeutic agents. Future research should prioritize canine preclinical models, physicochemically standardized formulations, long-term follow-up, and the simultaneous assessment of safety, inflammatory responses, tissue regeneration, and microbial control. Such an approach will be essential to determine whether the substantial body of experimental evidence accumulated on GO can ultimately be translated into safe and effective clinical applications in canine veterinary medicine.

CONCLUSIONS

The accumulated scientific evidence supports classifying the use of graphene oxide (GO) and its hybrid nanocomposites in canine veterinary dentistry as a disruptive preclinical technology at the proof-of-concept stage, characterized by high bioactive potential but still insufficient clinical validation.

Multifunctional Bioactive Platform: Findings from in vitro studies and laboratory animal models confirm that GO extends beyond the role of a passive mechanical reinforcement. It acts as an instructive microenvironment capable of modulating osteogenesis, promoting regenerative immunomodulation through M1-to-M2 macrophage polarization, preventing multispecies biofilm formation, and controlling the release kinetics of growth factors (e.g., BMP-2 and VEGF) and antimicrobial agents.

Limited Canine Preclinical Evidence: Although early in vivo milestones have demonstrated the efficacy of GO-based matrices for preservation of post-extraction alveolar bone, regeneration of the periodontal ligament and cementum in furcation defects, and attenuation of marginal bone loss in experimental peri-implantitis, the dog-specific literature is

characterized by marked temporal discontinuity and lacks contemporary studies published after 2022.

Biological and Methodological Gap: A critical disconnect persists between the expansion of GO biomaterial synthesis and its in vivo validation. The physicochemical heterogeneity of the nanomaterial (C/O ratio, lateral sheet size, and purity), the lack of standardized therapeutic doses, the absence of long-term immunosafety and clearance data, and the divergence between human bacterial models and interactions with the canine periodontal microbiome, particularly Porphyromonas gulae, represent the principal barriers to establishing clinically applicable protocols.

Declaration of conflict of interest

The author declares no conflict of interest related to this research.

Declaration of authorship contribution

Segundo Fabricio Mera Juaregui: Conceptualization, data curation, formal analysis, investigation, methodology, project administration, validation, visualization, writing – original draft, and writing – review and editing.

Artificial Intelligence Usage Statement

The author declare that they used Artificial Intelligence as support for this article, and also that this tool does not replace the intellectual task or process in any way. After rigorous reviews with different tools in which it was verified that there is no plagiarism as evidenced in the evidence, the authors state and acknowledge that this work was the product of their own intellectual work, that it has not been written or published on any electronic or AI platform.

REFERENCES

Ahn, J. H., Kim, I. R., Kim, Y., Kim, D. H., Park, S. B., Park, B. S., et al. (2020). The Effect of Mesoporous Bioactive Glass Nanoparticles/Graphene Oxide Composites on the Differentiation and Mineralization of Human Dental Pulp Stem Cells. *Nanomaterials*, *2020;10(4)*: 620. https://doi.org/10.3390/nano10040620

Alazab, M. H., Abouelgeit, S. A., & Aboushelib, M. N. (2023). Histomorphometric evaluation of 3D printed graphene oxide-enriched poly(ε-caprolactone) scaffolds for bone regeneration. *Heliyon*, *2023;9(5)*: e15844. https://doi.org/10.1016/j.heliyon.2023.e15844

Al-Noaman, A., & Rawlinson, S. C. F. (2023). A novel bioactive glass/graphene oxide composite coating for a polyether ether ketone-based dental implant. *Eur J Oral Sci*, *2023;131(2)*: e12915. https://doi.org/10.1111/eos.12915

An, N., Yan, X., Qiu, Q., Zhang, Z., Zhang, X., Zheng, B., et al. (2024). Human periodontal ligament stem cell sheets activated by graphene oxide quantum dots repair periodontal bone defects by promoting mitochondrial dynamics dependent osteogenic differentiation. *J Nanobiotechnology*, *2024;22(1)*: 133. https://doi.org/10.1186/s12951-024-02422-7

Biru, E. I., Necolau, M. I., Zainea, A., & Iovu, H. (2022). Graphene Oxide–Protein-Based Scaffolds for Tissue Engineering: Recent Advances and Applications. *Polymers*, *4 de marzo de 2022;14(5)*: 1032. https://doi.org/10.3390/polym14051032

Bousiakou, L. G., Qindeel, R., Al-Dossary, O. M., & Kalkani, H. (2022). Synthesis and characterization of graphene oxide (GO) sheets for pathogen inhibition: Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa. *J King Saud Univ - Sci*, *2022;34(4)*: 102002. https://doi.org/10.1016/j.jksus.2022.102002

Castro, J., Payan-Valero, A., Valencia-Llano, C., Insuasty, D., Rodríguez, J., Ordoñez, A., et al. (2024). Evaluation of the Antibacterial, Anti-Cervical Cancer Capacity, and

Biocompatibility of Different Graphene Oxides. *Molecules*, *5 de enero de 2024;29(2)*: 281. https://doi.org/10.3390/molecules29020281

Cheng, Q., Lu, R., Wang, X., & Chen, S. (2022). Antibacterial activity and cytocompatibility evaluation of the antimicrobial peptide Nal-P-113-loaded graphene oxide coating on titanium. *Dent Mater*, *J. 2022;41(6)*: 905–15. https://doi.org/10.4012/dmj.2022-094

Costa, M. C. F., Marangoni, V. S., Ng, P. R., Nguyen, H. T. L., Carvalho, A., & Castro, A. H. (2021). Accelerated Synthesis of Graphene Oxide from Graphene. *Nanomaterials*, *22 de febrero de 2021;11(2)*: 551. https://doi.org/10.3390/nano11020551

Cunha, E., Tavares, L., & Oliveira, M. (2022). Revisiting Periodontal Disease in Dogs: How to Manage This New Old Problem?. *Antibiotics*, *2022;11(12)*: 1729. https://doi.org/10.3390/antibiotics11121729

Dai, P., Qi, G., Zhu, M., Du, Q., Wang, K., Gao, Y., et al. (2024). Periodontal ligament stem cell tissue engineering scaffolds can guide and promote canine periodontal tissue regeneration. *Front Vet Sci*, *2024;11*: 1465879. https://doi.org/10.3389/fvets.2024.1465879

Du, T., Huang, B., Cao, J., Li, C., Jiao, J., Xiao, Z., et al. (2022). Ni Nanocrystals Supported on Graphene Oxide: Antibacterial Agents for Synergistic Treatment of Bacterial Infections. *ACS Omega*, *2022;7(22)*: 18339–49. https://doi.org/10.1021/acsomega.2c00508

Erdoğan, B. G., & Saritaş, Z. K. (2024). Farklı Restoratif. *Mater*, *yallerin Tükrük ve Serum Kortizol Seviyesi Üzerine Etkilerinin Değerlendirilmesi. Vol. 3. 2024;3(2)*: 27–37. DOI: https://orcid.org/0000-

Fani, K., Farahpour, M. R., & Tabatabaei, Z. G. (2022). SeO32−/ graphene oxide hybridized to multicomponent biopolymer based the scaffold to accelerate bone defect regeneration. *Ceram Int*, *2022;48(24)*: 37212–22. https://doi.org/10.1016/j.ceramint.2022.08.298

Gao, Y., Wang, X., & Fan, C. (2024). Advances in graphene-based 2D materials for tendon, nerve, bone/cartilage regeneration and biomedicine. *iScience*, *2024;27(7)*: 110214. https://doi.org/10.1016/j.isci.2024.110214

Gutiérrez-Cruz, A., Ruiz-Hernández, A. R., Vega-Clemente, J. F., Luna-Gazcón, D. G., & Campos-, J. (2022). A review of top-down and bottom-up synthesis methods for the production of graphene, graphene oxide and reduced graphene oxide. *J Mater Sci*, *2022;57(31)*: 14543–78. https://doi.org/10.1007/s10853-022-07514-z

Harvey, C. E. (2005). Management of Periodontal Disease: Understanding the Options. *Vet Clin North Am Small Anim Pract*, *2005;35(4)*: 819–36. https://doi.org/10.1016/j.cvsm.2005.03.002

Harvey, C., Crowder, S. E., Clarke, D. E., Goldschmidt, S., Stepaniuk, K. S., Hoyer, N., et al. (2023). Day one core competencies in veterinary dentistry. *J Am Vet Med Assoc*, *2023;261(12)*: 1880–6. https://doi.org/10.2460/javma.23.05.0242

Hosseini, F. S., Kan, H. M., Whitfield, T., Argyrou, C., Abedini, A. A., Allen, N. S., et al. (2025). Graphene Oxide in Bone Regenerative Engineering: Current Challenges and Future Perspectives. *ACS Bio Med Chem Au*, *2025;5(3)*: 350–64. https://doi.org/10.1021/acsbiomedchemau.4c00152

Huang, R., Gu, Y., Yuan, Y., Wang, Y., Pan, Y., Li, B., et al. (2024). A self-assembling graphene oxide coating for enhanced bactericidal and osteogenic properties of poly-ether-ether-ketone. *Front Bioeng Biotechnol*, *2024;12*: 1378681. https://doi.org/10.3389/fbioe.2024.1378681

Inchingolo, F., Inchingolo, A. M., Latini, G., Palmieri, G., Di, C., Trilli, I., et al. (2023). Application of Graphene Oxide in Oral Surgery: A Systematic Review. *Mater*, *ials. 2023;16(18)*: 6293. https://doi.org/10.3390/ma16186293

Jahan, N., Roy, H., Reaz, A. H., Arshi, S., Rahman, E., Firoz, S. H., et al. (2022). A comparative study on sorption behavior of graphene oxide and reduced graphene oxide towards

methylene blue. *Case Stud Chem Environ Eng*, *diciembre de 2022;6*: 100239. https://doi.org/10.1016/j.cscee.2022.100239

Jeong, J. O., Jeong, S. I., Lim, Y. M., & Park, J. S. (2022). Effective BMP-2 Release and Mineralization on a Graphene Oxide/Polyvinylpyrrolidone Hydrogel Forming Poly (ε-Caprolactone) Nanofibrous Scaffolds. *Mater*, *ials. 2022;15(23)*: 8642. https://doi.org/10.3390/ma15238642

Jiříčková, A., Jankovský, O., Sofer, Z., & Sedmidubský, D. (2022). Synthesis and Applications of Graphene Oxide. *Mater*, *ials. 2022;15(3)*: 920. https://doi.org/10.3390/ma15030920

Kabore, B. A., Njoroge, W. K., Masika, E., Minjauw, M. M., Dendooven, J., Detavernier, C., et al. (2026). Comparative analysis of structural, physicochemical, and electrochemical properties of graphene oxide derived from commercial graphite and rice husk biomass for sensing applications. *Results Mater*, *marzo de 2026;29*: 100867. https://doi.org/10.1016/j.rinma.2025.100867

Kawamoto, K., Miyaji, H., Nishida, E., Miyata, S., Kato, A., Tateyama, A., et al. (2018). Characterization and evaluation of graphene oxide scaffold for periodontal wound healing of class II furcation defects in dog. *Int J Nanomedicine*, *2018;Volume 13*: 2365–76. https://doi.org/10.2147/IJN.S163206

Kumari, S., Singh, D., Srivastava, P., Singh, B. N., & Mishra, A. (2022). Generation of graphene oxide and nano-bioglass based scaffold for bone tissue regeneration. *Biomed Mater*, *2022;17(6)*: 065012. https://doi.org/10.1088/1748-605X/ac92b4

Kwak, J. M., Kim, J., Lee, C., Park, I., Lee, M., Min, D., et al. (2022). Graphene Oxide as a Biocompatible and Osteoinductive Agent to Promote Implant Osseointegration in a Rabbit Tibia Model. *Adv Mater Interfaces*, *2022;9(28)*: 2201116. https://doi.org/10.1002/admi.202201116

Li, Y., Yang, L., Hou, Y., Zhang, Z., Chen, M., Wang, M., et al. (2022). Polydopamine-mediated graphene oxide and nanohydroxyapatite-incorporated conductive scaffold with an

immunomodulatory ability accelerates periodontal bone regeneration in diabetes. *Bioact Mater*, *2022;18*: 213–27. https://doi.org/10.1016/j.bioactmat.2022.03.021

Lima, R., Neves, J. G., Navarro, D. a. D., Prado, D. a. M. H., Hirata, M. C., Menezes, L., et al. (2026). Treatment of dental implant surfaces with graphene oxide nanoparticles: Synthesis, characterization, and assessment of antimicrobial potential. *Proc Inst Mech Eng [H]*, *2026;240(3)*: 246–56. https://doi.org/10.1177/09544119261416733

Lin, L., Zheng, Y., Wang, C., Li, P., Xu, D., & Zhao, W. (2023). Concentration-Dependent Cellular Uptake of Graphene Oxide Quantum Dots Promotes the Odontoblastic Differentiation of Dental Pulp Cells via the AMPK/mTOR Pathway. *ACS Omega*, *2023;8(6)*: 5393–405. https://doi.org/10.1021/acsomega.2c06508

Liu, L., Zhu, C., Fan, M., Chen, C., Huang, Y., Hao, Q., et al. (2015). Oxidation and degradation of graphitic materials by naphthalene-degrading bacteria. *Nanoscale*, *2015;7(32)*: 13619–28. https://doi.org/10.1039/C5NR02502H

Liu, J., Chen, S., Liu, Y., & Zhao, B. (2022). Progress in preparation, characterization, surface functional modification of graphene oxide: A review. *J Saudi Chem Soc*, *2022;26(6)*: 101560. https://doi.org/10.1016/j.jscs.2022.101560

Liu, Y., Niu, C., Chu, M., Liu, M., & Chi, Y. (2025). The preparation and characterization of graphene oxide- multiwalled minocycline coatings on ultrafine-grained titanium implants for enhanced performance studies. *Front Oral Health*, *2025;6*: 1565325. https://doi.org/10.3389/froh.2025.1565325

Martuci, R., Oliveira, S. J., Martuci, M., Reis-Campos, J., & Figueiral, M. H. (2025). Antimicrobial Effect of Graphene in Dentistry: A Scoping Review. *Dent J*, *2025;13(8)*: 355. https://doi.org/10.3390/dj13080355

Mashhadi, M., Taghvaei, H., Noroozi, R., Eskandari, V., Arif, Z. U., Bodaghi, M., et al. (2024). Biological and Mechanical Response of Graphene Oxide Surface-Treated Polylactic Acid

3D- Printed Bone Scaffolds: Experimental and Numerical Approaches. Adv Eng. *Mater*, *2024;26(3)*: 2301260. https://doi.org/10.1002/adem.202301260

Miyaji, H., Kato, A., Takita, H., Iwanaga, T., Momose, T., Ogawa, K., et al. (2016). Graphene oxide scaffold accelerates cellular proliferative response and alveolar bone healing of tooth extraction socket. *Int J Nanomedicine*, *2016;2265*. https://doi.org/10.2147/IJN.S104778

Narváez-Romero, A. M., Rodríguez-Lozano, F. J., & Pecci-Lloret, M. P. (2025). Graphene-Based. *Mater*, *ials for Bone Regeneration in Dentistry*: A Systematic Review of In Vitro Applications and Material Comparisons. Nanomaterials. 8 de enero de 2025;15(2):88. https://doi.org/10.3390/nano15020088

Naseri, M., Abbasi, M., Rezaei, E., Amirian, R., Mavaei, M., Mohammadi, G., et al. (2026). Smart biomaterials in the dimensional revolution of bioprinting: From 2D to 6D for advanced biomedical applications. *Biomed Pharmacother*, *2026;201*: 119718. https://doi.org/10.1016/j.biopha.2026.119718

Nishina, Y., & Eigler, S. (2020). Chemical and electrochemical synthesis of graphene oxide – a generalized view. *Nanoscale*, *2020;12(24)*: 12731–40. https://doi.org/10.1039/D0NR02164D

Park, J., Park, S., Kim, J. E., Jang, K. J., Seonwoo, H., & Chung, J. H. (2021). Enhanced Osteogenic Differentiation of Periodontal Ligament Stem Cells Using a Graphene Oxide-Coated Poly(ε-caprolactone) Scaffold. *Polymers*, *2021;13(5)*: 797. https://doi.org/10.3390/polym13050797

Pradyumn, Sharma, N., Barman, P. B., Sil, A., & Hazra, S. K. (2026). Ambient-driven functional group modulation for p–n switching in reduced graphene oxide. *Mater*, *2026;13*: 102783. https://doi.org/10.1016/j.nxmate.2026.102783

Purbantoro, S. D., Taephatthanasagon, T., Purwaningrum, M., Hirankanokchot, T., Peralta, S., Fiani, N., et al. (2024). Trends of regenerative tissue engineering for oral and

maxillofacial reconstruction in veterinary medicine. *Front Vet Sci*, *2024;11*: 1325559. https://doi.org/10.3389/fvets.2024.1325559

Qi, X., Jiang, F., Zhou, M., Zhang, W., & Jiang, X. (2021). Graphene oxide as a promising material in dentistry and tissue regeneration: A review. *Smart Mater Med*, *2021;2*: 280–91. https://doi.org/10.1016/j.smaim.2021.08.001

Qian, W., Qiu, J., & Liu, X. (2020). Minocycline hydrochloride-loaded graphene oxide films on implant abutments for peri-implantitis treatment in beagle dogs. *J Periodontol*, *2020;91(6)*: 792–9. https://doi.org/10.1002/JPER.19-0285

Qin, W., Li, C., Liu, C., Wu, S., Liu, J., Ma, J., et al. (2022). 3D printed biocompatible graphene oxide, attapulgite, and collagen composite scaffolds for bone regeneration. *J Biomater Appl*, *2022;36(10)*: 1838–51. https://doi.org/10.1177/08853282211067646

Qiu, Z., Lin, X., Zou, L., Fu, W., & Lv, H. (2024). Effect of graphene oxide/ poly-L-lactic acid composite scaffold on the biological properties of human dental pulp stem cells. *BMC Oral Health*, *4 de abril de 2024;24(1)*: 413. https://doi.org/10.1186/s12903-024-04197-7

Radunovic, M., Pavic, A., Ivanovic, V., Milivojevic, M., Radovic, I., Di, R., et al. (2022). Biocompatibility and antibiofilm activity of graphene-oxide functionalized titanium discs and collagen membranes. *Dent Mater*, *2022;38(7)*: 1117–27. https://doi.org/10.1016/j.dental.2022.04.024

Shahriari, S., Sastry, M., Raman, R. S., & Panjikar, S. (2026). Graphene-oxide surface chemistry governs protein adsorption: molecular docking insights. *Carbon*, *2026;254*: 121472. https://doi.org/10.1016/j.carbon.2026.121472

Souza, A. P., Neves, J. G., Navarro, D. a. D., Lopes, C. C., Moraes, M., Correr-Sobrinho, L., et al. (2023). Chitosan/Xanthan/Hydroxyapatite-graphene oxide porous scaffold associated with mesenchymal stem cells for dentin-pulp complex regeneration. *J Biomater Appl*, *abril de 2023;37(9)*: 1605–16. https://doi.org/10.1177/08853282231155570

Suhaimin, N. S., Hanifah, M. F. R., Azhar, M., Jaafar, J., Aziz, M., Ismail, A. F., et al. (2022). The evolution of oxygen-functional groups of graphene oxide as a function of oxidation degree. *Mater*, *Chem Phys. 2022;278*: 125629. https://doi.org/10.1016/j.matchemphys.2021.125629

Sánchez-Cepeda, A., Pazos, M. C., Leonardo, P. A., Ingrid, S. C., Correa-Araujo, L. S., María, D. e. C. G., et al. (2024). Functionalization of 3D printed poly(lactic acid)/graphene oxide/β-tricalcium phosphate (PLA/GO/TCP) scaffolds for bone tissue regeneration application. *RSC Adv*, *2024;14(54)*: 39804–19. https://doi.org/10.1039/D4RA05889E

Tavakoli, M., Emadi, R., Salehi, H., Labbaf, S., & Varshosaz, J. (2023). Incorporation of graphene oxide as a coupling agent in a 3D printed polylactic acid/hardystonite nanocomposite scaffold for bone tissue regeneration applications. *Int J Biol Macromol*, *2023;253*: 126510. https://doi.org/10.1016/j.ijbiomac.2023.126510

Uslu, C., Tatar, B. E., Uyanıkgil, Y., Tomruk, C., Yılmaz, B., Demirkol, N., et al. (2024). Evaluation of graphene oxide-doped poly-lactic-co-glycolic acid (GO-PLGA) nanofiber absorbable plates and titanium plates for bone stability and healing in mandibular corpus fractures: An experimental study. *J Plast Reconstr Aesthet Surg*, *2024;92*: 79–86. https://doi.org/10.1016/j.bjps.2024.02.063

Ward, E. (2022). A Review of Tissue Engineering for Periodontal Tissue Regeneration. *J Vet Dent*, *2022;39(1)*: 49–62. https://doi.org/10.1177/08987564211065137

Wei, Y., Wang, Z., Han, J., Jiang, X., Lei, L., Yang, X., et al. (2022). Modularized bioceramic scaffold/hydrogel membrane hierarchical architecture beneficial for periodontal tissue regeneration in dogs. *Biomater Res*, *2022;26(1)*: 68. https://doi.org/10.1186/s40824-022-00315-0

Williams, A. G., Moore, E., Thomas, A., & Johnson, J. A. (2023). Graphene-Based. *Mater*, *ials in Dental Applications*: Antibacterial, Biocompatible, and Bone Regenerative Properties.

Seifalian A, editor. Int J Biomater. 2023;2023:1–18. https://doi.org/10.1155/2023/8803283

Wu, S., Gan, T., Xie, L., Deng, S., Liu, Y., Zhang, H., Antibacterial, o. f. f. o. r. i. n. A. d. v., et al. (n.d.). . https://doi.org/10.1016/j.bioadv.2022.213121

Xi, M., Wan, R., Luo, W., He, Y., Feng, X., Wang, T., et al. (2026). Graphene Oxide–based Biomaterials in Sports Medicine. *BIO Integr*, *2026;7(1)*. https://doi.org/10.15212/bioi-2025-0172

Xie, Q., Wang, T., He, L., Liang, H., Sun, J., Huang, X., et al. (2024). Biological and structural properties of curcumin-loaded graphene oxide incorporated collagen as composite scaffold for bone regeneration. *Front Bioeng Biotechnol*, *2024;12*: 1505102. https://doi.org/10.3389/fbioe.2024.1505102

Yang, H., Wu, X., Ma, Q., Yilihamu, A., Yang, S., Zhang, Q., et al. (2019). Fungal transformation of graphene by white rot fungus Phanerochaete chrysosporium. *Chemosphere*, *febrero de 2019;216*: 9–18. https://doi.org/10.1016/j.chemosphere.2018.10.115

Yang, J., Shojaei, S., & Shojaei, S. (2022). Removal of drug and dye from aqueous solutions by graphene oxide: Adsorption studies and chemometrics methods. *Npj Clean Water*, *2022;5(1)*: 5. https://doi.org/10.1038/s41545-022-00148-3

Yang, Y., Li, M., Zhou, B., Jiang, X., Zhang, D., & Luo, H. (2023). Graphene oxide/gallium nanoderivative as a multifunctional modulator of osteoblastogenesis and osteoclastogenesis for the synergistic therapy of implant-related bone infection. *Bioact Mater*, *2023;25*: 594–614. https://doi.org/10.1016/j.bioactmat.2022.07.015

Yang, F., Lin, Y., Shen, S., Gu, Y., Shuai, C., & Feng, P. (2024). Polydopamine chelating strontium on graphene oxide enhances the mechanical and osteogenic induction properties of PLLA/PGA bone scaffold. *Int J Bioprinting*, *2024;10(1)*: 1829. https://doi.org/10.36922/ijb.1829