REVIEW PAPER

Upgrading Rstorative Dentistry with Graphene Nanoparticles: a Review

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Abstract: *Graphene Nanoparticles (GNPs), an upshot of nanotechnology has attracted great interest in diverse research fields including dentistry for their unique properties. Graphene Nanoparticles are cytocompatible and when combined with other compounds, they possess improved synergistic antimicrobial and anti-adherence properties against oral pathogens. The cytotoxicity of graphene in the oral setting has been reported to be very limited in the scientific literature. Current applications of graphene include reinforcing Polymethylmethacrylate (PMMA) for the fabrication of dentures, improving properties of dental luting agents like glass ionomer cement, reinforcing restorative composites and ceramics, and improving osseointegration of titanium dental implants by coating with graphene. This paper reviews the nanoparticle 'Graphene' and its potential uses in the field of prosthetic dentistry.*

Keywords: *Graphene, Graphene Nanoparticle, Reinforced Polymethylmethacrylate (PMMA).*

1. INTRODUCTION

The oral cavity has a dynamic environment where rehabilitation of function and aesthetics with a prosthesis is challenging. When exposed to high temperature, masticatory and abrasive forces, the contiguity of dental materials with oral fluids leads to mechanical failures, requiring replacement over a period, with added cost [1].
This necessitates continuous research for newer materials with improved properties. Previous research has shown that the combination of two or more materials with different compositions, morphology, and characteristics can lead to products with customized physical, chemical, and biological properties.
Nanotechnology is a field of research and innovation concerned with building 'things'- in the scale of atoms and molecules. It is said to be able to massively increase manufacturing production at significantly reduced costs. Products of nanotechnology will be smaller, cheaper, lighter yet more functional. Reinforcing dental materials with "Nanoparticles" is an innovative technology in this direction. Graphene nanoparticle, owing to its exceptional properties, is being considered the new material of choice in various applications of prosthetic dentistry. Graphene was discovered by Andre Geim and Konstantin Novoselov at the University of Manchester in 2004 for which they were awarded the Nobel prize in 2010.
Graphene is one of the allotropes of elemental carbon. It is a one-atom-thick, sp2 hybridized, two-dimensional sheet of carbon atoms arranged in a honeycomb lattice. Graphene can be transformed in zero-dimensional (0D) nanomaterials (such as fullerenes), rolled into one-dimensional (1D) nanotube, or manipulated in 3D graphite. Graphene sheets exist in bi-layers and multilayers. As the number of layers increases, the properties of the material get modified [2].
The interlayers are re-arranged through weak Van

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der Waal forces, accounting for the softness of the material. Being regarded as "the thinnest material in the universe", graphene has attracted great interest in diverse research fields including dentistry for its unique mechanical properties, flexibility, non-toxicity and impermeability to liquids and gases, relative to its physical size [3]. Graphene exists in different forms such as graphene powder, graphene sheets, graphene nanoflakes, graphene nanoplates and graphene foam (Fig. 1).

1.1. Graphene Derivatives (Fig. 2):

Different graphene derivatives are as follows:
Graphene oxide (GO) is a highly oxidized form of graphene prepared by the oxidation of graphite. Its chemistry is further changed by removing the oxygen-containing groups to obtain reduced Graphene oxide (rGO) through thermal, chemical, or ultra-violet exposure processes.

1.2. Synthesis

The market for graphene applications is essentially driven by progress in the production of graphene with properties that are appropriate for its specific application. Currently, Graphene can be synthesized by two approaches i.e., Top-down and Bottom-up (Table 1).

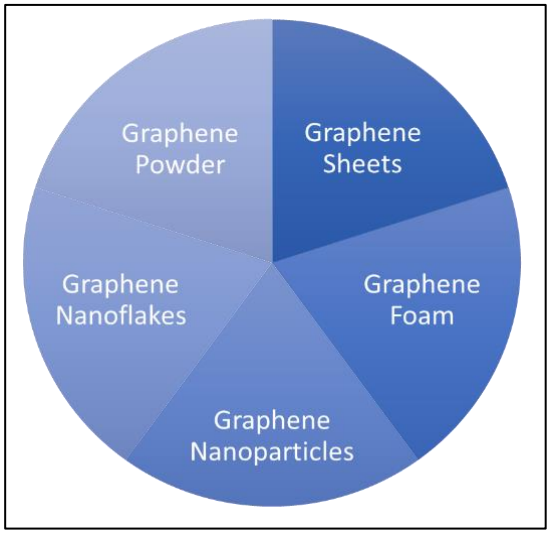

Fig. 1. Different forms of Graphene

Among these, Chemical Vapour Deposition (CVD) has emerged as an important and the most successful method to produce largescale and high-quality graphene sheets for various applications and mechanical exfoliation suffers from low yields, making it difficult to apply to industrial-scale production [4-6].

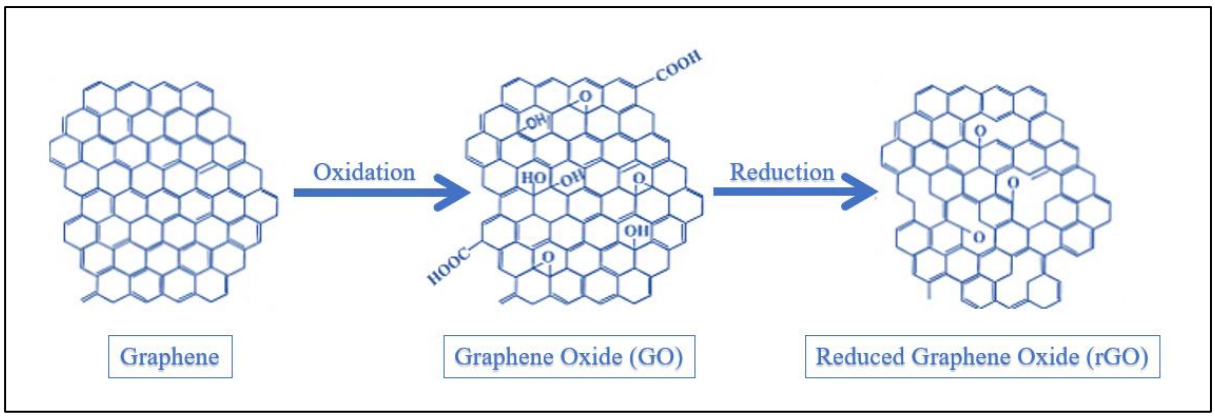

Fig. 2. Different derivatives of Graphene

Table 1. Various methods for fabrication of Graphene nanoparticles

Top-Down Approach	Bottom-Up Approach
• Separation • Peeling • Cleavage • Mechanical and Electrochemical solvent-based exfoliation of graphite or its derivatives	• Chemical Vapour Deposition (CVD) • Pyrolysis • Chemical Synthesis • Arc discharge • Decompression of Carbon Nanotube (CNT) • Solvothermal • Epitaxial Growth • Electrically Assisted synthesis

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1.3. Properties of Graphene

The properties of Graphene have been summarised in Table 2 [7-12].

1.3.1. Biocompatibility and cytotoxicity

For a safer development of Graphene Nanoparticles (GNPs), it is necessary to understand the interaction of graphene and its derivatives with living systems and their toxicity in vivo and in vitro. Evidence suggests cytotoxicity of GNPs cannot be generalized as it depends on various factors such as morphology (size, shape, and sharp edges), surface charge, surface functionalization, dispensability, state of aggregation, number of layers, purity, and methods of synthesis [13]. Graphene is recognized as a hydrophobic material while GO is slightly hydrophilic due to the presence of oxygen-containing functional groups on its basal plane. Compared to hydrophobic Graphene, hydrophilic GO may be more cytocompatible. Greener exfoliating methods suggested by Peng et al. produce a high-purity GO that contains lower (Mn^{2+} and Fe^{2+}) and hence they have significantly lower cytotoxicity than traditionally prepared GO. A study conducted by Olteanu et al. assessed the cytotoxic potential of GO, Thermally reduced graphene oxide (TRGO), and Nitrogen-doped graphene (N–Gr), on human dental follicle stem cells. The result showed that GFNs, especially GO, increased the intracellular ROS generation in a concentration and time-dependent manner. Studies have suggested that 50 ug/ml is the toxicity threshold for GO in normal mammalian cells. Concentration higher than 40 ug/ml harms human fibroblast cells and T lymphocytes, reduces cell viability, and alters mitochondrial membrane potential. While at low concentrations (4 ug/mL), they exhibit a good safety profile providing a high antioxidant defense [14].

1.3.2. Antimicrobial property

GFNs are a potential antibacterial agent, but a thorough understanding of the antimicrobial mechanism is still in its infancy. A study conducted by He et al. evaluated the antibacterial activity of GO nanosheets against the common types of oral microflora (Streptococcus mutans, Porphyromonas gingivalis, Fusobacterium nucleatum) and found that GO nanosheets were highly effective in inhibiting the growth of dental pathogens.[15] Multiple mechanisms have been proposed in this regard by Radhi A. et al. (Table 3) [16]. Currently, graphene and its derivatives have attracted much attention in caries research as a preventive, cariostatic, and remineralizing material. Research has well demonstrated that they are significant in inhibiting cariogenic bacteria, preventing dental hard tissue demineralization, and facilitating remineralization. They have been also effective in the management of periodontal diseases (Table 4, 5) [17].

Table 2. Physical properties of Graphene and its derivatives

	Values	**Inference**
Atomic width	0.335 nm	Single atom thick
Electron mobility	200,000 $cm^2/(Vs)$	Maximum (>100x higher than silicon)
Strength	42 N/m	Strongest material ever tested (100 times stronger than steel)
Toughness and stretchability	Comparatively brittle, up to 25%	Highly relevant for flexible electronics
Stiffness	Same as diamond	
Impermeability	Even the minimum atom (Helium) cannot pass through a sheet of graphene	
Electrical resistivity	1×10^{-8} Ωm	Among the lowest of any known material at room temperature (35% less than copper)
Transparency	Almost transparent	Absorbs only 2.3% of light
Surface area	2600 $m^2 g^{-1}$	Large
Thermal conductivity	5000 W/mK	Exceptional
Electrical conductivity	1 X lO^8cmW.s	High
Density	0.77 mg/m^2	Extremely light material
Fracture strength	130 GPa	High
Young's modulus • Single layer graphene • Bilayer graphene	 2.4 ± 0.4 TPa 2.0 ± 0.5 TPa	Highly resistant material

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Table 3. Different mechanisms of anti-microbial action

Physical Damage	Chemical Damage	Electron Transfer
Piercing through the microbial cell membrane Blade-like graphene materials pierce through the cell membrane causing leakage of intracellular substance leading to cell death. **Wrapping and photothermal ablation** Provoke bacterial cell damage by enclosing the bacterial cells providing a barrier to isolate growth medium, inhibiting bacteria proliferation, and decreasing microbial metabolic activity and cell viability.	**Primary oxidative stress** Intracellular reactive oxygen species (ROS) accumulation could cause intracellular protein inactivation, lipid peroxidation, and dysfunction of the mitochondria, leading to disintegration of the cell membrane and eventual cell death.	Interrupting electron transport in the respiratory chain and leading to the destruction of microbial integrity and cell death.

Table 4. Properties of graphene and its derivatives for the management of dental caries

Graphene and Its Derivatives	Properties
GRAPHENE	
Graphene	Inhibits cariogenic biofilm
Graphene-silver nanoparticles	Inhibits cariogenic biofilm
Graphene-zinc nanoparticles	Inhibits cariogenic biofilm
Graphene-zinc oxide nanoparticles	Inhibits cariogenic biofilm
Graphene-fluorine	Inhibits cariogenic biofilm Promotes enamel and dentin mineralization
GRAPHENE OXIDE	
Graphene oxide	Inhibits cariogenic bacteria and fungi Inhibits cariogenic biofilm Promotes enamel and dentin mineralization
Graphene oxide-silver nanoparticles	Inhibits cariogenic bacteria
Graphene oxide-bioactive glass	Inhibits cariogenic bacteria Promotes enamel and dentin mineralization
Graphene oxide-silver-calcium fluoride	Inhibits cariogenic bacteria
Graphene oxide-carnosine-hydroxyapatite	Inhibits cariogenic bacteria
Graphene oxide-copper	Inhibits cariogenic biofilm
Graphene oxide-polyethyleneimine	Promotes enamel and dentin mineralization
Graphene oxide-poly-methyl methacrylate	Inhibits cariogenic bacteria
Graphene oxide-nanoribbon	Inhibits cariogenic biofilm
Reduced Graphene Oxide	
Reduced graphene oxide	Inhibits cariogenic bacteria
Reduced graphene oxide-silver nanoparticles	Inhibits cariogenic biofilm Promotes enamel and dentin mineralization
GRAPHENE OXIDE QUANTUM DOTS	
Graphene oxide quantum dots-bioactive glass	Promotes enamel and dentin mineralization

1.4. Applications

1.4.1. Dentures and composite restorations-reinforcement of poly methyl methacrylate

Polymethyl methacrylate (PMMA) was introduced by Dr. Walter Wright in 1936. It is the most common component of the acrylic resins used for denture bases in prosthetic dentistry which primarily involves replacement of missing teeth using artificial substitutes. Due to its biocompatibility, relative lack of toxicity, and excellent aesthetics, it has been used for several decades now. However, wear of PMMA dentures, their poor mechanical properties, volume shrinkage after polymerization, and poor antimicrobial (anti-adhesion) effects often lead to early cracks and fractures in clinical use, which have posed major drawbacks lately. Researchers have attempted to improve the material with

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many modifications [18].

Resin composites are commonly used materials for dental restorations because of their aesthetics. However, the strength and polymerization shrinkage of composites still remain a concern, and methods to minimize them are being actively studied by current researchers in dentistry. Papageorgiou et al. in their study found that the presence of graphene even at very low loadings can provide significant reinforcement to the final material. The addition of graphene structures to composites determines strong chemical interactions in the matrices and their homogenous dispersion generates uniform stress distribution, leading to an increase in mechanical strength of the composites [19].

Graphene loading varies from 0.5 to 20% in literature; however, the best results are observed with low filler contents. Tripathi et al. exhibited that the addition of GO beyond 1% resulted in mechanical strength deterioration attributed to the filler agglomeration effect and poor stress transfer characteristics [20]. Reinforcing resin polymer matrices with graphene gold nanoparticles as fillers showed improvement in the degree of conversion and surface properties, offering a good solution to improve the physicochemical properties of dental nanocomposite [21]. Shakeri et al. outlined a 32% improvement in flexural strength when PMMA denture bases are loaded with 0.05% Ag nanoparticles, without the graphene addition but Bacali C et al. reported an increase in the modulus of rupture by 174% by adding 2% Gr-Ag to the PMMA resin [14, 22]. By using PMMA mixed with carbon nanotube or graphene, polymerization shrinkage can be reduced. Carbon nanotubes have the intrinsic property of adhesion with the polymer which results in stress transfer from polymer to carbon nanotubes resulting in reduced dimensional changes. Graphene oxide may act as a polymerization inhibitor by premature chain termination. However, the association of PMMA with carbon-graphite fibre decreases water absorption at high fibre loading, due to the increase in the ratio of insoluble composites [23].

1.4.2. Graphene nano-reinforced biopolymer CAD/CAM (G CAM) disk [24-29]

G-CAM is a graphene nano reinforced biopolymer disc for fixed dental prosthesis using CAD/CAM drilling technology marketed by Graphenano Dental, Spain. They are highly aesthetic, lightweight, radio-opaque, and waterproof CAD/CAM disks designed to prevent plaque build-up and discoloration. These discs have surface hardness like that of the natural teeth and balance the weight of natural dentition [24-29]. Thus, can be used to create a dynamic and flexible prosthesis that withstands the masticatory forces.

G-CAM disc-like monolithic material does not require sintering or thermal processing hence, the working time is reduced. The manufacturing time of a metal-ceramic or zirconium-ceramic is between 25 and 30 hours, but with a G-CAM disc, the time is reduced to 2 hours [24-29]. Therefore, the daily work in the dental laboratory becomes more effective, easier, and quick. This novel, durable, versatile material is PMMA nano-reinforced with graphene, which is a comprehensive solution for restorative dentistry due to its allied mechanical, physical, chemical, and biological properties (Table 5).

The G-CAM colorimetry, based on the VITA classic shade guide, is not only limited to the intermediate colours but also allows for more shades through the make-up of photopolymerizable surfaces in the laboratory. They are available in the following dental shades: BL2, A1, A2, A3, A3.5, B2, and C2 in addition to Transpa and Pink (Fig. 3). The thickness of G-CAM discs can be 14, 16, 18, 20, 22, 24 and 26 mm.

These discs' different shades and thicknesses are indicated in the manufacturing of various dental prostheses as given below such as individual dental crowns, 3-unit dental bridges, dental bridges of more than 2 implants, dental veneers, direct rehabilitations and dental implants, and complete dental prosthesis (Fig. 4).

1.4.3. Dental luting agents- reinforcement of glass ionomer cement

Glass ionomer cement (GIC) represent a group of cements with extensive applications in dentistry and medicine i.e., luting of crowns and bridges, restoration of deciduous and permanent teeth, and minimally invasive restorative techniques. Conventional GICs (CGIC) possess poor mechanical properties such as wear resistance, that limits their usage in areas with low stress. Efforts have been made to improve the properties of GIC, by the inclusion of metallic particles,

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resin components, and microfibers, among others. Also, additives like chlorhexidine and zinc are used to improve the antimicrobial properties of GIC [30].

Table 5. Graphene and its derivatives for the management of periodontal disease

Graphene and Its Derivatives	Properties
GRAPHENE	
Graphene	Inhibits oral fungi biofilm
	Inhibits oral periodontal pathogenic bacteria
	Increases bone regeneration
	Increases bone regeneration
	Inhibits periodontal pathogenic bacteria
Graphene-hydroxyapatite	
Graphene-titanium	
GRAPHENE OXIDE	
Graphene Oxide	Inhibits periodontal pathogenic biofilms
	Increases bone regeneration
	Increases periodontal tissue regeneration
	Increases bone regeneration
	Inhibits periodontal pathogenic biofilms
Graphene Oxide-polyetheretherketone	Increases bone regeneration
Graphene Oxide-chitosan	Increases bone regeneration
	Increases bone regeneration
Graphene Oxide-hydroxyapatite	Increases bone regeneration
Graphene Oxide-silk fibroin	Inhibits oral bacteria
Graphene Oxide-titanium	Increases bone regeneration
Graphene Oxide-chitosan-hydroxyapatite	Inhibits oral bacteria
	Increases bone regeneration
Graphene Oxide-lysozyme-titanium	Increases bone regeneration
Graphene Oxide-minocycline hydrochloride-titanium	Increases bone regeneration
Graphene Oxide-dexamethasone-titanium	
Graphene Oxide-bone morphogenetic protein 2-titanium	
REDUCED GRAPHENE OXIDE	
Reduced Graphene Oxide	Increases bone regeneration
Reduced Graphene Oxide-chitosan	Increases bone regeneration
Reduced Graphene Oxide-hydroxyapatite	Increases bone regeneration
Reduced Graphene Oxide-titanium	Increases bone regeneration
Reduced Graphene Oxide-dexamethasone-titanium	Increases bone regeneration
GRAPHENE OXIDE-QUANTUM DOTS	
Graphene Oxide Quantum Dots	Living cell labelling
Graphene Oxide Quantum Dots-curcumin	Inhibits periodontal pathogenic bacteria

Fig. 3. Various available shades of the G-CAM disc

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Fig. 4. Various indications of G-CAM disc

Glass ionomer cement is a tooth-coloured material and a major drawback of using rGO in dental materials such as GIC is its dark colour. Sun et al. attempted the addition of fluorinated graphene (FG) (Fig. 5) to conventional GIC which resulted in a compound that is white with significantly improved mechanical, tribological, anti-bacterial properties, enhanced Vickers hardness number (VHN), Compressive strength, and improved oral solubility [31]. The existence of fluoride ion (F^-) leads to the production of fluorapatite which because of its stable structure resists acid dissolution better than hydroxyapatite. Pure GICs have the highest accumulated F^- releasing in the early stage (during the first month), which is because of their fastest dissolution rate. The addition of FG decreased the solubility of the GICs composites, so F-releasing was relatively lower, The GICs/FG (4 wt%) composite had similar performance with other FG-added groups at initial stage, but then the F− release increased rapidly due to the accelerating of dissolution. Soon the GICs/FG (4 wt%) composite has the most F^- releasing[31].
Sharafeddin et al in their study reported that the addition of GO to CGIC has no significant effect on its flexural strength. However, altered mixing time and powder-liquid ratio may affect the mechanical properties of the cement [32].
In addition, (F^-) can inhibit the bacterial plaque formation and growth of Streptococcus mutans and Staphylococcus aureus.

1.4.4. Ceramics- reinforcement of composite ceramics

Zirconia is a potential material for dental restorations owing to its aesthetics, high hardness, strength, excellent wear resistance, and biocompatibility. However, the brittleness is still an obstacle for ZrO_2-based ceramics. Hence, more effort is being directed to improve the mechanical properties of ZrO_2-based ceramics. Carbon nanotubes (CNTs) are widely used as one of the reinforcing materials of choice in polymers, metals, and ceramics. The CNT-reinforced ceramics have been found to exhibit excellent biocompatibility and mechanical properties.
Researchers have found that the addition of GO is beneficial for enhancing the density and mechanical properties of Yttria stabilized Tetragonal Zirconia Polycrystal (Y-TZP) ceramics, especially in fracture toughness.

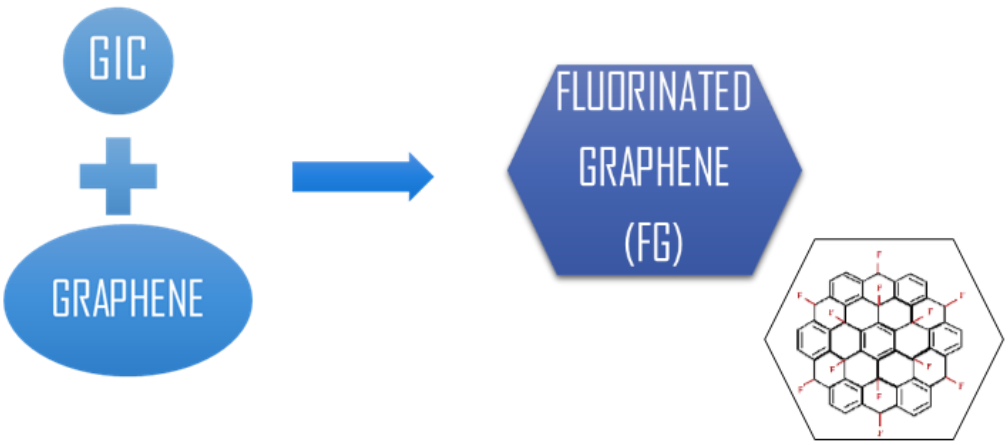

Fig. 5. Process of fabrication of Fluorinated Graphene

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Zeng et al. fabricated a rGO toughened ZrO_2 ceramic with good mechanical properties. They found that the fracture toughness improved up to 175% at 0.09 wt% rGO compared to the raw zirconia ceramic [33]. The excellent mechanical properties of graphene-containing zirconia are attributed to their ability to form a Zr–C–O bond. Owing to self-lubricating properties, the addition of GO reduces the friction coefficient, and wear rate and lowers surface roughness. The low contact angle of composites leads to better wetting properties, with GO sheets and an increase in bending strength and fracture toughness by up to 200%.

1.4.5. Dental implants– improved osseointegration with graphene coated implants

Titanium implants are currently considered the best replacement for natural teeth as they have favourable biocompatibility and are reliable and predictable. Owing to their inherent inertness in inducing fibrous tissue growth, there can be a failure at times, making them the best option but with additional improvements.

Lack of seal at the interface after fibro-osseous integration may lead to bacterial contamination and colonization, which may eventually impair osteogenesis and induce bone loss. Studies have proved graphene to be an excellent implant coating material. The transfer of graphene to the titanium surface can be carried out by wet and dry methods as given by Liang X. et al [34].

Due to their potential osteogenic and antibacterial ability, graphene results in better osseointegration in many ways. Graphene possesses an osteogenic property that enhances the expression of osteogenic genes like RUNX2, COL-I, and ALP, which boosts osteocalcin gene and protein expression and consequently increases the deposition of mineralized matrix [35]. When combined with the antibiotic minocycline hydrochloride, the antibacterial activity is improved as a result of the GO-Coating against facultative anaerobic or aerobic bacteria like *Porphyromonas gingivalis* and *Streptococcus mutans* due to the synergic effect of minocycline release-killing and GO contact-killing [36].

GO can be used as a carrier for BMP2, osteoinductive, and Substance P, an MSC recruitment agent, resulting in new bone formation on titanium implants [35]. Graphene oxide/chitosan/hydroxyapatite-titanium (GO/CS/HA-Ti) is produced through electrophoretic deposition method by incorporating GO and chitosan (CS) into a hydroxyapatite-titanium substrate, which presents with better bioactivity by improving the adhesion, proliferation, and differentiation of BMSC cells and superior osseointegration [37]. A suitable environment for the attachment, proliferation, and differentiation of Periodontal Ligament Stem cells (PDLSCs) is provided by the GO-Ti substrate [38, 39]. Graphene has many potential benefits in implant dentistry, however, the nanoparticles with which it is combined and the environmental factors such as an individual patient's oral cavity must be considered before any further measure is taken.

Ahmad Al-Noaman et al. prepared a novel composite coating of bio-active glass and GO on Polyether ether ketone (PEEK) implants and quantified the adhesive strength using a pull-off test. They found that the bio-active glass/GO coating had few microcracks and micro-porosities and adhered strongly to the implant [40].

1.4.6. Dental restorative adhesives

The use of Graphene in restorative dentistry as a reinforcing agent in various types of dental resins to strengthen bonding and adhesive strength has been in the practice of late [41].

In this regard, Orthodontic bonding resins have been formulated by mixing fluorinated graphite and bioactive glass to improve antibacterial properties and increase remineralization effects that aid in preventing white spot lesions on the enamel surfaces [42]. Silanized GO (SGO) nanoparticles have also been used in orthodontic bracket adhesives and dental adhesives, and researchers found that the addition of 0.25 wt% of SGO in commercial Transbond XT adhesive displayed excellent antimicrobial and mechanical properties.

No significant difference in cell toxicity of human gingival fibroblasts was found between the control Transbond XT adhesive and the SGO-modified adhesive, thus indicating that the addition of SGO nanoparticles did not affect the overall cytotoxicity of the material [43]. Functionalized graphene and hydroxyapatite fillers were used as reinforcing particles for light-cured adhesives for dental applications.

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Table 6. Various properties of G-CAM disk

Physical	Mechanical	Chemical	Biological
Wide chromatic range (aesthetic)	High Elastic modulus	Chemically inert	Non-Irritant
High glass transitional temperature	High deformation resistance	Insoluble in oral fluids	Non-Toxic
Improved dimensional stability	High-stress limit	Does not absorb water or saliva	Bacteriostatic
High electrical conductivity	High impact resistance		Antiallergic
Radiopaque	Increased Hardness		
Translucent	Long-lasting		
Waterproof and stable			

Silver-doped hydroxyapatite (HA-Ag), silver-doped graphene (Gr-Ag), and graphene and silver-doped hydroxyapatite (HA-Ag-Gr) nanofillers were investigated as main components in bis-GMA (2, 2- bis[4-(2-hydroxy-3-methacryloxypropoxy) matrices for resin-based dental adhesives [44].

Another study was conducted to improve polyetheretherketone (PEEK) nanofillers for bone restoration in orthopedic and orthodontic/dental applications. The researchers created novel surface-porous nanofillers made of PEEK combined with hydroxyapatite (HA) and graphene oxide via a heated injection mold process. It was found that the inclusion of the hydroxyapatite and graphene oxide significantly improved the overall cell adhesion and proliferation on the PEEK surface showing notable promise in improving tissue integration of PEEK nanofillers for orthopedic and dental/orthodontic applications [45].

2. CONCLUSIONS

Graphene and its nano-sized derivatives exhibit great versatility and peculiar properties. As they can be functionalized and combined with several biomaterials, these carbonaceous materials hold great potential to design bio-composites with fine-tuned physicochemical and mechanical properties.

The development of graphene as a biomedical material for use in restorative dentistry has become a highly interesting research field in the last few years. Meanwhile, this field of research is still in its infancy stage and requires proper future research directions to change it into a market-oriented research area. However, further research on bio-functionality is warranted to support the currently limited clinical evidence on Graphene's future scientific innovations.

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