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Review Article

Applications of graphene oxide and reduced graphene oxide in advanced dental materials and therapies

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الملخص

اكتسبت عائلة الجرافين من المواد النانوية الاهتمام في مجال طب الأسنان بسبب خصائصها الجذابة. يعد أكسيد الجرافين وأكسيد الجرافين المخفض من مشتقات الجرافين الرئيسية المستخدمة على نطاق واسع في تطبيقات طب الأسنان. إن خواصه الميكانيكية الممتازة، وتوافقه الحيوي الفائق، وخصائصه المضادة للبكتيريا الجيدة، وثباته الكيميائي الشديد، وخصائصه القبلية المفضلة تجعل هذه المادة لا مفر منها في طب الأسنان. تسمح الطبيعة البرمائية لـ أكسيد الجرافين بإجراء تعديلات تساهمية وغير تساهمية مناسبة للتطبيقات الطبية الحيوية. يمكن أن يؤثر الجرافين على تمايز الخلايا الجذعية لب الأسنان ويعزز خصائص المواد الحيوية الأخرى. هنا تمت مراجعة تطبيقات طب الأسنان لـ أكسيد الجرافين أو أكسيد الجرافين المخفض في النشاط المضاد للميكروبات، وتوصيل الأدوية العلاجية، وطب الأسنان الترميمي، وزراعة الأسنان، وتجديد اللب، وتجديد العظام، وتجديد أنسجة اللثة، وأجهزة الاستشعار الحيوية، وتبييض الأسنان.

الكلمات المفتاحية: أكسيد الجرافين؛ طب الأسنان الترميمي؛ المواد الحيوية؛ زراعة الأسنان؛ تجديد اللب

Abstract

The graphene family of nanomaterials acquired significant attention in the field of dentistry due to a range of interesting properties. Graphene oxide (GO) and reduced graphene oxide (rGO) are the major graphene derivatives that are widely used in dental applications. These derivatives exhibit excellent mechanical properties, superior biocompatibility, good antibacterial properties, extreme chemical stability, and favorable tribological characteristics, thus representing highly materials for dentistry. The amphiphilic nature of GO allows covalent and noncovalent modifications that are favorable for biomedical applications. Graphene can influence the differentiation of dental pulp stem cells (DPSCs) and enhance the properties of other biomaterials. Here, we review the dental applications of GO or rGO with regards to antimicrobial activity, therapeutic drug delivery, restorative dentistry, implants, pulp regeneration, bone regeneration, periodontal tissue regeneration, biosensors, and tooth whitening.

Keywords: Biomaterial; Graphene oxide; Implants; Pulp regeneration; Restorative dentistry

Introduction

Maintaining oral health has superior importance for individuals and the well-being of society. The Global Oral Health Status Report (2022), published by the World Health Organization, (WHO) reported that almost 3.5 million people suffer from oral disorders. However, oral problems are usually preventable in the initial stages.[1] At present, the maintenance of oral health remains a notable challenge. Even though several methods and techniques have been implemented to manage oral diseases, there is still no perfect method. Several biomaterials have been applied in dentistry in an attempt to improve therapeutic applications.

The application of biomaterials to treat dental conditions is challenging since these materials need to be situated within the mouth and therefore exposed to various environmental factors, including moisture, temperature, pressure, different types of food, and abrasion from toothbrushes. The extreme conditions to which dental materials can be exposed can

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induce mechanical failure or degradation, thus resulting in treatment failure and the need for further work, inconvenience and additional cost. The primary concerns associated with the development of dental materials are their biocompatibility, mechanical strength, flexibility, ease of modification, and their ability to improve environmental conditions within the oral cavity.[2]

The graphene family of nanomaterials possess unique and versatile chemical, mechanical, thermal, and biological properties that have garnered significant attention in the field of dental research and other biomedical applications. Graphenes are two-dimensional (2-D) carbon materials with unique planar structures of sp^2-hybridized carbon atoms with specific chemical, electrical, mechanical, and thermodynamic properties. Graphene is one of the strongest and thinnest materials in nature and offers an ultrahigh surface area (2600 m^2/g).[3] Graphene has various derivatives that exhibit distinct forms, chemical compositions, and morphologies. Graphene oxide and reduced graphene oxides are the primary forms under investigation for biomedical and non-biomedical applications. The potential application of graphene sheets remains limited due to low water dispersibility, hydrophobicity, high surface area, and high surface energy.[4]

Graphene oxides are oxygenated forms of graphene that consist of an abundance of functional oxygen groups, including carboxyl, hydroxyl, carbonyl, and epoxides. The presence of oxygen functionalities means that these forms of graphene are compatible with water and dispersible. However, the oxidation of graphene leads to defects and a reduction in mechanical properties. To counteract this, graphene oxide (GO) can be reduced chemically or thermally to reduced graphene oxide (rGO) as this can improve mechanical properties and retain hydrophilicity. In addition, rGO can retain the high surface area and strength of pristine graphene and the hydrophilicity of GO, at least in part[5]

GO and rGO exhibit unique properties that differ from pristine graphene, thus allowing for applications in technical fields, such as chemical and microelectronic devices, energy storage, biomedicine,[6] and composite materials.[7] Graphene can affect the differentiation of stem cells and improve the properties of other biomaterials. In addition, graphene can rapidly and powerfully combine with other biomaterials in terms of regenerative, antimicrobial, and restorative dentistry and can exert synergistic effects. The outstanding mechanical characteristics, chemical stability, biocompatibility, antibacterial activity, and favorable tribological characteristics of graphene can facilitate reductions in friction and wear.

Graphene-related composites (particularly GO) possess a number of advantages, including excellent ductility and elasticity, a large surface area, outstanding mechanical strength, and significant biocompatibility. Furthermore, graphene can easily be functionalized (covalently and non-covalently) with other functional materials to improve the effects desired for dental applications. While the research and development of dental biomaterials with graphene remain at an embryonic stage, their specific characteristics and potential to be functionalized with biomaterials provide us with significant options for numerous clinical applications.

In this review, we discuss the potential applications of GO and rGO in dentistry by considering existing literature and recent developments. Initially, we summarize the properties of GO and rGO along with the methods used for their synthesis. Then, we outline biocompatibility concerns and the existing literature relating to biocompatibility.

Properties of graphene oxide

Physicochemical properties

GO is a 2-D material with a random allocation of oxidized areas with oxygen functionalities and non-oxidized carbon atoms in sp^2-hybridization. Carboxyl functional groups are situated on the edges of graphene sheets, while hydroxy and epoxy groups are situated on the basal plane. The interplanar spacing of GO is greater following oxidation. The structure and oxidation level of GO are purely based on the oxidation conditions, energetic input, and physicochemical properties of the graphite used. The properties of GO can all be influenced by altering these parameters. The reduction of GO can lead to significant changes in behavior which allow GO to regain its graphene-like properties. Structurally, rGO is similar to graphite with few oxygen functionalities. The structures of GO and rGO are shown in Figure 1.[8,9]

The 2-D structure of GO provides an extensive surface area and good colloidal stability. However, rGO exhibits poor colloidal stability due to fewer oxygen functionalities.[10] A form of rGO with good colloidal stability can be prepared by changing the reducing agents. In nature, GO is highly hydrophilic and can form stable aqueous dispersions over a wide concentration range. Similarly, organic solvents, such as dimethyl formamide, tetrahydrofuran, ethylene glycol, and n-methyl-2-pyrrolidone, can also form stable dispersions with GO.[11]

The stability of GO in a solvent is based on the cohesion parameters of the solute and solvent and particularly, interactions on the GO/solvent interface. The solubility of GO is generally based on the hydrogen bonding between the functional groups in GO and the molecules in solvents. Tertiary alcohols are the major functional groups that participate in hydrogen bonding; epoxides only play a minor role in this process. rGO exhibits hydrophobicity due to its increased carbon/oxygen ratio.[12]

Mechanical properties

Graphene is one of the most robust materials in nature. Knowledge about the mechanical strength of graphene and its derivatives is vital for dental applications. Graphenes exhibit attractive mechanical properties with nearly 1.0 TPa Young's modulus and 130 $\pm$ 10 GPa. The introduction of oxygen groups can affect various characteristics of graphene. The mechanical strength of GO is less than that of pristine graphene.[7,13] Several groups have investigated the mechanical properties of GO, although these properties can vary across different samples. The layer thickness is an important factor to note when considering the mechanical behavior of GO and is inversely proportional to Young's modulus. The graphene monolayer has a very high Young's modulus when compared to thick GO.[7]

Figure 1: Diagrammatic representation of GO and the synthesis of GO.

Electrical properties

GO and rGO can exhibit different electrical properties depending upon the number of functional oxygen groups present. GO is almost an electrical insulator due to the fact that it possesses more oxygen functional groups on the basal plane and edges. Oxidation disrupts the π conjugation and induces bandgaps, thus preventing the movement of charge carriers. Graphene is a significant conduction; rGO is also a good conductor but is not as efficient as graphene due to the residual functional groups remaining after reduction.[8]

Optical properties

Both GO and rGO exhibit assertive absorption behavior in the ultra-violet (UV) and visible light regions because of the presence of $\pi-\pi^*$ transitions within the oxygen functionalities. The λ_{max} of GO and rGO is usually observed at ~230 nm and ~260 nm, respectively. Regaining the π conjugated structure of rGO has been shown to result in increased transparency in the UV visible and near infra-red (NIR) regions. Fluorescence quenching ability, photoluminescence, and plasmonic effects can all be tailored for various applications such as sensing and photothermal therapy.[14,15]

Synthesis of GO and rGO

Synthesis of GO

The unique properties of graphene nanomaterials have led to a significant increase in demand and generated a search for the more scalable synthesis of graphene and its derivatives. Existing methods for the synthesis of graphene, such as graphite exfoliation[16,17] and chemical vapor deposition,[18,19] are unsuitable for industrialization. Thus, GO was considered as an alternative as this can be easily synthesized from graphite by oxidation.[20–22] GO is the oxidized version of graphene and features several functional oxygen groups (e.g., carbonyl, hydroxyl, carboxyl, and epoxide groups) on its basal plane. These oxygen groups can be introduced into graphene by chemical oxidation. The incorporation of oxygen makes graphite more hydrophilic, weakens the interaction between layers, and increases dispersibility in a variety of solvents.[23–25] This method results in the easy exfoliation of graphite into the single-layered graphene oxide.

There are five established methods for the preparation of graphene oxide: the Brodie method,[26] the Staudenmaier method,[27] the Hofmann method,[28] the Hummers' method[29] and the Tour method.[30] Modifications of these methods have also been reported.[31–36] All these methods involve the initial treatment of graphite with a strong acid, such as sulfuric acid (H_2SO_4), hydrochloric acid (HCl), and nitric acid (HNO_3), followed by a reaction with an alkali metal compound such as potassium permanganate ($KMnO_4$). The Brodie, Staudenmaier, and Hofmann methods use potassium chlorate ($KClO_3$) as an oxidizing agent, whereas the Hummers' method uses $KMnO_4$ as an oxidizing agent, thus reducing the risk of explosion during reaction. However, the Hummers' method oxidizes only the surface of the graphite; this results in partial oxidization and creates difficulty in terms of exfoliation. Therefore, the Hummers' method was modified further to enhance synthetic efficiency.[26–29]

In 2010, Tour et al. developed a novel method for the synthesis of graphene oxide which entirely avoided the use of sodium nitrate ($NaNO_3$). Instead, these authors increased the amount of $KMnO_4$ and performed the reaction in an H_2SO_4/H_3PO_4 mixture (9:1 ratio). These changes improved the efficiency and extent of oxidation.[30] The advantages and disadvantages of the various methods used to synthesize GO and rGO are given in Table 1 and Table 2, respectively.

Synthesis of rGO

rGO is synthesized by the reduction of GO; this represents the most acceptable way to produce rGO on an industrial scale.[9,37,38] This reduction procedure enables the production of a structure that is similar to graphene that contains oxygen atoms, heteroatoms, and structural flaws. As a result of this reduction, most oxygen atoms will be removed and the aromatic conjugation will also be restored, thus providing rGO with graphene-like properties. Several methods can be used to reduce graphene oxide, including chemical, electrochemical, thermal, hydrothermal, and photocatalyst reduction methods. Of these, the most widely used method is chemical reduction.[39]

One of the best ways to produce rGO is by chemical reduction; this method can enable the large-scale synthesis of rGO.[40] A wide variety of reducing agents has been used for this type of reduction, including sodium borohydride ($NaBH_4$)[41]; strong alkali solutions, such as sodium hydroxide (NaOH) or potassium hydroxide (KOH)[42]; and various organic and inorganic solvents.[41,43–45] Thermal reduction is a convenient method for the reduction of GO reduction and does not leave reagent residues after synthesis. Thermal reduction can be accomplished by the rapid heating (>2000 °C/min) of GO in an inert vacuum, atmosphere or in a reducing atmosphere.[46–48]

Table 1: Advantages and disadvantages of various models used to synthesize GO.

Method	Advantage	Disadvantage	Reference
Brodie method	- Scalable process	- Longer reaction time - Multiple oxidation process - Generation of toxic gases - Explosion chances	19
Staudenmaier method	- The addition of an H_2SO_4 aliquot and additional $KClO_3$ improved the extent of oxidation - Simple method when compared to the Brodie method - Scalable process	- Time consuming - Hazardous - $KClO_3$ addition lasted for one week - Inert gas is required to remove the chlorine dioxide evolving - Risk of explosion	20
Hofmann method	- H_2SO_4 and HNO_3 are used together to reduce the fumes produced by HNO_3 - Scalable process	- An inert gas is required to remove the chlorine dioxide evolved - Risk of explosion - Hazardous	21
Hummer's method	- The use of H_2SO_4, $NaNO_3$, and $KMnO_4$ - No chlorine dioxide fumes - Simple method - Scalable process	- Use of corrosive H_2SO_4 - Generation of toxic gas - Difficult to control temperature	22
Tour's method	- More efficient method - Improved yield - No toxic gas is generated - Scalable process		23

Table 2: The advantages and disadvantages of various methods used to synthesize rGO.

Method	Advantage	Disadvantage	Reference
	Reduced graphene oxide synthesis		
Chemical reduction	- Widely accepted method - Wide variety of chemical reductants are available - Scalable process	- Removal of reductant residue is difficult - Presence of residual oxygen and hetero atoms - Structural flaws	31–40
Thermal reduction	- No harmful residues are left - Simple method	- Higher temperature - Release of CO and CO_2 gases cause structural defects as this methods is performed in a vacuum - Produces minuscule and wrinkled graphene	41–43
Microwave and photo reduction	- Fast and energy efficient method - The concurrent reduction and doping of GO and the preparation of hybrid materials are possible - Widely used method for reduction and exfoliation	- Not scalable - Risk of explosion	44–46
Photo catalyst reduction	- Facile, clean, and versatile method	- The use of harmful radiation (UV) makes this method less user-friendly - Not scalable	47–50
Solvothermal/ hydrothermal reduction	- Very effective method; significantly reduces flaws and defects - High-quality graphene can be obtained	- Relatively higher pressure required - Not suitable with all solvents - Maintaining super critical conditions can be tedious	51–53
Electrochemical reduction of GO	- No additives required - Room temperature reaction	- Requires expensive equipment - Waste disposal is difficult due to hazardous nature	54–56

Microwave or photo-irradiation is one of the prominent methods used to reduce GO and represents an rapid and energy-efficient technique. Microwave irradiation can directly transfer energy to a reactant and cause a sudden rise in the internal temperature of the reaction mixture, thus improving the reaction efficiency and reducing the reaction time.[31,49,50] Photocatalysts, such as titanium dioxide (TiO_2), can also be used to reduce GO and act by removing functional groups from the GO, thus creating a reducing mechanism to form rGO.[51–53]

The solvothermal/hydrothermal reduction of GO is performed at a lower temperature but with a higher pressure. This represents a transformation or chemical reaction that takes place in a solvent with supercritical conditions or close to a pressure–temperature domain arising from heating.[54–56] A conventional electrochemical apparatus can also be used to reduce GO films to rGO at ambient temperature using a buffered aqueous solution; reduction occurs via the exchange of electrons between the electrodes and GO.[57–59]

Biocompatibility

It is important to determine the biocompatibility of GO when considering biomedical applications. Researchers continue to investigate the impact of GO on biological systems and develop new strategies to make GO safer and more effective. The biocompatibility of graphene material is highly dependent on its size, shape, level of oxidation, particulate state, surface functionalization, the number of layers, the method of preparation, and the biological context in which GO is utilized.[60] The size and exfoliation of GO mostly depends on the intensity and time of sonication.[61]

A previous study investigated the toxicity of GO and rGO on human red blood cells (RBCs) and fibroblasts. GO and graphene sheets (GS) were synthesized by 5-h bath sonication (bGO), 5-min probe sonication (p-GO 5), 30-min probe sonication (p-GO 30), and hydrothermal treatment (GS), respectively. The smallest GO exhibited the greatest hemolytic activity, whereas the aggregated GS exhibited less hemolysis. The coating of GO with chitosan almost eliminated hemolysis. The clear cytotoxicity on fibroblast cells suggested that compacted graphene sheets caused more toxicity than less extensively packed sheets. The effect of GO and rGO was found to depend on environment exposure and the type of interaction with cells. The hemolytic effects of GO, GS, and chitosan/GO are depicted in Figure 2(A-E).[61]

Multiple studies have suggested that an increased level of reactive oxygen species (ROS) is a key factor responsible for cytotoxicity, as ROS can oxidize DNA, proteins, and lipids.[60] In a previous study, the biocompatibility of GO was estimated on human fibroblasts and mice. In fibroblast cells, 50 μg/mL of GO led to the induction of apoptosis and a reduction in the extent of adhesion. GO-injected mice revealed no signs of toxicity at a low dose (0.1 mg) and mid-dose (0.25 mg). However, at a high dose (0.4 mg), lung granuloma formation was observed and the kidneys were unable to clear the GO.[62]

GO derivatives exhibit less stability in biological media; this phenomenon is known as reversible aggregation. Ionic salts in the biological media can neutralize the charge of the surface oxygen groups and result in aggregation. Hence, modification of the surface of GO is a vital requirement prior to biological applications.[61] GO and rGO surface modification can be achieved through both covalent and non-covalent modifications. Covalent modification of GO is possible through the bond formation of polymers or molecules with oxygen-containing functional groups and cycloaddition reactions. Non-covalent modification can be performed easily with GO and rGO through π–π interactions and van der Waals forces. In addition, aromatic molecules can efficiently adsorb onto the surface of GO or rGO via π–π interactions.[63]

Biocompatibility challenges and the adhesion of non-specific biomolecules were addressed in several previous studies by modification with PEG. Theis modification improved the solubility and biocompatibility of GO as it PEG was able to cap the surface functionalities and reduce hazards. Several studies have reported that PEG functionalization reduced toxicity in various cell lines and animal models.[63–65] For example, GO was found to induce complete fatality when administered to mice at a dose of 80 mg/kg;

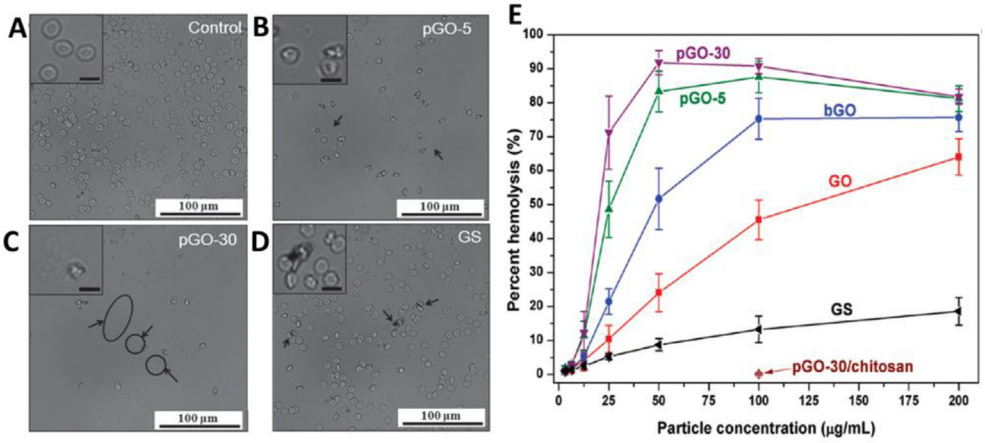

Figure 2: (A–D). Optical micrographs of hemolytic activity. RBCs with PBS **(A),** pGO-5 **(B),** pGO-30**(C),** and GS **(D),** and the percentage hemolysis of RBCs with GO, GS, and chitosan/GO **(E).** Reprinted with permission from ACS 2011.[61]

however, a 100% survival rate was recorded when mice were administered with PEG-grafted GO.[65]

The surface charge of GO or rGO can be altered by surface functionalization. For example, positively charged and surface-modified GO nanoplatelets were found to induce higher pulmonary inflammogenicity in a previous study.[66] A GO biodistribution study further reported greater levels of accumulation in organs that were rich in the reticuloendothelial system (RES) and that GO was cleared rapidly from the blood circulation. The accumulation of GO varied according to size; large GO accumulated preferentially in the lungs, whereas small GO accumulated preferentially in the liver.[67] The alteration of surface charge by surface functionalization has also been shown to influence the biodistribution of GO. The administration of GO with Tween-80 was previously shown to change the pattern of biodistribution by reducing liver accumulation and enhancing lung accumulation.[67,68]

Graphene is known to exhibit immunological properties. The interaction of graphene with immune cells is based on factors such as the type of graphene derivative, the dimensions of the graphene sheet, concentration, incubation time, and notably the nature of interacting cells. Previous studies have reported that graphene with a more irregular surface can induce greater levels of toxicity in immune cells. GO has also been shown to increase the production of pro-inflammatory cytokines (IL-1, IL-6, IL-10, and TNF-alpha) in vitro due to the activation of Toll-like receptors in macrophages.[69] The effect of GO- and GO-AgNPs coated nickel-titanium (Ni—Ti) alloy on the viability and expression of IL-6 and IL-8 were previously investigated in human pulp fibroblasts. The coated alloy exhibited good biocompatibility with fibroblasts and also upregulated the levels of IL-6 and IL8, as determined by enzyme-linked immunosorbent assay (ELISA)[70]

GO can also exhibit dose-dependent toxicity to cells and animals, such as the induction of cellular apoptosis, the formation of lung granuloma, and difficulty in kidney clearance. When graphene derivatives are used for in vivo applications, their biocompatibility must be investigated; effects are predominantly related to dose, physicochemical characteristics, and the biological context in which GO is utilized.

Applications of GO in dentistry

Multidisciplinary research is required to develop a suitable biomaterial for dental applications. The design of dental materials is very important since the oral and maxillofacial tissues, and the teeth, exhibit different physical, chemical and biological characteristics. A single component material cannot provide all of the required properties for dental applications. Therefore, the application of composite materials with other components are necessary to meet the specific requirements of dental applications. Therefore, researchers have exploited the antimicrobial properties, ultra-high surface area, the presence of functional groups, and the ease of modification of graphene in combination with other biomaterials and biomolecules to assist the development of new dental applications. The potential application of GO and rGO in the dental field is discussed at length in the next section.

Antimicrobial activity

Graphene exhibits intrinsic antimicrobial activity. The physicochemical interaction of graphene with microbial cells is the underlying reason for this phenomenon. The size, shape, and generation of ROS are the primary factors contributing to the antimicrobial activity of graphene. Previous research has shown that microbes and dental problems are closely related. Furthermore, the microbial colonization of the oral cavity maintains a strict balance with the oral microenvironment. This balance is essential to keep the oral cavity safe. Dental caries and periodontal disorders are known to arise from unbalanced microbial activity. *Streptococcus mutans* is an important cariogenic microorganism that produces high amounts of organic acids, thus reducing the pH of the oral cavity. *Fusobacterium nucleatum* and *Porphyromonas gingivalis* are anaerobic Gram-negative bacteria associated with infection of the root canal and periodontitis.[71]

The antimicrobial properties of graphene nanomaterials have gained increasing popularity due to their ability to disrupt cell membranes and generate ROS. The chemical and physical properties of graphene-based nanomaterials, such as morphology, flake size, concentration, and surface functionalities, are known to play a main role in antimicrobial activity. However, these factors will further depend on the microbial cell and stages of maturity.[72]

The effect of GO (20, 40, and 80 μg/mL) on dental pathogens was previously analyzed using three different types of bacteria (*P. gingivalis, S. mutans, and F. nucleatum*) that can cause dental caries, along with periapical and periodontal diseases. These results suggested that GO is highly efficient in killing dental pathogens, and that the activity increases with increasing concentrations of GO. Transmission electron microscopy (TEM) images of microorganisms treated with GO further revealed the loss of membrane integrity, cell wall damage, and, thereby, the leakage of cell contents (Figure 3A).[71]

Similarly, Zhao et al..[73] evaluated the activity of plain GO with varying degrees of oxidation and concentration. These authors evaluated the antimicrobial effects of GO against *S. mutans* in biofilm and planktonic forms; results revealed the concentration-dependent effect and the efficient activity of GO at higher concentrations (80 μg/mL). This study confirmed that the functional groups of GO plays a crucial role in antimicrobial activity; higher levels of toxicity were observed with more extensive oxygen functionality.

The soft pulp of teeth is more prone to pathogenic infection. Root canal therapy (RCT) is the method of choice to save affected teeth and does so by removing the infected pulp and by disinfecting and filling the cavity with an inert obturating material. The application of obturating material is critical for the success of RCT and relies upon antimicrobial properties, biocompatibility, and sealant effects. For the first time, researchers have developed a biocompatible, adhesive, antimicrobial and graphene-based endodontic obturating material for RCT.

rGO nanoplatelets represent an embedded nanocomposite that is made by the reversible addition—fragmentation chain-transfer (RAFT) polymerization of ethylene glycol dimethacrylate (EGDMA) and methyl

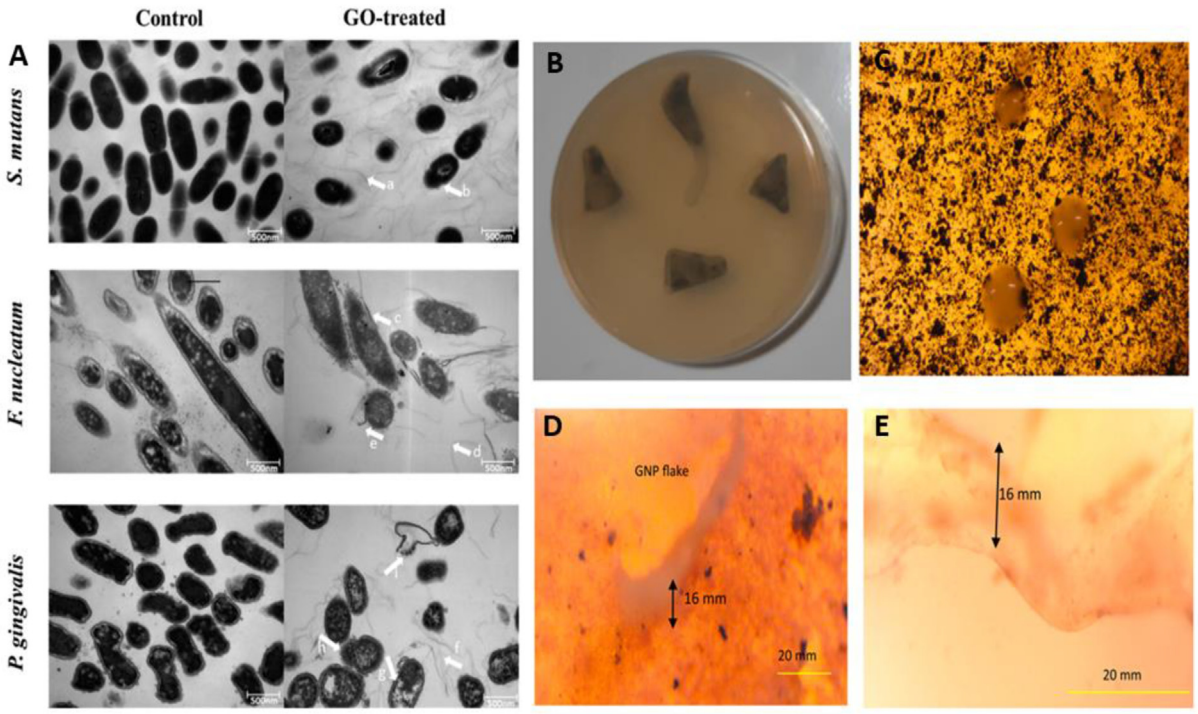

Figure 3: **(A)** Transmission electron microscopy (TEM) images of control and GO-treated microorganisms at 80 μg/mL. Reprinted with permission from ACS 2015.[71] The antibacterial activity of GNP after 24 h of incubation of **(B)** *Escherichia coli*, **(C)** *Staphylococcus aureus* growth on GNP-2, **(D)** the zone of inhibition of GNP-2 when *E. coli* was present in the nutrient medium, and **(E)** a magnified image of the zone of inhibition of GNP for *E. coli*. Reprinted with permission from Elsevier 2021.[74]

methacrylic acid (MAA). This nanomaterial (GNP) exhibits tensile strength and elongation breaks that are similar to gutta-percha (GP-C), the most commonly used obturating material. The scattering of rGO nanoplatelets over the surface of GNP produces crystalline spikes that can improve the adhesion of GNP to biosurfaces. Compared to GP-C, GNP exhibits a 95% increased ability to inhibit bacterial colonization without disturbing the integrity of nearby cells (Figures 3B–E and 4).[74]

As an alternative to sodium hypochlorite root canal irrigant, a silver nanoparticle (Ag-NP) and aqueous GO matrix (Ag-GO) was designed to improve the disinfection protocol applied during RCT. Compared to sterile saline, 2% chlorhexidine (CHX), 17% ethylene diamine tetra acetic acid (EDTA), and 1% sodium hypochlorite (NaOCl), Ag-GO particles (0.25%) were found to enhance the antimicrobial and biofilm disruption. However, 2.5% NaOCl was found to be superior to the Ag-GO particles.[75] Similarly, another study described the development of an endodontic irrigant with graphene-silver composite nanoparticles and evaluated its performance against *Enterococcus faecalis* in comparison with saline and 3% NaOCl. Analysis showed that graphene silver composite nanoparticles exhibited a greater level of antimicrobial activity than 3% NaOCl.[76]

In a recent study, the antimicrobial efficiency of GO, double antibiotic paste (DAP), and their combination, was investigated against *E. faecalis* during RCT. *E. faecalis* is a highly resistant pathogen that persistently complicates endodontic treatments. Human mandibular premolars contaminated with 3-week-old *E. faecalis* were subjected to this treatment for 1, 7, and 14 days; colony-forming units (CFUs) were eradicated within 1 day of the combination (GO-DAP) treatment being administered. A significant antimicrobial effect was also observed for GO and double antibiotic paste alone after 7 and 14 days of treatment.[77] Another study investigated *S. mutans* and the relative effects of zinc oxide (ZnO) and GO- added composite resin and successfully confirmed the antimicrobial activity of GO and its combination with ZnO.[78]

Ni–Ti alloys have proven to be very useful for the manufacture of endodontic devices. The use of GO and silver nanoparticles (AgNPs) can improve the application of Ni–Ti alloy in dentistry. In a previous study, a GO-AgNPs hybrid material was coated on Ni–Ti alloy via electrophoretic deposition to improve antibacterial and antibiofilm properties. A biofilm formation study evaluated the effects of GO-AgNPs-coated Ni–Ti alloys against *S. mutans* and reported that the deposition of GO-AgNPs exhibited significant antibiofilm activity over time.[79]

Furthermore, the GO-AgNPs coating can also influence the corrosion of Ni–Ti alloy. In another study, the resistance to corrosion of bare and coated Ni–Ti alloy was evaluated by potentiodynamic polarization and electrochemical impedance spectroscopy in a 3.5% solution of NaCl. These results suggest that the GO and GO-AgNPs coating of Ni–Ti can significantly reduce the extent of corrosion. A coating of GO and GO-Ag-NPs was shown to reduce the release of Ni and Ti ions when compared to a bare Ni–Ti alloy. Hence, this coating also improved biocompatibility by reducing the release of metal ions into the human body.[70]

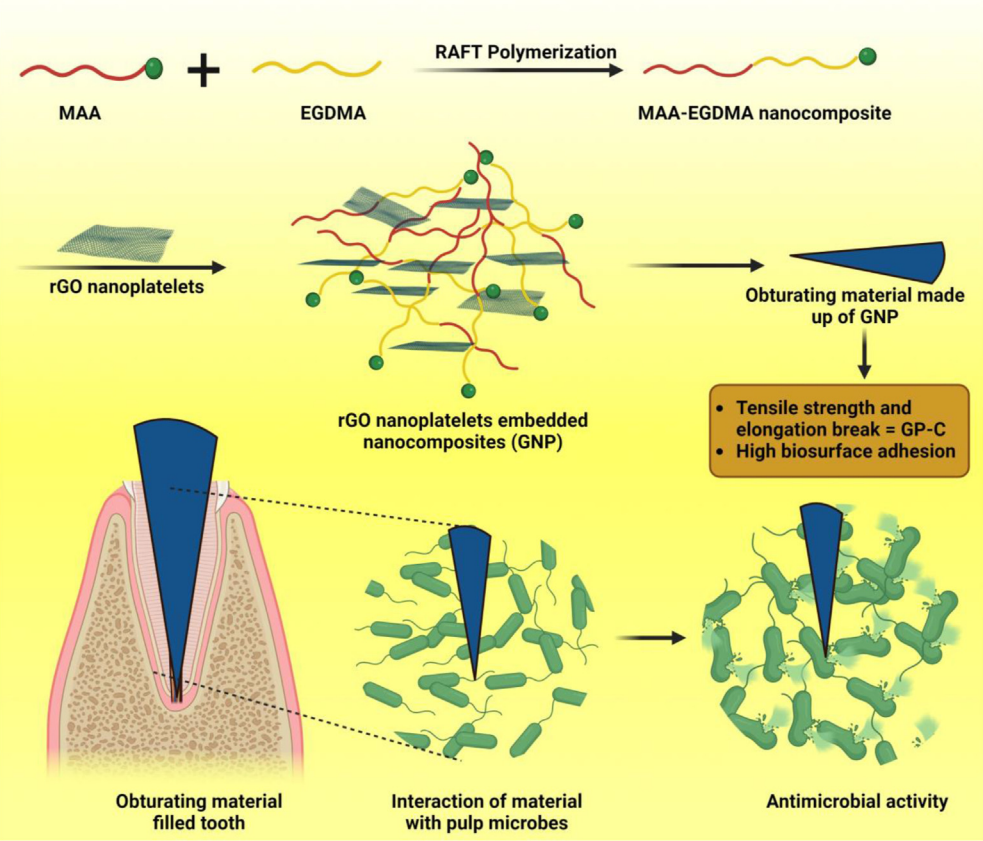

Figure 4: The antibacterial activity of rGO nanoplatelet-embedded nanocomposite obturating material during root canal treatment.

The delivery of therapeutic molecules

Graphene is one of the best platforms for the delivery of therapeutic molecules. The ionized group on the graphene helps to bind molecules by both covalent bonding and electrostatic interaction. The hydrophobic surface of graphene allows molecules to attach physically through $\pi-\pi$ stacking. The ultra-high surface area of graphene can support significant loading of molecules.[4]

A previous study reported the design of an amoxicillin (AMOX) drug carrier with graphene oxide to control the infections caused by *Enterococcus faecalis*. This GO-AMOX combination was dispersed in a hydrogel containing an enzyme responsible for the release of AMOX. Enzymatic activity was shown to effectively control the release of AMOX from GO by breaking the peptide linker. Subsequently, the released AMOX dispersed into the hydrogel and penetrated the site of action to exhibit activity.[80]

In a previous study, Geun La et al.[81] used GO as a carrier for bone morphogenetic protein-2 (BMP-2) to facilitate the regeneration of bone. Negatively ($GO\text{-}COO^{-}$) and positively ($GO\text{-}NH_3^{+}$) charged sheets of GO was coated layer-by-layer on the surface of Ti substrates. BMP-2 was loaded on the outer negatively charged surface of GO and coated on Ti. The loading of BMP-2 on GO, and its coating on Ti implants, was shown to permit the sustained delivery of loaded molecules and, thereby, bone formation on the implant. The in vitro osteogenic differentiation of human bone marrow-derived mesenchymal stem cells (BMSCs) was higher when treated with Ti substrate coated with BMP-loaded GO. In vitro studies showed that the delivery of BMP-2 enhanced alkaline phosphatase activity in bone-forming cells, and in mouse models of calvarial defects; 8 weeks after the implantation of BMP-2-GO-Ti, there was a greater extent of bone formation when compared to Ti and GO-Ti (Figure 5).

Geun La et al.[82] loaded substance P (SP) with BMP-2 to improve bone regeneration. The capacity of mesenchymal stem cells to recruit SP was found to promote the formation of bone. Dual delivery using GO-coated Ti was found to enhance bone regeneration in a mouse model of calvarial defects.

Restorative dentistry

Restorative dentistry aims to restore the oral and dental tissues. Generally, restorative dentin materials make direct contact with pulp cells. Therefore, these materials exhibit suitable antimicrobial properties, mechanical properties, and the ability to enhance the differentiation of odontoblasts. Nahorny et al..[83] synthesized a multi-walled carbon nanotubes (MWCNT)-GO hybrid nanomaterial combined with nanohydroxyapatite (nHAP) to prevent the erosion of dentin. The integrity of the coating was investigated before and after treatment with acidulated phosphate fluoride gel (APF).

In another study, GO nanosheets (nGO) were combined with polymethyl methacrylate (PMMA), a widely used dental material, to overcome the poor antimicrobial or antiadhesion

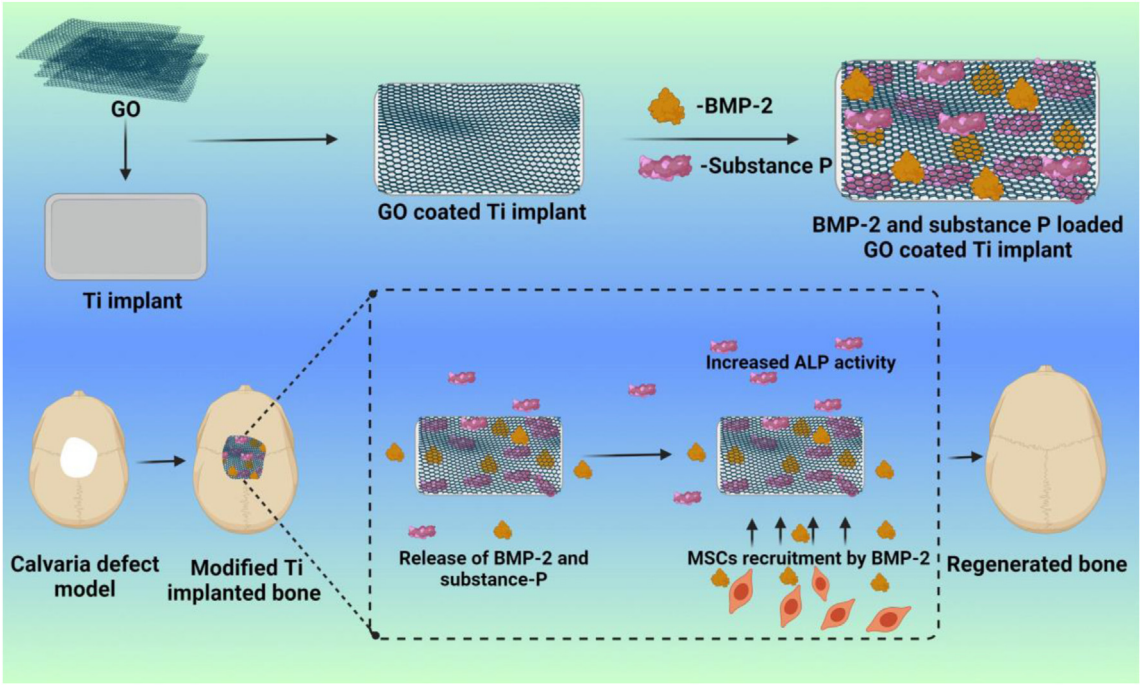

Figure 5: The bone regeneration ability of BMP-2 and a substance P-loaded GO-coated Ti implant.

effects of PMMA when used as orthodontic devices or provisional restorative materials. Increased nGO incorporation enhanced the hydrophilicity and surface roughness of PMMA and thereby sustained antimicrobial and adhesive effects (Figure 6C and D).[84] A clinical case report further revealed that the use of GO-incorporated PMMA for maxillary rehabilitation over a period of 8 months did not result in any aesthetic, mechanical, or biological complications. Good stability and health were also observed in the soft tissues. Consequently, these authors suggested using GO-PMMA resins for prosthetic rehabilitation.[85]

Ti is a well-established material that is used to seal pulp in dentistry applications due to its biocompatibility, mechanical properties, and processability. Previous researchers fabricated a Ti-based material via micro-arc oxidation (MAO) incorporated with self-assembled GO for odonto-integration in pulp sealing. The micro- and nano-structure of the material coating on pulp provided a suitable environment for the odontogenic differentiation of human dental pulp stem cells (HDPSCs) as well as the antimicrobial effect of GO. Characterization and the evaluation of activity for this material revealed better cell adhesion, mineralization, odontoblast differentiation, and antibacterial activity when used for odonto-integration and pulp sealing with 1.0 mg/mL of Ti-MAO and GO treatment (Figure 6A and B).[86]

One primary reason underlying the failure of restorative dental treatment is the loss of adhesion between the pulp and the restorative material. A previous study reported the design of an experimental adhesive resin to improve adhesion; this resin contained 5% HAP (hydroxyapatite) incorporated with varying amounts of GO (0.5 wt% of GO and 2 wt% of GO). Teeth were prepared to construct bonded specimens using the three adhesive bonding agents to assess micro-tensile bond strength (μTBS), with and without thermocycling (TC). These adhesives were applied twice on the dentin with a micro-brush, then by photo-polymerization and air thinning. Compared to adhesive containing 0.5 wt % of GO, an adhesive containing 2 wt% of GO was associated with a better μTBS, better dentin interaction with hybrid layer establishment, and durability for thermocycled and non-thermocycled samples. The authors concluded that the incorporation of nano-GO particles improved the mechanical and adhesion properties of the experimental adhesive.[87]

Another study evaluated the degree of conversion, dentin bond integrity, and bond strength of dental adhesives with GO. Compared to the control, adhesives incorporating GO nanoparticles were associated with a better μTBS. A thermocycled adhesive containing 2% GO was associated with a μTBS that was almost identical to non-thermocycled samples. Furthermore, the degree of conversion was found to be high with control. Enhanced GO content was associated with an increase in the μTBS of the adhesive to dentin but a decrease in the degree of conversion.[88]

Bioactive calcium silicate cements are widely used in the cementing of prosthetics, the induction of mineralization, and the management of tooth perforation. A previous study incorporated graphene nanosheets (GNS) into two bioactive cements (biodentine and endicem Zr) to overcome specific disadvantages, such as modest create any difference in terms of composition, but did reduce the setting time and improve hardness.[89]

Zirconia (ZrO_2) has emerged as an excellent aesthetic restorative material. However, the long-term attachment of ZrO_2 to resin composites remains a major issue despite surface pre-treatments. In a previous study, two commercial silane primers were infused with GO to enhance the shear bond strength of ZrO_2 to a resin composite. Pre-treated ZrO_2 was silanized with Relyx-GO and monobond-GO primers and resin stubs were bonded onto the surface of ZrO_2. Long-

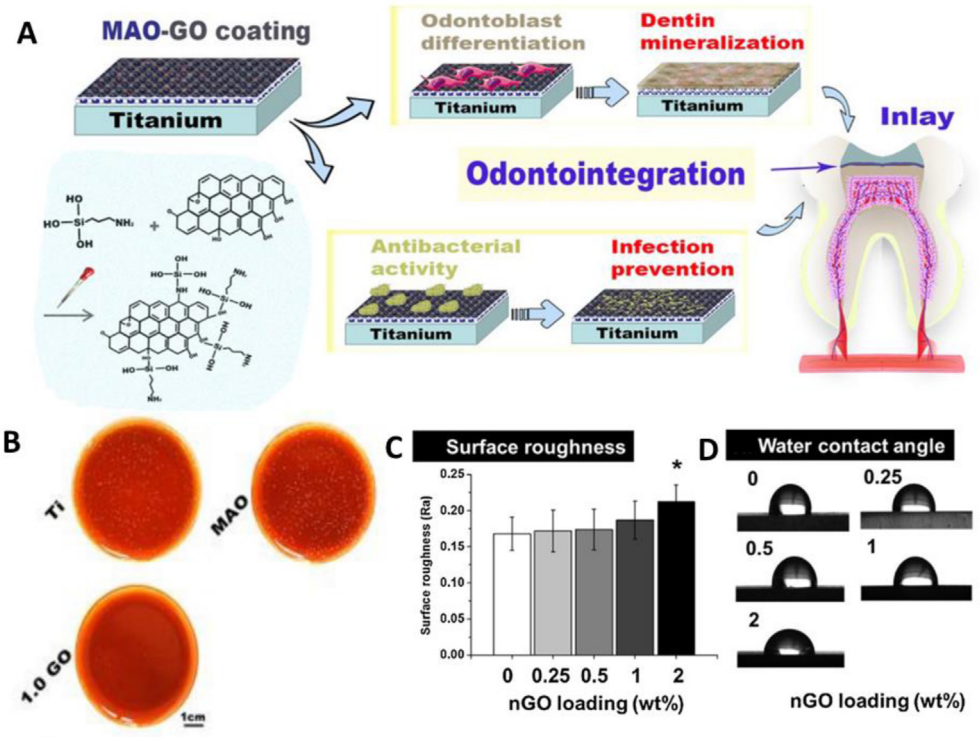

Figure 6: **(A)** The fabrication of GO-coated porous Ti for pulp sealing along with its antibacterial and odontointegration activity, **(B)** images of the bacterial spread plate test, reprinted with permission from RSC 2020,[86] **(C)** surface roughness and **(D)** water contact angles of nGO at different levels of nGO incorporation. Reprinted with permission from Elsevier 2018.[84]

term storage analysis revealed that the primers blended with GO and increased the surface roughness, water contact angles, and shear bond strength between ZrO_2 and the resin composite. Furthermore, GO acted as a shock-absorbing elastic layer between ZrO_2 and the resin composite.[90]

Sun et al.[91] attempted to enhance the antimicrobial, tribological, and mechanical properties of a glass ionomer cement (GIC) by incorporating fluorinated graphene (FG). FG was fabricated from GO by a hydrothermal reaction. Subsequently, GIC powder was mechanically blended with various weight ratios of FG (0.5, 1, 2, and 4 wt%). The authors suggested that an increased FG content increased the Vicker's microhardness and compressive strength. Furthermore, antibacterial activity was also higher in the presence of FG-incorporated GIC. In addition, this composite did not affect solubility, color, or fluoride-releasing properties.

Implants

Recently, GO and its unique properties have gained much attention with regards to dental implant applications. GO has been used to design composite implant materials or implant coating due to its biocompatibility, surface functionality, mechanical and tribological properties, and corrosion resistance. Mahmoodi et al.[92] reported a GO-reinforced hydroxyapatite on a tantalum substrate via the electrophoretic deposition technique (EPD) for application in dental implantology. Traditional tantalum (Ta) implants are associated with certain disadvantages, including low abrasion resistance and bone regeneration, which can induce local inflammatory reactions. EPD was conducted at two different times (10 and 5 min) at a constant 30 V. The presence of GO/hydroxyapatite (GO/HA) composite on the Ta substrate led to an 18-fold increase in hardness and resistance to plastic deformation while also improving bio-tribological behavior, fracture toughness, an 8-fold increase in stiffness, a lower friction coefficient, and a significantly higher corrosion current density when compared to uncoated Ta. Analysis also increased antimicrobial activity against *E. coli* and *S. aureus* cells on the surface of Ta (Figure 7A).

Titanium (Ti) implants are applied in a broad range of circumstances in the medical field, while Ti surface modification can strengthen implant osseointegration. Recently, researchers described the deposition of GO/hyaluronic acid (HA) on titanium alloy for dental implant applications. The EPD technique was used to deposit GO and HA on the surface of Ti. Subsequently, the biological examination of mouse BMSCs (bone marrow stromal cells) showed that proliferation and osteogenic differentiation were enhanced on GO/HA-modified surfaces, with slight enhancement in alkaline phosphatase activity and osteogenesis-related gene expression. Furthermore, improvements were identified for cell adhesion and cell diffusion along with increased roughness and hydrophilicity, thus confirming the attractiveness of this material for dental implant applications.[93]

A recent study reported the use of glass/GO composite-modified polyether ether ketone (PEEK) as a dental implant. PEEK cannot be used as a dental implant alone because it lacks antimicrobial activity and bioactivity. Therefore, a glass/GO composite coating was employed which helped to overcome these disadvantages. The

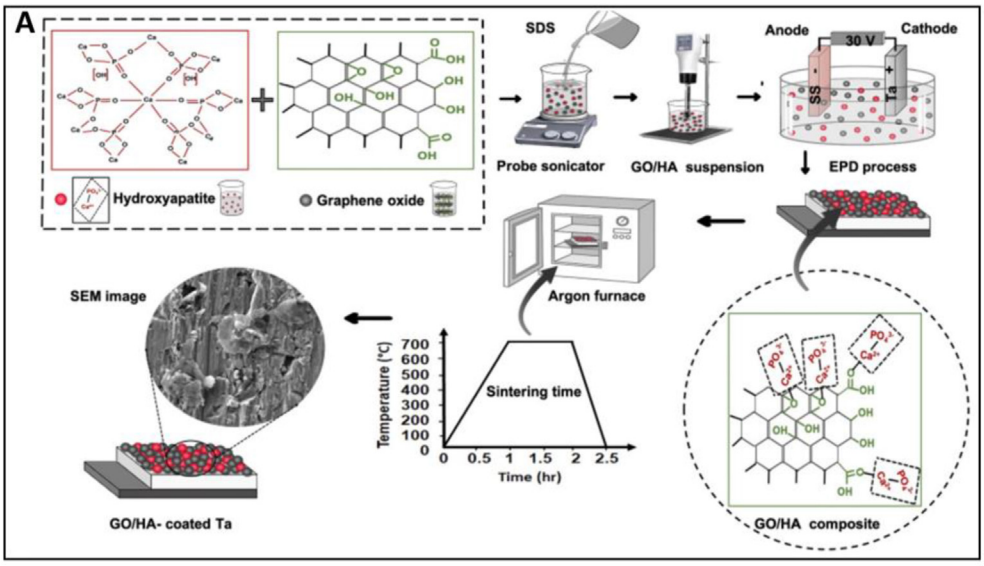

Figure 7: (A) Schematic illustration showing how GO/HA composite is coated on the surface of Ta using the EPD method. Reprinted with permission from Elsevier 2021.[92]

composite coating of a bioactive glass was created by the sol–gel route and doped with 0.75 wt% of GO. The adhesive strength of the coating was then tested. Next, gingival fibroblasts and osteoblast-like cells were used to evaluate the cytotoxicity of the implant. Analysis showed that the implant was not cytotoxic to either of these cell types; in addition, the wettability also improved to <20° following coating with the composite.[94]

Jang et al.[95] reported a study on graphene oxide-deposited zirconia (Zr-GO) implants for enhanced antimicrobial activity. The GO was coated onto zirconia using an atmospheric pressure plasma generator; a mixture of argon/methane gas was passed onto specimens of zirconia at a rate of 10 L/min (240 V). Both GO-coated and uncoated zirconia were evaluated for antimicrobial activity. Analysis revealed that *S. mutans* attachment and biofilm production was substantially reduced in the presence of Zr-GO when compared with that of Zr alone. However, MC3T3-1 osteoblasts did not show any significant differences in terms of cell attachment, whereas Zr-GO led to increased proliferation and differentiation when compared to Zr.

A previous study described the modification of flexible graphene with an $CaSiO_3$/acrylic polymer membrane as a substitute for heavy metal implants. As heavy metal implants produce allergic reactions and implant rejection, there is a significant need to identify an alternative compositive that is biocompatible, mechanically robust, flexible, and lighter. The hot-pressing method was used to deposit a polymer bioactive membrane with a mixture of CaSiO3 microparticles (CSOMPs) and an acrylic polymer (AP) on flexible graphene composites (FGCs). The elastic modulus of CSOMPs + AP)/FGC composite was 107% greater than that of bare FGC. A biocompatible hydroxyapatite formation was observed on the (CSO-MPs + AP)/FGC composite when submerged in body fluid for 21 days. The presence of the carboxylic acid group, OH group, in CSO-MPs + AP, along with its porosity, resulted in a high level of bioactivity. This composite can be used as a biosensor with high electrical and mechanical conductivity properties and good bioactivity, or as a biomaterial to promote osseointegration in dental implants.[96]

Wei et al.[97] reported the pristine behavior of a graphene oxide nanosheet towards BMSCs. In this study, BMSCs were treated with different concentrations of GO and analyzed based on proliferation, alkaline phosphatase activity, adhesion density, and mineralization; furthermore, osteogenic-related protein measurements were undertaken to confirm GO-induced osteogenesis. The pristine GO nanosheet inhibited cell proliferation for 3 days at a higher concentration (10 μg/mL). However, this nanosheet facilitated proliferation at a lower concentration (0.1 μg/mL) after 5 days with a sequential seeding method. Osteogenic differentiation was also promoted at a concentration of 0.1 μg/mL. Analysis revealed that cell proliferation and differentiation behaviors were dependent on the concentration of the pristine GO nanosheet. Collectively, these properties facilitate the application of GO in dental implantation.[97]

In another study, Raja et al.[98] reported the preparation of a one-dimensional (1-D) nano bioactive glass on rGO sheets and performed in vitro bioactivity studies with implant materials. 45S5 Bioglass® is a biomaterial that is uses commonly for dental implantations. In this study, the authors described the synthesis of 1-D bioactive glass nanorods (BGNR) of 45S5 composition by tuning the sol–gel parameters to incorporate 2-D rGO sheets. Analysis showed that these sheets had a combeite high mineral phase ($Na_{5.27}Ca_3Si_6O_{18}$), whereas nanohybrids exhibited a calcite mineral phase ($CaCO_3$) in combination with combeite when analyzed by X-ray diffraction (XRD). Next, the BGNR and BGNR with rGO were subjected to size and shape analysis by scanning electron microscopy (SEM). In addition, bioactivity (mineralization), hemocompatibility, antibacterial efficacy and cell proliferation were measured and found to be enhanced in comparison with BGNR. These results

identified the synergetic effects of rGO/BGNR nano-hybrids and their potential for application in dental implant materials.

Qin et al.[99] reported a study on previously contaminated titanium implants that had been treated with GO and investigated for osteointegration and antimicrobial activity. Previously infected titanium implants were cleaned by simple brushing (Group B) and then treated with different concentrations of GO (64, 128, 256, and 512 μg/mL) (Group G). Contamination was initially removed by brushing and then treated with various concentrations of GO (Group GB), and a group without any treatment (group C) was included. Intact clean (IC) Ti served as the control. Analysis demonstrated that high concentrations of GO (≥256 μg/mL) inhibited residual bacterial growth and biofilm reformation. Furthermore, the viability of BMSCs from the GB-256 and IC groups was higher than that of BMSCs from the GB-512, C, and B groups.

Gao et al..[100] reported a dental implant that exhibited a sandwich structure which was formed by coating the percutaneous part with GO which was then packaged under mineralized collagen. This distinctive coating technique ensured that the implant exhibited good photothermal stability and high photothermal conversion capability. This coating inhibited colony formation by *Streptococcus sanguinis*, *Fusobacterium nucleatum* and *Porphyromonas gingivalis*, and disrupted the cell membranes of free bacteria. The addition of dimethylaminododecyl methacrylate was shown to lead to further improvements in antibacterial properties. Soft tissue sealing was also improved due to significant enhancement in the cytoskeleton organization, adhesion, and proliferation of human gingival fibroblasts. These effects were achieved by the sandwich structure and the mineralized collagen outer layer which protected tissue cells from photothermal therapy and increased the recovery of cell activity. This design provides us with a convenient alternative technique for dental implant abutment surface modification and functionalization.

Pulp regeneration

The materials that are currently used for endodontic regeneration do not ensure the complete regeneration of the dentin-pulp complex, especially with regards to neovascular induction. Researchers have recently developed a copper and water-soluble form of GO (Cu-GO) nanocomposite and investigated the odontogenic and neovascularization properties of dental pulp stem cells (DPSC). Treatment with the Cu-GO nanocomposite led to the differentiation of odontoblasts, vascular endothelial growth factor (VEGF), and the secretion of glia-derived neurotropic factor. The subcutaneous transplantation of a Cu-GO-coated calcium phosphate cement scaffold (Cu-GO/CPC) with DPSCs into nude mice resulted in the generation of a dentin-pulp-like complex with dentin sialophospho protein, CD31, and GAP43. Compared to bare CPC, Cu-GO/CPC was associated with a larger number of blood vessels and a larger mineralized area (Figure 8).[101]

In another study, the in vitro biological effects of 3-D GO/sodium alginate (GOSA) and reduced GOSA (rGOSA) scaffolds were investigated in DPSCs. The effects of scaffold biomaterial composition, physicochemical properties, and

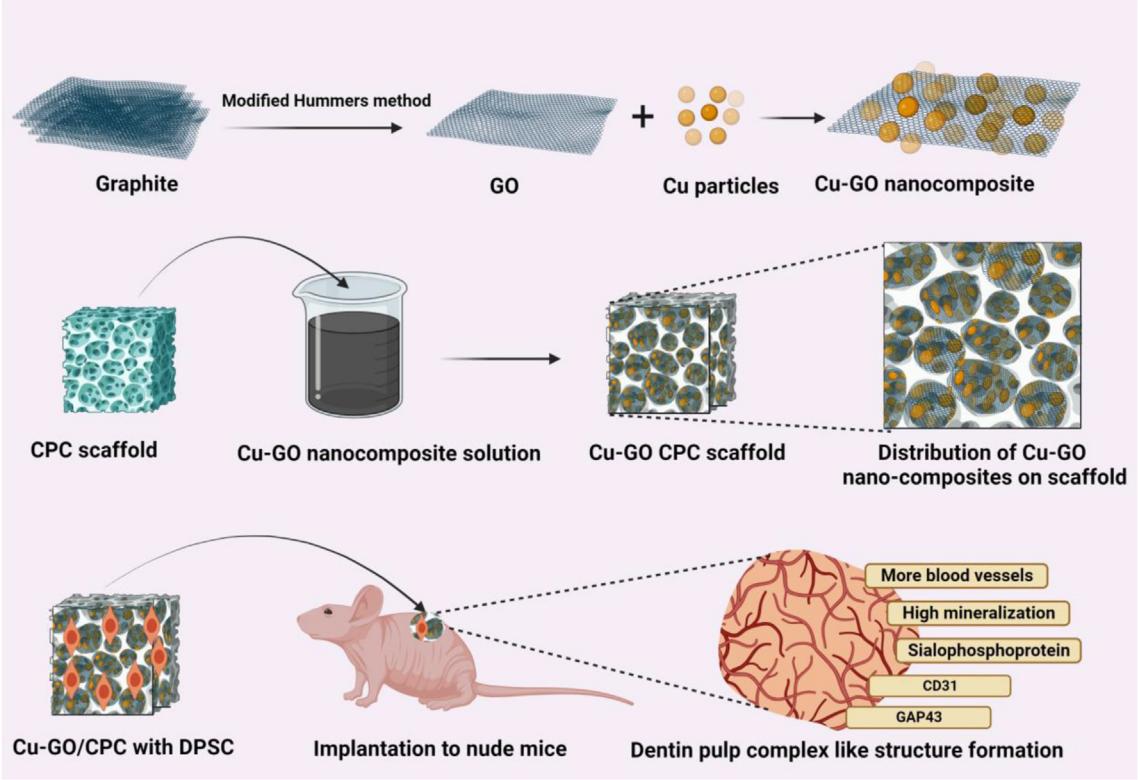

Figure 8: The application of Cu-GO-coated calcium phosphate cement scaffolds for the regeneration of pulp.

porous structure were also evaluated. The addition of GO enhanced the biodegradation properties of the scaffolds and enhanced the viability of DPSCs. Analysis also revealed that the 3-D structure supported the adhesion and migration of cells in a more pronounced manner than 2-D structures. Scaffold-based in vitro studies further showed that structures with greater porosity were more efficient in terms of reducing the activity of DPSCs.[102]

In another study, the odontoblastic and osteogenic differentiation of graphene synthesized by the CVD method was evaluated in DPSCs. Cells were seeded on graphene and glass and maintained for 28 days in a culture medium. Analysis suggested that graphene induced a greater level of mineralization and up-regulated the expression of osteogenic genes. The authors reported that graphene can potentially induce osteogenic DPSCs without chemical inducers but not odontoblastic differentiation.[103] Other authors investigated rGO functionalized 3-D printer polycaprolactone (PCL) scaffolds for pulp regeneration using rat DPSCs. rGO surface functionalization and surface etching of rGO, incorporating acid for additional roughness, dramatically improved both cell attachment and cell surface coverage invitro.[104]

Graphene and pulsed electromagnetic fields (PEMFs) can foster diverse cellular developmental programs. The combination of these effects exceeded the sum of their individual effects in terms of DPSC regeneration. During early neurogenesis, graphene induced the highest expression of transient receptor potential canonical cation channel type 1 (TRPC1), while PEMF exposure promoted in vitro differentiation by activating the TRPC1. Furthermore, when the timing of PEMF exposure coincided with the highest graphene-induced TRPC1 expression, there was enhanced expression of neurogenic genes and proteins. Thus, this combination strategy represents a promising new application in the field of pulp regeneration.[105]

Bone regeneration

The use of organic and inorganic bioactive materials as composites in bone regeneration were recently evaluated using the combination of silk fibroin (SF), nHAP, and SF and GO. Analysis revealed that bone regeneration and biocompatibility were highest with the SF and GO combination. Directional temperature field freezing technology was used to prepare a directed channel of SF/nHAP/GO scaffolds to yield the characteristics of natural bone tissue. Compared to traditional spongy scaffolds, this scaffold structure exhibited superior pore connectivity, a reduced degradation rate, flow resistance for osteogenesis, and the ability to induce bone-like apatite. BMSCs exhibited improved adhesion and proliferation in the presence of directed SF/nHAP/GO scaffold channels. The improved biological activity and stronger osteointegration ability of these directed channels provide significant potential for applications in bone regeneration.[106]

Ti and its alloy-based implants are often used as implants due to their bioinert and mechanical characteristics; however the clinical use of these implants is limited by reduced interaction with bone-associated cells and a lack of bone regeneration and bifunctionality. The surface modification of implant structures can improve the osseointegration of Ti. rGO was coated evenly over the surface of Ti by the meniscus-dragging deposition (MDD) technique. These rGO-Ti substrates exhibit strong effects in terms of promoting the osteogenic differentiation of hMSCs, and possess superior levels of bioactivity and osteogenic potential.[107] Similarly, the surface coating of rGO on conventional sandblasted, large-grit, and acid-etched (SLA) Ti implants has been shown to impart better biocompatibility and absorption ability for exogenous proteins. Therefore, this type of implant can support cell growth and osteogenic differentiation without the application of osteogenic factors. Therefore, rGO-coated implants are able to accelerate both osseointegration and bone tissue regeneration.[108]

A scaffold-free, magnetically controlled cell sheet technique was previously developed by Zhang et al.[109]; this technique allowed the incorporation of growth factors for complex tissue regeneration with Fe_3O_4 magnetic nanoparticles wrapped with nano-scale GO fragments (GO@Fe_3O_4). Analysis showed that GO@Fe_3O_4 could readily be absorbed by DPSCs; thus, MNP-labelled cells can be arranged by magnetic force to produce multi-layered cell sheets. Carboxyl groups on GO allow the easy binding of growth factors and their delivery for bone formation. This newly constructed growth factor immobilized cell sheets induced more bone formation, exhibiting promising potential in future regenerative medicines (Figure 9A).

In another study, researchers developed an injectable hydrogel consisting of a poly (*N*-isopropylacrylamide) (PNIPAAm) copolymer-GO composite, and varying ratios of chitosan (CS), to fabricate DPSC scaffolds for bone tissue engineering. These hydrogels were prepared with a PNIPAAm copolymer-GO composite fabricated from the free radical copolymerization of PNIPAAm, itaconic acid, and PEG in the presence of GO and CS. Analysis revealed the upregulated expression of RunX2 and osteocalcin in human DPSCs and enhanced osteogenic induction. Consequently, this study highlighted the use of this scaffold in bone tissue engineering for the transplantation of DPSCs (Figure 9B).[110]

Calcium phosphate bone cement (CPC) paste is used in many orthopedic applications. An injectable CPC-CS paste consisting of GO was previously used to enhance the orthopedic applications of CPC by reducing infections, imparting biocompatibility, and by increasing the viability of stem cells for bone regeneration. The incorporation of GO did not change the flexural strength of CPC-CS. However, the presence of GO in the paste successfully inhibited *S. aureus* (an important form of bacteria that causes osteomyelitis) biofilm formation and led to the excellent growth and viability of human umbilical cord mesenchymal stem cells (hUCMSCs) for bone regeneration.[111]

Recently, researchers developed a new nanocomposite made up of magnesium hydroxide ($Mg(OH)_2$) (inner), HAP (outer), and GO (middle) ($Mg(OH)_2$/GO/HA) on the surface of Mg−Zn−Ag. All three coatings exhibited hydrophilicity, resistance to corrosion, and a high bonding strength. In vitro results suggested an improvement of antibacterial action with $Mg(OH)_2$ and the osteogenic differentiation of MC3T3-E1 cells with GO/HA. In vivo findings confirmed that Mg alloys coated with nanocomposites helped to prevent bacterial invasion and improved bone regeneration ability.[112]

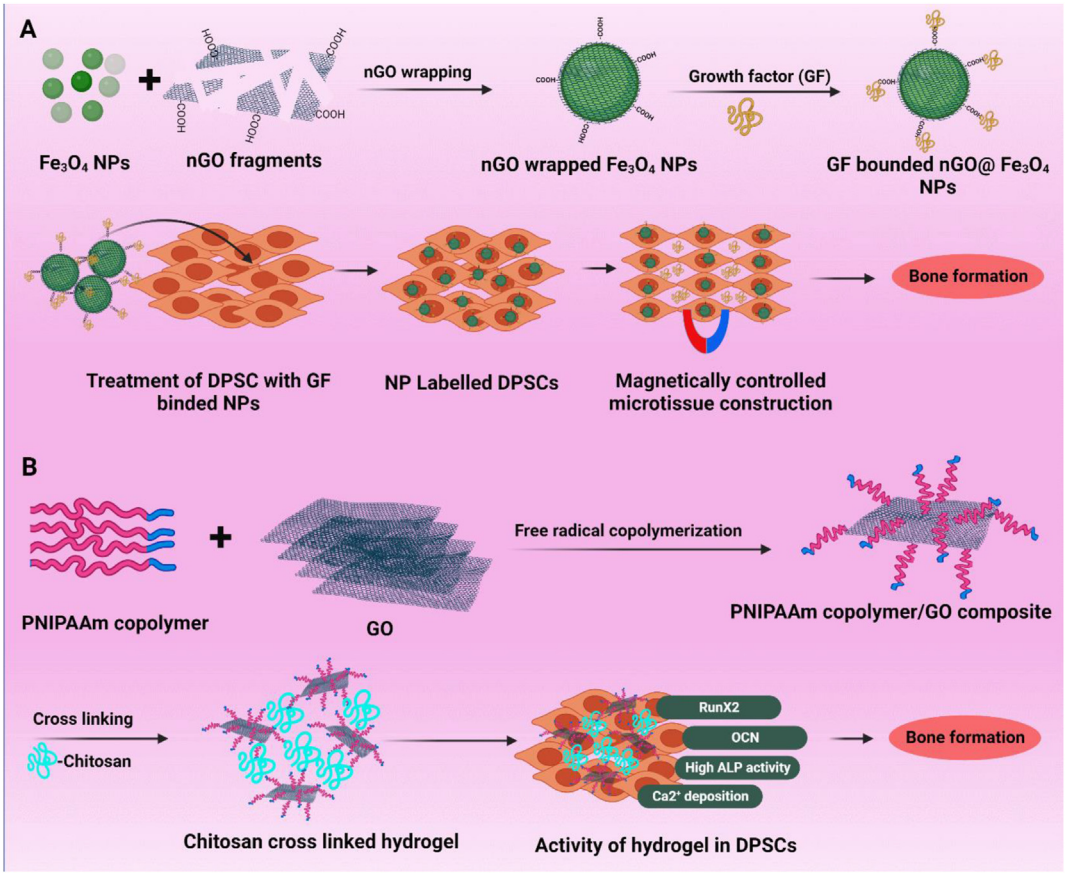

Figure 9: **(A)** The magnetically controlled cell sheet technique for bone formation, **(B)** injectable hydrogel fabrication for bone formation.

Periodontal tissue regeneration

Periodontitis is a chronic infectious disease that leads to the degradation of periodontal tissue. The removal of inflammation and the regeneration of periodontal tissues are the primary concerns for periodontal therapy. Tissue engineering scaffolds have gained increasing levels of attention in the field of periodontal tissue regeneration and 3-D scaffolds now play a key role in tissue repair during periodontal tissue engineering. A novel 3-D collagen scaffold, incorporating GO, was previously fabricated for the periodontal tissue healing of class II furcation defects in dogs. In this study, the surface of a collagen sponge was coated with GO suspension. Compared with the 3-D collagen scaffold, the GO-treated scaffold led to a significant increase in the formation of periodontal attachment, including periodontal ligament-like tissue, alveolar bone, and cementum-like tissue. These biocompatible GO-based scaffolds exhibited good capacity for periodontal tissue and bone tissue formation.[113]

In another study, researchers designed a new scaffold material by combining CS, PVA, GO, and astaxanthin (ASTA) nanofibers by an electrospinning method to support immunity and tissue regeneration in the host. ASTA is a fat-soluble keto-carotenoid that exhibits both antioxidant and anti-inflammatory properties and is known to exert impact on immune function. This preliminary research highlighted the potential use of these scaffolds in periodontal tissue engineering.[114]

The regeneration of periodontal tissues is very challenging in the inflammatory periodontal environment under diabetic conditions. Periodontal tissue regeneration was previously achieved by modulating the inflammatory microenvironment of diabetes with a poly dopamine-mediated GO (PGO) and nHAP-incorporated alginate/gelatin scaffold. nHAP and PGO provided osteoinduction and conduction pathways, respectively. This scaffold supported the regeneration of tissues by the transfer of electrical signals and the activation of calcium channels. PGO is known to exhibit ROS scavenging properties, anti-inflammatory activity, and immunomodulatory effects. This scaffold showed excellent regeneration in periodontal bone defects in diabetic rats via the synergistic action of all components.[115]

Biosensors

Innovative GO-based devices have gained significant attention due to their potential to improve diagnostic and monitoring processes in dentistry. These sensors are designed to detect specific biomolecules or analytes within the oral cavity, thus providing valuable information for dental professionals and patients. The well-known Au electrode uses impedance measurements from the in vitro and real-time tracking of growth in human and animal cells. However, this technique is limited by microbial growth on the Au electrode which can shield signal strength due to the small size of the microbial cells. Therefore, the use of rGO-CE as

an electrode may provide a large surface area, thus facilitating measurements from significant microbial growth by producing a strong impedance signal.[116] Three types of oral bacteria were previously cultured on the surface of rGO-CE: *S. mutans, Lactobacillus fermentum,* and *Actinomyces viscosus*. The recorded impedance for *S. mutans* and *A. viscosus* was 3.3- and 6.0-fold more potent than Au. A detectable signal on the graphene electrode was obtained in the case of *L. fermentum,* unlike the Au electrode at 1.17 KHz.[116]

The quantitative estimation of eugenol is highly significant as it can be hazardous in various ways following high intake. In a previous study, the concentrations of eugenol were investigated in dental samples by the application of a GCE/CrGO-β-CD/ADA-SPE/AuNPs (glassy carbon electrode/chemically reduced graphene oxide-β-cyclodextrin/1-adamantane carboxylic acid-soybean peroxidase enzyme/gold nanoparticles) biosensor. The biosensor was constructed using optimal conditions identified by response surface methodology. This biosensor had a sensitivity that lay in a linear range between 1.3×10^{-8} and 1×10^{-5} mol L^{-1} for the quantification of eugenol.[117]

Very recently, another biosensor was developed for the detection of diseases in teeth. This biosensor incorporated bismuth telluride (Bi_2Te_3) and a graphene layer was overlaid on a Ag metal layer-based surface plasmon resonance biosensor. This biosensor was made from a 35 nm, 1 nm and 0.5 nm thickened Ag layer, Bi_2Te_3, and graphene, respectively. These sensors exhibited high levels of sensitivity (79.310 deg/RIU, 118.88 deg/RIU, and 169.87 deg/RIU) for enamel, dentin, and cementum, respectively, with a single-layer Bi_2Te_3 and graphene component.[118]

Tooth whitening

The American Academy of Cosmetic Dentistry reports that the public response to tooth whitening is very high because more than half of all people are undergoing orthodontic treatment to change the color of their teeth. A previous study described the use of cobalt-tetraphenylporphyrin (Co-TPP) molecules that had been surface-modified with rGO for tooth whitening. Co-TPP was loaded onto the hydrophobic surface of GO by π−π interactions. Tooth specimens stained with dyes, tea, and betel nuts were bleached using H_2O_2, H_2O_2 with CoTPP, or H_2O_2 with CoTPP/rGO. The excited singlet state oxygen in H_2O_2 reduced Co-TPP (Co^{IV}) to Co^{III} chromogens, which are responsible for the decomposition of stains.[119]

Conclusion and prospects

The development of biomaterials for dental use is crucial due to the challenging oral environment. Graphene oxide (GO) and reduced graphene oxide (rGO) show significant promise in dentistry and offer a range of attractive physicochemical, biological, and mechanical properties. Despite their appeal and stability in the oral cavity, graphene-based dental research remains in its infancy. The graphene family of nanomaterials has proven to be highly beneficial for research in dental materials due to its antimicrobial properties and ability to combine and resolve the weaknesses of existing biomaterials.

However, biocompatibility remains a concern due to the fact that long-term studies have yet to be published. Since the physical and chemical nature of GO and rGO are known to exert an effect on toxicity, a standardized toxicity investigation is required for all future applications.

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Conflict of interest

The author has no conflicts of interest to declare.

Ethical approval

No ethical approval was required for this review.

Author contribution

AS designed the study, collected the data, wrote the initial and final draft of the manuscript, and is entirely responsible for the content and similarity index of the manuscript.

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