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Review Article

Mechanism and factors influence of graphene-based nanomaterials antimicrobial activities and application in dentistry



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ABSTRACT

The antimicrobial activity of graphene-based nanomaterials (GBNs) has recently gained significant attention in numerous biomedical science applications. GBNs exhibit excellent antimicrobial properties and have been adopted as antimicrobial nanomaterials due to their abilities to disrupt the integrity of bacterial cell membrane and produce reactive oxygen species (ROS). This review discussed the various mechanisms of GBNs' antimicrobial effects and factors underlying GBNs' antimicrobial activity, such as microbial cell morphology, GBNs' flake size and concentration, presence of functional groups, exposure to electromagnetic radiation, and effects of electrical conductivity. The potential applications of GBNs in clinical treatment were highlighted in this review to provide an in-depth understanding of the GBNs' antimicrobial effects in dentistry and provide directions for future studies. These applications included GBNs incorporation with acrylic resin to fabricate dentures, composite resin and cement in restorative treatment, adhesive materials in orthodontic treatment, and implants coating in dental implant treatment.

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1. Introduction

The oral cavity consists of multiple surfaces that are commonly covered with oral microorganisms. When dental

materials (e.g. dental restorative and implants) are placed within an oral cavity, they become exposed to various types of bacteria and fungi. These pathogenic microorganisms build plaque biofilm that is crucial for their growth and survivability. Additionally, the plaque biofilm serves as a protection

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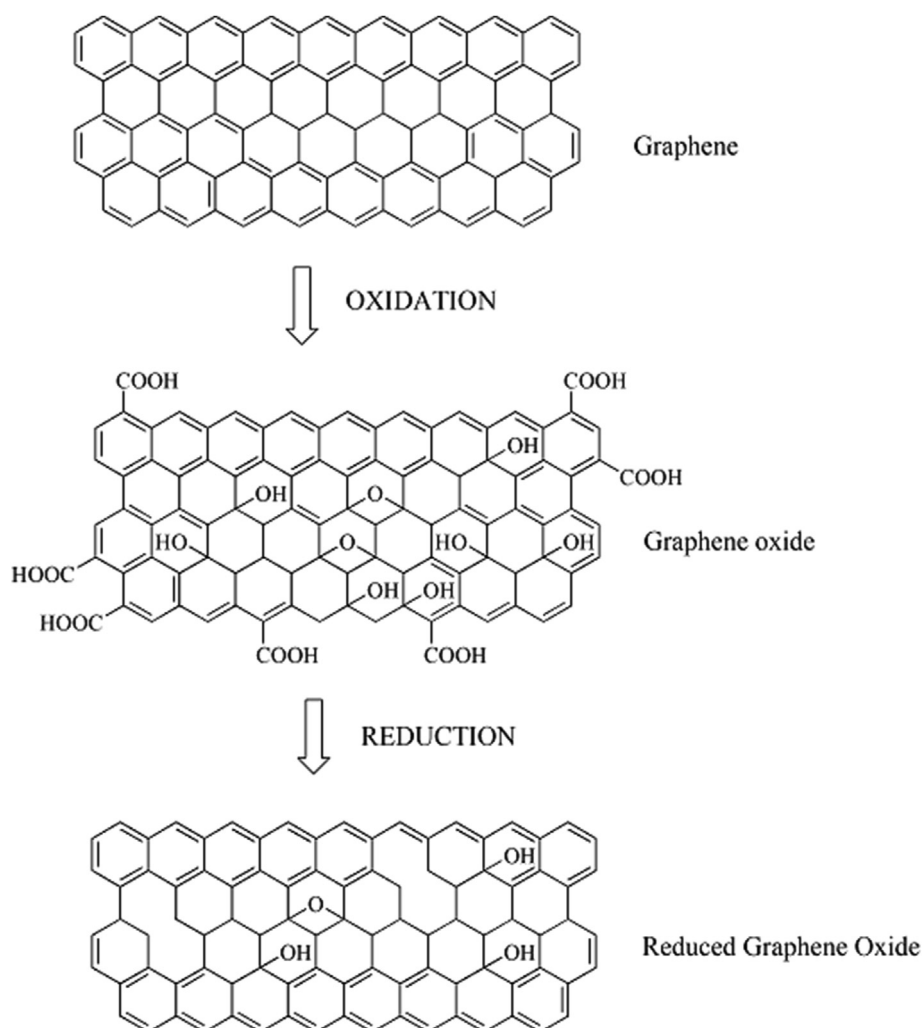


Fig. 1 – Structure of graphene, GO, and rGO. The GO was produced from graphene oxidation, while the rGO was produced from the chemical or thermal reduction of GO.

from external antimicrobial agents [1,2]. It is worthy to note that the plaque biofilm is the primary cause of dental caries, periodontitis, and other dental-related diseases.

Numerous antimicrobial dental materials have been developed to minimise dental treatment failures caused by microbial infections [3–8]. The primary approach is to embed or coat the dental materials with antimicrobial agents (e.g. chlorhexidine and quaternary ammonium compounds) as they enhance antimicrobial effects [9–11]. Unfortunately, there is often a trade-off between antimicrobial and mechanical properties of these agents. For instance, the mechanical performance of dental materials decreased with the addition of antimicrobial agents [12].

Alternatively, nanomaterials have been used widely as the biomaterials to improve the mechanical performance of dental materials [13–16]. In comparisons to other types of biomaterials, nanomaterials have a superior antimicrobial activity that does not compromise the mechanical performance of dental materials [17–21]. Graphene is one of the most promising nanomaterials that was discovered in 2004 [22]. It is a two-dimensional carbonaceous material and serves as a basic structural element of graphite [22,23]. Graphene

composes of a one-atom-thick layer of sp^2 carbon atoms arranged in a hexagonal honeycomb structure [24,25]. Additionally, graphene has remarkable characteristics, such as large surface area [26] and superior mechanical, electrical, and thermal properties [27]. Due to these characteristics, graphene has been used in many applications [28–30]. Nevertheless, the use of graphene may be limited to agglomeration and processing difficulty due to the single carbon component of pristine graphene [31,32]. Furthermore, chemical modification is required to produce graphene derivatives, such as graphene oxide (GO) and reduced graphene oxide (rGO), which are more versatile and applicable in various applications [32].

As shown in Fig. 1, GO (an oxidised derivative of graphene) has oxygen-containing functional groups, such as epoxy ($-COC-$) and hydroxyl ($-OH$) groups on its basal planes and carboxylic acid ($-COOH$) groups at its edges [32]. Due to these oxygen-containing functional groups, GO has better dispersity and stability in aqueous solutions than pristine graphene. The presence of these oxygen moieties also plays a significant role in antimicrobial activity [33]. For that, GO has a more potent antibacterial effect against *Escherichia coli* than graphite, graphite oxide, and rGO [34]. GO is also commonly used as a

precursor for preparing rGO [32,35]. The rGO is produced by removing the covalent functional groups of GO via thermal or chemical reductions [32]. The rGO exhibits the properties of pristine graphene and GO — high strength and moderate water dispersibility [32,36]. However, the properties of rGO may vary depending on the reduction process employed during its production [35].

The emergence of graphene and its derivatives has attracted significant attention to nanomedicine and tissue engineering. The excellent properties of graphene and its derivatives (e.g. biocompatibility, antimicrobial effect, low toxicity, and easy chemical functionalisation) have contributed to their popularity [37]. It was also due to these properties that graphene and its derivatives hold promise for their use as restorative materials and medical devices [37].

Bio-composites can be designed with the desired properties due to GO's abilities to function or combine with other biomaterials (e.g. polymer, ceramic, and metal) [38–40]. For instance, dental materials have been modified with graphene and its derivatives via colloidal dispersion, direct synthesis, sintering, and conjugation [41]. However, the current state of the dental materials' matrix needs to be considered before the modification process to ensure graphene and its derivatives are well dispersed and functionalised in the selected matrix.

Various microorganisms are present in oral cavities, dentures, and other removable dental appliances. The microorganisms commonly found in the oral cavity are *Streptococcus mutans* (*S. mutans*), *Streptococcus sanguinis* (*S. sanguinis*), *Staphylococcus aureus* (*S. aureus*), *Streptococcus salivarius* (*S. salivarius*), *Streptococcus sobrinus* (*S. sobrinus*), *Streptococcus parasanguinis* (*S. parasanguinis*), *Candida albicans* (*C. albicans*), *Porphyromonas gingivalis* (*P. gingivalis*), *Enterococcus faecalis* (*E. faecalis*) and *Fusobacterium nucleatum* (*F. nucleatum*) [42,43]. *S. mutans* and *E. faecalis* are examples of Gram-positive bacteria. *S. mutans* is the facultative anaerobic bacteria and the main aetiological factor in the initiation and development of cariogenic biofilms [43], whereas *E. faecalis* is commonly found in reinfected, root canal-treated teeth. On the other hand, *P. gingivalis* and *F. nucleatum* are Gram-negative anaerobic bacteria associated with periodontitis and root canal infection [44,45].

In recent years, several studies have examined the antimicrobial effects of GBNs on dental pathogens. For example, it has been reported that GO exhibited potent antimicrobial and antiadhesion activities on bacteria and fungi [39,46]. Additionally, GO was found to inhibit the growth of *S. mutans*, *S. aureus*, *E. coli*, and *C. albicans*, with minimal cytotoxicity [39,47]. The antimicrobial mechanism of GBNs involves physical and chemical actions. The mechanism principally kills pathogens by the sharp edges of the GBNs [48], wrapping and trapping bacterial membranes by the nanosheets [49], and the production of reactive oxygen species (ROS) [50]. Understanding the underlying antimicrobial mechanisms of GBNs can contribute to developing the next-generation of dental materials resistant to microbial infections.

The present review discussed the recent progress on GBNs application to improve the antimicrobial activity of biomaterials in dentistry. Additionally, the mechanism underlying GBNs' antimicrobial activity on several dental pathogens (including Gram-positive bacteria, Gram-negative bacteria, and fungi) were discussed. Possible factors influencing GBNs'

antimicrobial activities were also discussed. Lastly, we discussed the potential use of GBNs in several dental applications, including restorative dentistry, endodontics, periodontics, and dental implants.

2. Antimicrobial mechanisms of GBNs

Knowledge about the mechanism underlying GBNs' antimicrobial activity is currently limited due to its complexity. The antimicrobial mechanism of graphene and its derivatives may vary depending on their physical and chemical properties [37,51]. Recent findings showed that the physical and chemical properties of GBNs, such as surface functionality [52], morphology [53], flake sizes [54], and concentration [54], play a vital role in their antimicrobial activities. The mechanisms through which GBNs cause microbial inhibition and death are not only dependent on their intrinsic and extrinsic factors, but they also depend on the components and structure of the microbial cells and stage of maturity [55]. Also, there is a consensus among researchers that the bacteria's structure can affect the antimicrobial agents' activity [56,57].

Earlier publications reported that the Gram-positive bacteria were more susceptible to GO than Gram-negative bacteria [58,59]. The higher susceptibility of Gram-positive bacteria to GO compared to Gram-negative was greatly influenced by their cell wall structure [59]. Bacterial cells composed of a polymer known as peptidoglycan (PG). Gram-positive bacteria have a thick layer of PG, whereas Gram-negative bacteria have a thin layer of PG. The PG layer protects the bacterial cells from osmotic pressure changes and small molecule insults [34]. Additionally, the PG layer serves as a chelating agent due to its adhesive surface proteins (e.g. teichoic acids and adhesins). The PG layer of Gram-positive bacteria is attached to their surface by densely functionalised anionic glycopolymers — wall teichoic acids (WTA) and lipoteichoic acid (LTA). Deokar et al. suggested that Gram-positive bacteria interacted with carbonaceous nanomaterials through electrostatic or hydrogen bonding [60]. The reaction of GO to WTA, lipids, and amino acids may cause morphological deformations [58]. These morphological deformations include inhomogeneous thickening of the PG cell wall, expansion of cell size, and flaws in septal positioning and number. Alteration of WTA also caused cell growth delay and cell agglomeration in solution [61].

In contrast to the Gram-positive bacteria, Gram-negative bacteria interacted with the nanomaterial through direct physical contact only [60]. Gram-negative bacteria has an outer membrane essential for their protection in a hostile environment (e.g. in the presence of antibiotics). This outer membrane increases the bacterial resistance to antibacterial activity. It is also due to this outer membrane that the Gram-negative bacteria has lower antibacterial activity than the Gram-positive bacteria [62]. Lipopolysaccharide (LPS), a surface protein attached to the outer leaflet of the cells' outer membrane, is responsible for the repulsion of hydrophobic molecules [63,64]. It may also cause overall repulsive forces on the bacteria through steric repulsion [65]. The dissimilarity of the bacteria's structure determines the type of interactions that occur between the two classes of bacteria with GO [58,60].

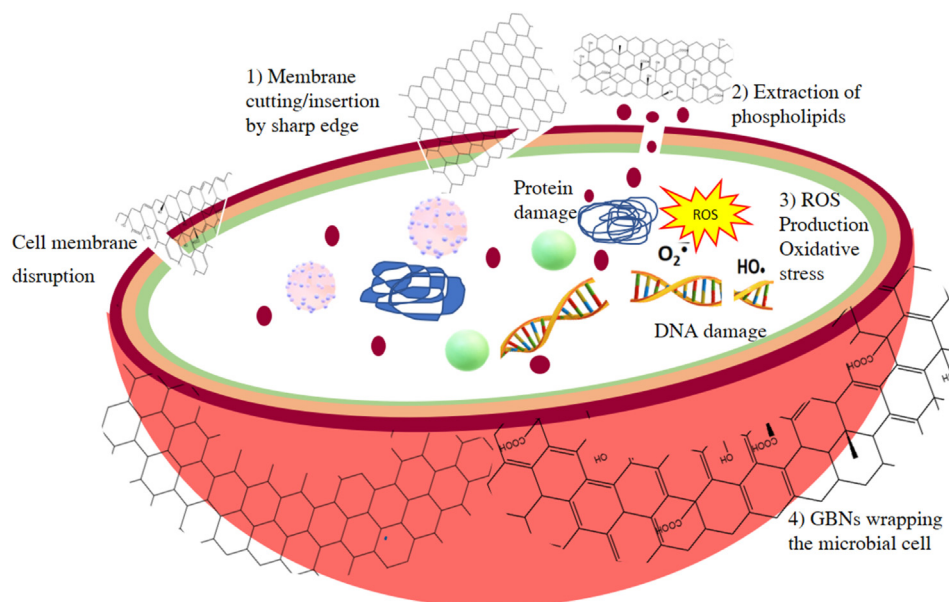


Fig. 2 – Antimicrobial mechanism of GBNs through physical and chemical interactions. 1) Membrane damaged by the sharp edge of GBNs, 2) extraction of phospholipids, 3) ROS generation for disruption of deoxyribonucleic acid (DNA) and proteins, and 4) wrapping of the bacterial cells by large surface area GO.

Moreover, the antimicrobial efficacy of GBNs depends on the four phases of bacterial growth curve: lag, logarithmic (exponential), stationary, and death. A study found that each phase of the bacterial growth curve exhibited a different susceptibility to GO [66]. Specifically, the study found that the cells of *E. coli* treated with 100 µg/mL of GO declined to less than 4% during the exponential stage. On the other hand, the bacteria cells' survivability in the stationary and death phases was more than 75% and 50%, respectively [66]. A recent study suggested that the biofilm's susceptibility to GO nanoparticles varied with age, which may be associated with changes in the cells' physiological state during maturation [55].

Most studies on antimicrobial properties of GBNs against a broad-spectrum of microbial suggested that the GBNs' antimicrobial activity can be explained through these three possible mechanisms: (i) cellular envelope stress (physical cutting, "nano-knives" effect, membrane insertion, and membrane components extraction), (ii) environmental isolation (wrapping effect), and (iii) oxidative stress [67]. These mechanisms coincided under most experimental conditions and caused a deadly effect [68]. Nevertheless, the mechanisms underlying GBNs' antimicrobial activity may vary depending on their applications. The antimicrobial activities of GBNs were restricted in a solid matrix, and most of the mechanisms mentioned above were less pronounced. Concerning this, a study proposed that the GO's antimicrobial activity in the solid matrix was due to the hydration layer formation on its surfaces [69]. Fig. 2 illustrates the possible mechanisms underlying GBNs' antimicrobial activity.

Cellular envelope stress associated with cutting mechanism and membrane insertion is commonly related to the GBNs' sharp edges. Experimental and simulation studies suggested that the GBNs' sharp edges can puncture the bacterial cells membrane, leading to cytoplasmic fluid leakage

[34,59,70]. This puncturing process is influenced by the GBNs' flake size, thickness, hydrophilicity, edge density, and oxygen functionalities [71–73]. Furthermore, the antimicrobial efficacy of GBNs is affected by their orientation angle during the interaction with microorganisms. Several speculations can be made from this interaction, such as insertion into the cellular envelope, lying parallel to the midplane of the lipid bilayer, or lying flat on the top of the lipid bilayer [73–75]. The insertion of GBNs nanosheets into bacterial cells membrane triggers the formation of pores in membranes or disrupts the membrane's phospholipids structure, but either way, these changes lead to cell death [73,74,76]. Moreover, the stronger bond between GBNs and membrane lipids than the attraction forces among lipid molecules within the membrane structures leads to the destruction of cell membranes [77].

It was observed that the Gram-negative bacteria treated with GO had hollows and dents on their membrane surface, indicating that the bacterial cells had corrugated membranes [58]. This morphological deformation was observed for *E. coli* [78] and *Pseudomonas aeruginosa* [79], which have been associated with dental implant failures [80] and periodontal diseases [81], respectively. Destructive extraction of lipid molecules reduces intracellular density that eventually leads to cellular membrane corrugations [78,82]. Direct physical contact between these bacteria (i.e. *E. coli* and *P. aeruginosa*) and GO lowers the bacterial cell membrane integrity and causes cytoplasmic fluids leakage [82]. It is important to note that these damages are irreversible [82]. Large surface bacteria (e.g. *P. aeruginosa*) have a cylindrical and elongated shape that increases their contact with GO. However, the contact between *P. aeruginosa* and GO may be limited by LPS that increase bacterial resistance. It has been reported that the *P. aeruginosa* cells were more affected by rGO due to its cylindrical and elongated shape [79]. On the other hand, the

spherical shape of Gram-positive bacteria (e.g. *S. aureus*) has a small surface area [79]. Thus, Gram-positive bacteria are less affected by rGO.

GO also displays broad-spectrum antimicrobial activity towards phytopathogenic fungi and bacteria. An antifungal study of GO showed that the GO's sharp edges could puncture the bacterial cells, leading to plasma membrane stress [83,84]. After being treated with GO nanosheets, the apical cells of conidia (*Fusobacterium graminearum* and *Fusobacterium oxysporum*) became swollen and stopped growing, although some remained unaffected [84]. Consequently, the GO nanosheets stopped germination and reduced conidia viability. The GO nanosheets also disrupted the germination cycle as spores cannot grow into mature mycelium to initiate the infection cycle [84]. These processes occur when the conidia interact with GO nanosheets, where the interaction forms spore-GO congeries and influences exchange of substance through the spore wall. As a result, spore germination is inhibited, resulting in morphological deformation. The growth eventually stopped due to cell swelling and lysis [84]. A study found that the fungicidal properties of GO on *C. albicans* were remarkable due to the insertion of GO nanosheets into the cell membrane, leading to cell lysis [85].

Wrapping mechanism is the second mechanism that inhibits bacterial activity. This mechanism is associated with the physical factors of GBNs [86]. An antibacterial study on graphene and GO nanosheets in suspension assays suggested that the wrapping mechanism could also cause bacterial cell damage [49,86]. The GBNs nanosheets wrap around the bacterial cells and isolate them from the environment, thus preventing a bacterium from proliferating [86]. This mechanical disturbance can rupture bacterial cells via electrostatic force, causing alteration of membrane potential, depolarisation, and disruption of bacterial cell membrane integrity [79]. These cellular changes later lead to osmotic imbalance, disturbed cellular respiration, cell lysis, and subsequently, cell death [79].

The wrapping mechanism has been observed for Gram-positive bacteria because their cells are usually found in clusters in the GO suspension. *S. aureus* and *E. faecalis* are commonly associated with dental implant failure and reinfection after root canal treatment, respectively [58]. These Gram-positive bacteria were found in clusters upon treated with GO suspension [58]. Additionally, Gram-positive bacteria have a large cell surface area as they are found in clusters. Consequently, Gram-positive bacteria cells are more exposed to GO nanosheets, causing them to trap easily [58]. An increased number of trapped cells resulted in a higher cell death rate [58].

In contrast to Gram-positive bacteria, Gram-negative bacteria are found as a single cell or paired cells. As such, they have small cell surface area that lowers their chance of exposure to GO nanosheets [58]. For this reason, it is harder for Gram-negative bacteria to trap [58]. Apart from bacteria, the wrapping mechanism also occurs in fungi. The mechanism involves GO nanosheets to trap and wrap the fungal spores, causing the fungal cells to agglomerate. Additionally, this mechanism hinders the absorption of nutrients into the fungal cells as the cell membrane is covered with the GO nanosheets [87].

Oxidative stress is the third mechanism that inhibits bacterial activity. In contrast to the wrapping mechanism, oxidative stress is associated with physicochemical properties of GBNs [88]. This mechanism involves oxidation of fatty acids by lipid peroxides formed by ROS [89]. The lipid peroxides accelerate chain reaction, induce cell lysis, and produce abundant ROS traces [89]. In bacterial cells, the reduction of molecular oxygen to water occurs via a series of proton-electron transfer reactions, in which adenosine triphosphate (ATP) is synthesised afterwards. However, the presence of superoxide anion and other oxygen-containing radicals interrupt the formation of water molecules, producing ROS traces in the cell's mitochondria [79]. These ROS traces damage the ribonucleic acid (RNA) and DNA. Additionally, the ROS traces impair the bacterial cells' ability to maintain their normal physiological redox-regulated function, thus collapsing the bacterial cell membrane integrity [79]. The disruption of the bacterial cell membrane through chemical oxidation leads to cell splitting and produces ROS traces that eventually cause cell death [90]. Additionally, abundant of ROS led to the accumulation of intracellular calcium, activation of transcription factors, and initiation of cytokines changes [91]. A study has shown that obligate anaerobes (e.g. *P. gingivalis* and *F. nucleatum*) are more susceptible to GO than facultative anaerobes (e.g. *S. mutans*) [70]. The bacteria's susceptibility to GO depends on their sensitivity to oxidative stress. The Transmission Electron Microscopy (TEM) analysis found that the *P. gingivalis* cells experienced a membrane leakage, in which all the cytoplasmic content flowed out, forming a circular cell wall. On the other hand, the cell wall of *F. nucleatum* was stripped down [70].

The interaction between microbial cells and solid substrates is greatly influenced by the substrates' surface wettability (i.e. hydrophilicity, hydrophobicity, and surface free energy (SFE)) [92–94]. Biological cells such as bacteria adhere to the solid surface effectively at a position with moderate hydrophilicity (i.e. water contact angles of 40°–70°) [95]. Incorporation of GBNs (e.g. GO or rGO) with a solid matrix, mainly that of polymers, creates a hydrophilic surface on the composite that attracts water molecules to form a hydration layer [69]. The hydration layer inhibits microbial adhesion and colonisation at the surface of the composites [96]. The GO composites' hydrophilicity and negative surface charge create a repulsive force against bacteria with a similar charge. This force resists the adhesion of most Gram-positive and Gram-negative bacteria, which carry a net negative charge. The negative charge of Gram-positive bacteria is attributed to the negative charge of the PG cell wall that consists mainly of phosphate groups. On the other hand, the negative charge of Gram-negative bacteria is attributed to the negative charge of outer membrane that consists mainly of phospholipids and LPS [97].

3. Factors influencing antimicrobial activities of GBNs

It has been established that the physicochemical properties of GBNs are closely related to their antimicrobial activities. Therefore, the presence of any external factors that can alter

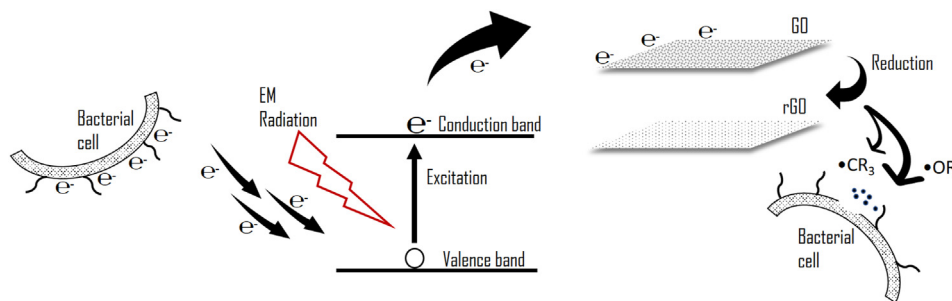


Fig. 3 – Effect of electromagnetic radiation on GBNs and possible mechanism of GBNs' antimicrobial activity.

the physicochemical properties of GBNs would directly affect their antimicrobial activities. Herein, we discussed several of these factors, including intrinsic and extrinsic parameters that could significantly affect GBNs' antimicrobial activities.

3.1. Electromagnetic radiation

GBNs (notably GO) have been used extensively as photocatalytic materials and GO's excellent photocatalytic activity has been well-documented [98]. Several studies have shown that electromagnetic radiation with energy ranging from ultraviolet (UV) to infrared can photochemically reduce GO owing to its strong optical absorbance [98]. The tunability of oxygen to carbon ratio of GO through chemical reduction is an effective technique to tailor its bandgap. GO acts as an electrical insulator when it is fully oxidised but acts as a semiconductor when it is partially oxidised [99]. In the semiconductor form, GO consists of the valence band and conductive band with bandgap energy ranging from 3.26 to 3.39 eV [98,99]. Irradiation of GO with photon energy above its bandgap energy generates electron–hole pairs at its surface [98]. Transformation of physicochemical features (i.e. semiconductor behaviour and catalytic reactivity of GO upon exposure to photon energy) alters GBNs' antibiotic effects [100].

It has been shown that GO can act as a semiconductor photocatalyst and produces electron–hole pairs that are responsible for the reduction of GO to rGO [101,102]. Fig. 3 illustrates GO's antimicrobial response under a specific electromagnetic radiation wavelength. Upon irradiation with adequate photon energy, an electron from the valence band rises to the conduction band, thus creating electron–hole pairs. Since microbial cells are present in the system, their electrons naturally fill the electron–holes. This process prevents the recombination of electron–hole pairs. Therefore, the electron–holes created during the excitation process are responsible for the oxidation of biomolecules. At the same time, the light-induced electrons are captured by the oxygen-containing groups. This process is responsible for the reduction of GO. Additionally, oxygen-centred radicals or carbon-centred radicals were produced during GO reduction [102]. These radicals enhance GO's antimicrobial activities against microbial [102]. As shown in Fig. 4, the survival percentage of *E. coli* cells treated with 25 µg/mL of GO exposed to simulated sunlight was reduced to $24.9 \pm 5.9\%$. Meanwhile, *E. coli* cells

treated with GO but were not exposed to simulated sunlight had a higher survival percentage of $68.8 \pm 9.2\%$. This finding suggested that the antibacterial activity of GO was enhanced by the simulated sunlight [102].

As mentioned above, external light does not only create electron–hole pairs, but it also enhances the formation of ROS that disrupts the bacterial cell membrane. A study showed that GO significantly sensitises the formation of singlet oxygen (1O_2) upon irradiation with ultra-low doses (65 mW cm^{-2}) of 630 nm light [103].

3.2. Electrical conductivity

Considering the application of GBNs as coating materials in which nanosheets merge to form large-area of film, the antimicrobial effect due to the sharp edge of nanosheet is expected to be significantly restricted. The antimicrobial action of graphene film deposited onto a large and flat area of substrates is therefore much dependent on the substrates or surrounding medium, which can be classified by electrical characteristics (i.e. conductor, semiconductor, and insulator) [104,105]. These studies have shown that large-area graphene films produced via the chemical vapour deposition (CVD) on copper (Cu), germanium (Ge), and silicon dioxide (SiO_2) exert different microbial activity against Gram-positive and Gram-negative bacteria [104]. The antimicrobial activity of graphene grown on Cu was more pronounced than that grown on SiO_2 . It has been observed that the antimicrobial activity of graphene films coated onto the substrate was greatly influenced by the substrate's ability to transfer its charge [104]. The transfer of charge follows the order of $\text{Cu} > \text{Ge} > \text{SiO}_2$ [104].

A similar study has been performed for GO deposited onto several substrates with different electrical conductivity [105]. The study aimed to provide an in-depth understanding of the charge transfer mechanism and its relationship with antimicrobial effects. It was evident from the study that the antimicrobial effects of GO depended on the conductive nature of the substrate onto which a graphene film was deposited in the order of $\text{GO/Zn} > \text{GO/Ni} > \text{GO/Sn} > \text{Go/Steel}$ [105]. However, the non-conducting substrate (i.e. GO/Glass system) did not show any antimicrobial activity. These findings suggested that substrates with good electrical conductivity facilitate electron transfer from the bacterial membrane to electron-withdrawing or electropositive surfaces. The transfer of electron subsequently alters the surface potential of the bacterial

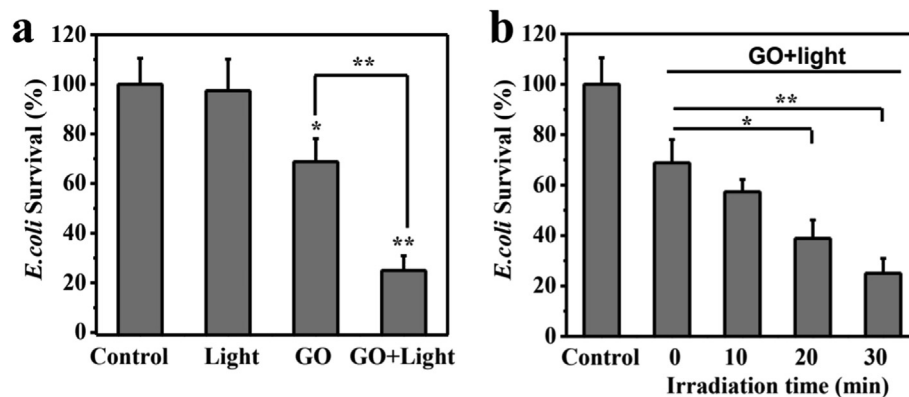


Fig. 4 – Evidence of GO nanosheets killing *E. coli* with or without exposure to simulated sunlight. (a) Antibacterial activity by GO under various conditions as assessed by numbers of colony-forming units. Cultured *E. coli* cells were treated by isotonic saline (as a control), simulated sunlight, or GO (with or without exposure to simulated sunlight). (b) Antibacterial activities of GO were influenced by the duration of exposure to simulated sunlight. The data shown are mean values and standard deviations from a representative of three independent experiments. *P* values were calculated using the student's *t*-test. A single asterisk (*) indicates *P* values that are less than 0.05 ($p < 0.05$). Double asterisks () indicates *P* values that are less than 0.01 ($p < 0.01$). Reproduced with permission from [102]. Copyright (2017), American Chemical Society.**

cell membrane, resulting in the loss of cell viability. Therefore, it can be said that the antimicrobial activities improve with increased conductivity [105].

A recent study on GO-coated onto zinc phthalocyanine (ZnPc) reached a similar conclusion to the relationship of charge transfer with antimicrobial activities [106]. Specifically, the study found that the death of *E. coli* was due to the dissipation of electron from bacterial cell membrane to the electron-deficit GO. As mentioned earlier, the deposition of GO onto a substrate causes GO to lose its electrons. The development of ROS-dependent oxidative stress in bacterial cells and the interaction mechanism between bacterial cells and GO/substrate are illustrated in Fig. 5.

3.3. Morphology

Wrinkle patterns is an emerging method for surface topography, and the presence of the wrinkles may affect the final

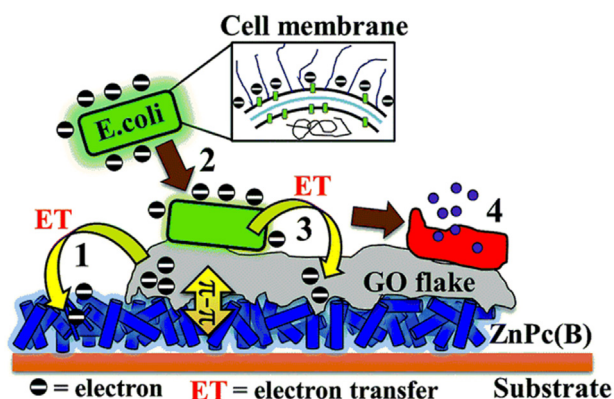


Fig. 5 – Schematic diagram illustrating the proposed interaction mechanism between *E. coli* cells and ZnPc(B)–GO [106]. Reproduced with permission from [106]. Copyright (2019) Royal Society of Chemistry.

properties of the film. Interfacial interactions between a material and its environment can be controlled by patterning the material's surface topography. In principle, the effect of the wrinkles depends on the final application. The effect may either be desirable or otherwise. GBNs wrinkled surfaces that consist of peaks and valleys are suitable for biological interactions (i.e. anisotropic cell growth and antimicrobial activity) [107]. A study demonstrated that the peak's sharp edge on the wrinkled GO surface inhibited bacterial cells' adhesion [53].

Moreover, the sharp edges facilitate the charge transfer in the GO nanosheets and disintegrate the bacterial cell membrane. On the other hand, bacteria of matching diameter with the terrains will be trapped inside, resulting in a strong interaction between GO and bacterial cells. This phenomenon causes the direct oxidation of cellular components [53]. Fig. 6 shows the effect of wrinkled GO surfaces on the bacterial cell membrane.

3.4. Functional groups

The physical properties of GBNs are greatly affected by their surface functionalities. A study found that GO with epoxy and hydroxyl rich surface functionalities exhibited smaller nanosheet size, smoother, less porous, and thinner film than GO with a carboxylic rich group [108]. The superior properties of GO with epoxy and hydroxyl groups inhibited bacterial cell proliferation and prevented biofilm formation. Conversely, GO with the carboxylic rich group is prone to bacterial cell adhesion owing to GO's higher surface roughness and non-uniform thickness [108].

Other studies also demonstrated that GO moieties (epoxy, hydroxyl, and carboxyl) could react with the bacteria's biomolecules [88]. This reaction disrupts redox reactions that may continue to affect cell growth and metabolic system adversely. Apart from that, these surface functional groups had different oxidative levels in which bacterial killing

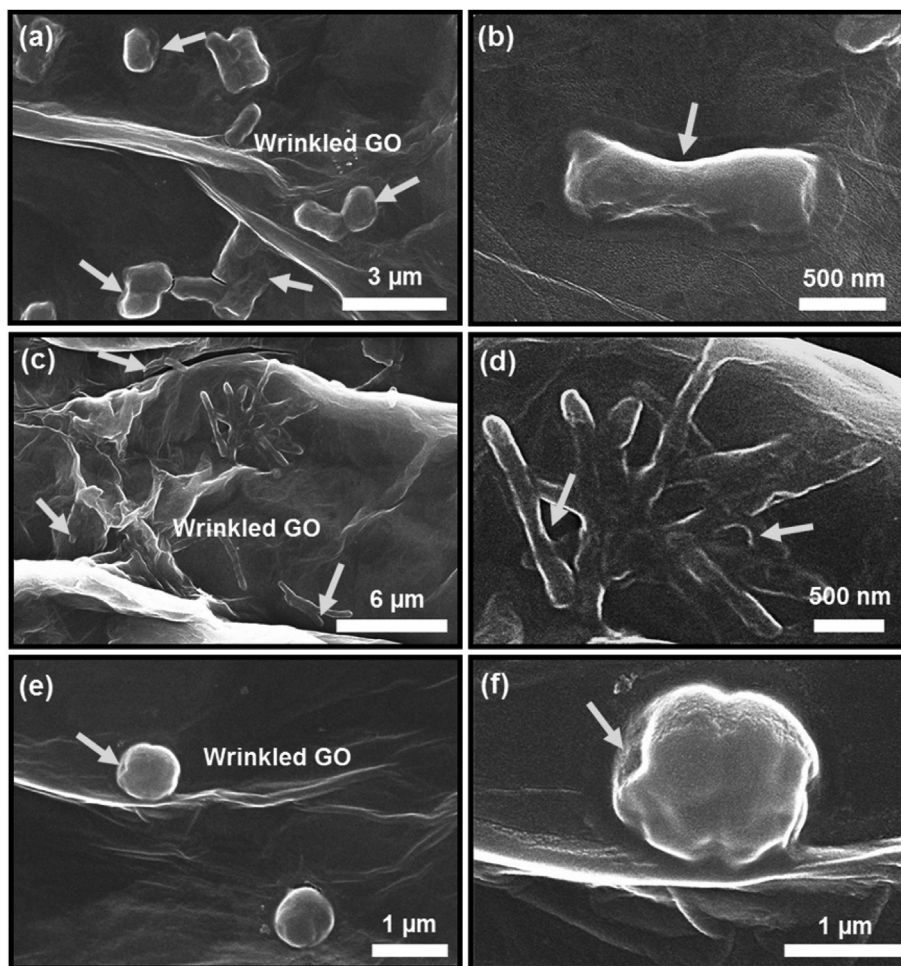


Fig. 6 – Scanning electron microscope (SEM) images of *E. coli* (a and b), *M. smegmatis* (c and d), and *S. aureus* (e and f) after the drop test on the wrinkled GO surfaces. Bacterial cells of each species exposed to the wrinkled GO films were completely enveloped with thin GO nanosheets. The arrows on the images point to local disruptions of the bacterial cell membrane caused by the exposure to GO nanosheets. Reproduced with permission from [53]. Copyright (2019), American Chemical Society.

efficacy varied [88]. The functional groups associated with carbon radicals were found to exert a high antimicrobial activity. For instance, a study showed that the high density of carbon radicals on the GO surface enhanced the oxidative level by increasing lipid peroxidation of the bacterial cell membrane, thus heightening the antimicrobial activity [34].

As mentioned above, GO with high oxygen-containing functional groups showed potent antimicrobial activity, mainly due to oxidative stress damage [52,109]. Nevertheless, in suspension form, findings from an antibacterial study of GO against *S. mutans* suggested that the primary mechanism underlying bacterial inhibition was due to the direct contact of the bacteria with the extremely sharp edges of GO, and not due to the oxidative stress damage [70]. GO with high oxygen-containing functional groups exhibited good dispersity and wrapping or trapping ability, thereby offering a larger surface area for interactions with bacterial cells [34,49]. This finding was further supported by Nanda et al. [48], who also found that high attachment of bacteria to GO nanosheets' surface

leads to better bacterial entrapment [48]. Collectively, these results implied that surface functionality exhibited several different modes on the antimicrobial mechanism.

A study reported that the mature biofilm of *S. mutans* was insensitive to GO isotonic saline dispersions at a concentration of 80 g/mL [110]. However, the mature biofilm of *S. mutans* was sensitive to GO dispersed in deionised water [111]. This contradictory finding can be explained by GO's stability in solutions, whereby GO is unstable in solutions containing electrolytes and forms aggregation, hence decreasing its antibacterial activity [111].

3.5. Flake size and concentration

Studies have shown that the antimicrobial activities of GBNs depend on their flake size and concentration [34,58,112]. A recent study on the relationship between GO size and exposure time with antimicrobial activity showed that the elongated-normal cells of *S. mutans* changed to sphere-like

cells with a crumpled structure when exposed shortly to GO suspensions for 10 s [112]. This structural change indicated the cutting effect of GO on bacterial cell morphology [112]. However, this observation was only noted in GO suspensions with small sizes (1295 and 2015 nm), but not in the GO suspensions with large sizes (3074 and 4544 nm). Although GO nanosheets with the smallest size had a promising cutting effect, they cannot wrap and trap the cells, resulting in fewer cell entrapment [112].

In contrast to short exposure, prolonged exposure of bacterial cells to GO suspensions showed different findings. Large-size GO suspension (3074 nm) had a great cell entrapment effect but a weak cutting effect. No cutting effect was observed for GO suspension with the largest size (4544 nm). Interestingly, GO suspension (2015 nm) revealed the highest antibacterial efficacy on *S. mutans* due to enhanced cutting and cell entrapment effects [112]. Although there are some inconsistencies in this study, flake size has been considered to influence GO's antibacterial activity significantly [112]. Moreover, GO's efficacy depends strongly on its applications and external parameters.

Another study on the antimicrobial activity of GO-coated surfaces, in which the GO sheets immobilised the substrate, revealed that smaller GO nanosheets exhibited higher antimicrobial activity than larger GO nanosheets [86]. More distorted bacterial cells were found on the surfaces coated with smaller GO nanosheets than on the surfaces coated with larger GO nanosheets. GO's antimicrobial activity was associated with oxidative stress induced by higher defect density of the smaller GO nanosheets that allowed more oxygen to be absorbed on its surface, explaining the higher oxidative potential [86]. For GO that was applied in suspension, its antimicrobial activity increased with increasing nanosheet area. Specifically, the increased antimicrobial activity was due to the aggregation and cell wrapping mechanism, whereby the large GO nanosheets completely wrapped the bacterial cells and isolated them from the environment [113].

Antibacterial activity of GO against the dental pathogen (i.e. *S. mutans*) has been investigated in planktonic [44,70] and biofilm [33] forms. The findings showed that GO exerted a strong bactericidal effect on planktonic and biofilm forms, and the susceptibility was observed in a concentration-dependant manner for both forms [33]. However, biofilm had greater survivability than planktonic cells due to its highly ordered structure in the surfaced-attached bacterial communities that made biofilm more resistant [114,115].

4. Application of GBNs in dentistry

GBNs have been successfully employed as an alternative material in biomedical, dental, and implants application [116]. GBNs are mainly used as an anti-corrosion coating and antimicrobial agent [116]. Additionally, GBNs have been used for drug and therapeutics delivery [117,118]. In this section, we emphasised on the potential of GBNs as an antimicrobial agent in dental applications. As discussed above, the mechanism underlying GBNs' antimicrobial activity includes cell membrane cutting, isolation of microbes via cell wrapping, oxidative stress, and hydration layer. However, the

antimicrobial mechanism of GBNs in the solid matrix or immobilised on the surface differs from that in colloidal suspension [108]. Therefore, the antimicrobial activity of GBNs incorporated with dental materials should be evaluated as per application. Herein, we summarised the effect of GBNs on dental pathogen based on their application in dentistry.

4.1. Restorative materials

The use of GBNs and nanoparticles composites as dental restorative materials has been intensively investigated [119,120]. Studies have shown that certain aspects of the physical and mechanical properties of the dental ceramic, dental adhesive, and dental resin were significantly improved with GBNs. Nonetheless, less is known about the biological effects (e.g. antimicrobial and antibiofilm) of GBNs on dental restorative materials.

Several studies were performed to incorporate GO-based nanomaterial with commercial glass ionomer to enhance GO's antimicrobial properties [121,122]. An in vitro study showed that the incorporation of reduced graphene-silver nanoparticles into conventional glass ionomer cement (GIC) significantly inhibited the growth of *S. mutans* [123]. The antibacterial efficacy of the GIC improves with the increasing amount of the graphene-silver nanoparticles, and its addition at about 2.00 wt.% exhibited excellent antibacterial activity without sacrificing their mechanical properties [123]. To maintain the aesthetic quality of the GIC, white-coloured fluorinated graphene (FG) was synthesised and added to the conventional GIC to create a composite with significant improvement in mechanical, physicochemical, and antibacterial properties. The composites of GIC/FG exhibited excellent performance in inhibiting *S. mutans* and *S. aureus* with efficacy rates that almost doubled the pure GIC [121].

Incorporation of GBNs in teeth filling inhibits antimicrobial growth and interferes with biofilm formation. In 2019, a study evaluated the antibacterial activity of GO, hydroxyapatite, and zirconia teeth filling nanoparticle against the biofilm-forming ability of *E. coli* and teeth chromogenic bacteria (i.e. *Enterobacter ludwigii*) [38]. The antibacterial activity was evaluated based on the growth and survivability of both bacteria against these nanoparticles. It was demonstrated that GO was the most efficient filler for infectious teeth with chromogenic bacteria since it can diminish the bacteria load efficiently [38]. Apart from tooth filling, graphene nanoplatelets were also used as a filler to the conventional polymer dental adhesive. Incorporation of this nanomaterial as filler in dental adhesives significantly inhibited the adhesion and growth of *S. mutans* while maintaining the same viscosity as the conventional ones [124].

Apart from acting as antimicrobial agents, GO nanocomposites can also be used as a treatment agent to prevent dentin demineralisation by covering the dentin surface. Nizami et al. [125] successfully synthesised five types of nanocomposite (i.e. GO-Ag, GO-CaF₂, GO-Zn, GO-Ca₃(PO₄)₂ and GO-Ag-CaF₂) to evaluate the efficacy of these nanocomposites on the bactericidal and anti-demineralisation activities. They found that GO-Ag, GO-Ag-CaF₂, and GO-CaF₂ nanocomposites prevented demineralisation without tooth discolouration. Additionally, GO-Ag and GO-Ag-CaF₂ inhibited

S. mutans growth without intoxicating the epithelial cells, except at a high concentration (0.1 w/v) [125]. Aside from that, GO can be used to coat dentin surfaces. Immersion of dentin block in diluted GO suspension successfully covered the dentin surface with few nanometres of GO film. The combination effect of GO film and near-infrared (NIR) irradiation markedly showed photothermal and bactericidal effects against *S. mutans*, thus enabled bacterial sterilisation [126].

A recent study demonstrated that GBNs did not only enhance the mechanical properties of polymethyl methacrylate (PMMA) denture resin but also displayed antimicrobial properties against dental pathogen [39]. Antimicrobial properties of GO nanosheets incorporated with PMMA were studied using four different microbial species (i.e. *E. coli*, *C. albicans*, *S. aureus*, and *S. mutans*). Incorporation of GO nanosheets (up to 2 wt.%) increased the PMMA's hydrophilicity and antimicrobial effects with minimal toxicity to human cells [39]. It was suggested that the possible mechanism for the effective antibacterial activity of GO incorporated with solid matrix was due to the increased hydrophilicity [39,127]. Hydrophilic forms a hydration layer on the composite surface and creates a tightly bound water layer. Hence, it has been proposed that the presence of the hydration layer or energetic barrier on the surface is the key mechanism underlying antimicrobial activity that prevents microbial adhesion [33,127]. Bacali et al. further incorporated graphene-silver nanoparticles with PMMA denture resin to enhance the composite's mechanical, biocompatibility, and antimicrobial activity [128]. It was found that the addition of graphene-silver nanoparticles improved the antibacterial activity of PMMA resin against *E. coli*, *S. aureus*, and *S. mutans* strains [128].

4.2. Endodontics

The use of photodynamic therapy (PDT) is more favourable than the sodium hypochlorite in root canal treatment owing to its ability to disinfect the root canal while preserving the stability of dentin. The principle of PDT is based on a nontoxic photosensitiser (PS), such as indocyanine green (ICG) that forms cytotoxic ROS following photoactivation by specific electromagnetic radiation [129,130]. Studies have suggested developing nano-PS conjugated to increase the production of ROS during photoactivation [131,132]. GO has been chosen to act as the nanocarrier and incorporated with ICG due to its large specific surface area that enables efficient functionalisation of the photosensitisers via various surface functional groups [133–135]. Besides, strong UV emission of GO also paved the way for the applications in PDT [136].

A study has investigated the antimicrobial activity of rGO-phthalocyanine complex against Gram-positive and Gram-negative bacteria upon photoactivation [133]. The study showed that Gram-positive bacteria (*S. aureus*) were more susceptible to pure GO and GO composites than Gram-negative bacteria (*P. aeruginosa* and *E. Coli*). It has been suggested that the lack of inner membrane was the main factor responsible for Gram-positive bacteria's susceptibility to GO [133]. Another study also showed that the incorporation of ICG with GO (at one-fifth of its concentration in conventional PD) significantly reduced the ability of *E. faecalis* to form biofilm [134]. Incorporation of GO with ICG enhances the ICG loading

and aqueous stability, thus improving its antimicrobial and antibiofilm effects against *S. mutans*. The antimicrobial and antibiofilm results showed that the incorporation of GO with ICG could significantly decrease *S. mutans* survivability up to 86.4% and suppressed biofilm formation up to 63.8% [135]. It was also suggested that incorporating ICG with GO could be a new approach to adjuvant treatment of endodontic infections [135].

Bioactive materials have been rapidly used in the field of endodontics for regeneration, repair, and reconstruction. Dubey et al. [137] have conducted a study investigating the potential of graphene nanosheets (GNS) (1357 wt.%) to improve two bioactive cements: Biodentine (BIO) and Endocem Zr (ECZ). The results showed that GNS did not interfere with the pH release profile [137]. The pH release profile plays crucial roles in bioactivity and antibacterial properties of the bioactive cements [137].

Apart from bioactive materials, the use of metal or metal oxide nanoparticles in dental materials offer several advantages, such as improved physical, mechanical, antimicrobial, and antibiofilm properties. However, the aggregation of these nanoparticles remains a major challenge. The use of GO as a matrix in GO-silver nanocomposite improved the stability and aggregation of the silver nanoparticles, which lead to the high binding capability [138]. Furthermore, synergistic antimicrobial activity from both silver and GO made the GO-silver nanocomposite more advantageous. Considering these advantages, Ioannidis et al. [139] have successfully synthesised silver nanoparticles on an aqueous GO matrix to study their efficacy against endodontics biofilm. Ex vivo study on the infected tooth model showed that the Ag-GO nanocomposite successfully killed the microbes and disrupted the biofilm formation. The use of Ag-GO under ultrasonic activation also selectively improved microbial killing efficacy in the lateral canal [139].

A recent study has modified titanium for pulp sealing with antibacterial and dentino-inductive materials via micro-arc oxidation (MAO) and self-assembling GO of varying content [140]. Incorporation of 1.0 mg/mL of GO with titanium-MAO showed excellent cell adhesion, mineralisation, and antibacterial properties [140]. Furthermore, bacterial colonies were nearly absent. The reduction ratio of bacteria was $93.25\% \pm 2.47\%$, which could be explained by the highest ROS level associated with the abundant oxygen-containing functional groups [140].

4.3. Periodontics

Researchers have extensively investigated the potential use of GO in tissue engineering therapy and created GO composites scaffolds. For instance, Nishida et al. [141] fabricated GO-scaffold where its implantation exhibited high tissue compatibility and facilitated the healing of tooth extraction sockets and periodontal defects [141,142]. Furthermore, Chen et al. [143] suggested that zinc oxi/carboxylated GO nanocomposites induced bone tissue regeneration. Moreover, GO has attracted significant attention due to its potential in gene and drug deliveries. GO's ability to ionically bond to cationic polyethyleneimine (PEI) polymers offer other advantages in gene delivery [144]. Antisense walR (ASwalR) RNA was

reported to inhibit the biofilm formation and sensitised *E. faecalis* to calcium hydroxide medication. GO-PEI complex has been used to load ASwaR plasmid RNA to enhance transformation efficiency in *E. faecalis* cells using similar approaches [145]. The results showed that the GO-PEI-ASwaR complex significantly reduced virulent-associated gene expressions, suppressed biofilm aggregation, improved bactericidal effects in the infected canal, and reduced periapical lesion size.

4.4. Dental implants

Titanium has been recognised as the gold standard in implant dentistry owing to its high corrosion resistance, long-term performance, and good biocompatibility with excellent osseointegration [146,147]. Despite these advantages, the growth of microbial biofilms in dental implants is the primary cause of implant diseases and implants' failure. Therefore, the development and modification of titanium implant with good osteogenic, antibacterial, and antibiofilm properties are vital. For that reason, several surface treatments have been developed over the last few years to improve the antibacterial activity of titanium implants, including nanotechnologies with antimicrobial properties (e.g. graphene). Graphene coating is widely used in dental and implants applications as it prevents metallic biomaterials surface from corruptions [116]. Anti-corrosion properties of GBNs make them feasible to be used in orthodontics, endodontics, and prosthodontics [148,149]. Several techniques, such as plasma treatment [150], CVD [151], wet and dry transfer [152] and electrophoretic deposition method [153], and have been employed to coat titanium substrate with GO-based material. The effective coating of titanium onto an object without changing the basic graphene properties gives advantages to the implants' performance in terms of their durability, osteogenic, and antimicrobial properties.

Numerous studies have been carried out to evaluate the performance of GBNs as a coating and anti-corrosion material to prevent implants from the corrosive environment [153,154]. Furthermore, the addition of GO improves the mechanical properties of the coating [155] and promotes cell adhesion and proliferation, which are facilitated by the hydrophilic functional groups (e.g. carboxyl, carbonyl, and hydroxyl) [153,154,156,157]. The superior performance of GBNs has inspired researchers to explore the use of GBNs in the treatment of peri-implantitis [158]. A recent study showed that implants coated with GO exhibited good therapeutic effects [158]. Nevertheless, studies investigating the antibacterial properties of GBNs in implant coating are relatively limited. Although the antibacterial properties of the GBNs have been well-documented, less is known about their antibacterial properties as coating materials, especially in dental implants. Also, the antimicrobial properties of the GBNs upon coated onto implants may differ from pristine [86]. The changes in the physicochemical properties of GO such as morphology and flake size may have different effect on antimicrobial activity when applied on surface [86].

Another recent study showed that titanium surfaces coated with six different graphene nanoplatelets exhibited different antimicrobial activities against *S. aureus* [159]. The

graphene nanoplatelets were produced using different techniques [159]. Comparisons on osteogenic and antibacterial properties between uncoated and graphene-coated titanium implants via dry transfer technique showed a significant decrease in the biofilm formation of *S. mutans* and *E. faecalis* on the surface of graphene-coated titanium implants [160]. The study further confirmed that the mechanism of bacterial and biofilm inhibition was mainly due to the surface properties and not due to the release of diffusible compounds from the surface (i.e. electron transfer) [160]. This finding is further supported by Agarwalla et al. [161], who suggested that graphene has altered the surface and wettability of the graphene-coated titanium. Compared to uncoated titanium with SFE of 38.3 mN/m, graphene-coated titanium with lower SFE of 13.8 mN/m exhibited a significant reduction in the biofilm formation of *S. mutans*, *E. faecalis*, *P. aeruginosa*, and *C. albicans* [161]. These findings contradict a previous study suggesting that the antimicrobial properties of GBNs are influenced by the electron transfer between GBNs and underlying metal substrate coated with GBNs [105].

Incorporation of GBNs with metal nanoparticles has also gained substantial interest in coating fabrication. Graphene or GO were incorporated with other nanoparticles (e.g. zinc oxide or silver) to enhance the antimicrobial properties of coating materials [162,163]. The negative charges of carboxyl, hydroxyl, and carbonyl groups on the GO surface permitted the deposition of the positive charges of metal ion, resulting in a more uniform distribution of metal nanoparticles on its surface [162]. Therefore, implants coated with a higher concentration of GO would have a higher concentration of metal nanoparticles deposited on the GO surface. The deposition of metal ions on the GO surface inhibits microbial and biofilm adhesion. A study using graphene/zinc oxide nanocomposites (GZNC) as a coating material for dental implants showed a good antibacterial and anti-biofilm properties of GZNC against *S. mutans* [163]. GZNC-coated acrylic tooth surfaces successfully inhibited 85% of *S. mutans* biofilm formation [163]. Furthermore, the low toxicity of GZNC made it an effective coating agent for dental implants [163].

The presence of a functional group on GO surface endows good bio-functionalisation with an antibacterial agent [164,165]. A multi-functional coating that consists of minocycline hydrochloride (MH) and GO has been developed to coat titanium implants [158,166]. The effect of MH and GO to inhibit bacterial growth and induce bone tissue regeneration for the improvement of bone-implants osseointegration was evaluated using *S. aureus* and rat bone mesenchymal stem cells (rBMSCs) [158,166]. The effect of MH was studied *in vitro*, whereas the effect of GO was examined *in vivo*. The results showed that the MH loaded-GO films on titanium surfaces exhibited excellent antibacterial and enhanced osteogenic activity. Therefore, MH loaded-GO films may potentially be used in clinical applications due to their superior ability in bone-implants osseointegration [158,166]. Another study on bio-functionalisation of GO showed that the fabrication of multilayer coating integrating lysozyme (Lys), tannic acid (TA), and GO had a significant effect on killing *E. coli* and *S. aureus* [167]. Observations on bacterial cell morphology showed that the bacterial cell membrane collapsed and bacterial structure deformed. Different antibacterial mechanisms

of Lys, TA, and GO produce a coating layer that kills bacteria differently [167].

Nevertheless, the multilayer coating of Lys-TA-GO appears more effective against *E. coli* than *S. aureus*. This differential effect is due to the nature of *S. aureus* that tends to clone aggregately, making them less susceptible to GO immobilised on implants substrate. This finding contradicts an earlier study [58], which stated that the bacteria in a cluster form tend to be trapped easily, thus they are more susceptible to GO suspension. The heterogeneity of these findings can be explained by the different mechanism of GBNs' antimicrobial action in immobilised and suspension forms.

5. Conclusion

The present study discussed current advances of GBNs in the dental application. Mainly, we focussed our discussion on GBNs' antimicrobial properties and mechanisms. Incorporating GBNs into dental materials improves the antimicrobial properties of the dental materials without compromising their mechanical properties. The mechanism of GBNs' antimicrobial effects is highly determined by various intrinsic (i.e. size, shape, surface chemistry) and extrinsic (i.e. electromagnetic radiation, underlying substrate) parameters. Although the mechanism of GBNs' antimicrobial effects is still in debate, many researchers believed that GBNs could potentially be used in dentistry to improve the dental materials' antimicrobial activity and mechanical properties. A growing number of dental materials incorporated with GBNs has been investigated extensively for their potential in restorative, endodontic, orthodontic, periodontal, and implant treatments. However, further research on the biocompatibility and bifunctionality are warranted to support the currently limited clinical evidence on GBNs application in dentistry.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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