IMPLICATION OF GRAPHENE MATERIAL IN DIVERSE BIOMEDICAL ARENA

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Abstract

Recent strides in nanotechnology have propelled significant advancements in characterizing, designing, synthesizing, delivering, and applying large-scale multifunctional materials measuring under 100 nm in one dimension. Nano-sized materials hold considerable promise in therapeutic applications owing to their precise dimensions, optical properties, expansive surface area, and customizable configurations, leading to innovative structures and formulations across various scientific disciplines. Clinical research has explored a spectrum of nano-materials, including quantum dots, carbon nanotubes, nano shells, pharmacogenetic nanoparticles, and others. Notably, graphene oxide (GO) has emerged as a standout candidate for biomedical purposes due to its optimized functional groups and expansive specific surface area, making it well-suited for therapeutic interventions. The field of graphene-based research achieved a significant milestone with the highlighting the diverse applications of GO, including drug delivery. Incorporating graphene into drug formulations has shown promise in enhancing therapeutic efficacy without necessitating dosage escalation, especially in cancer treatment. The unique attributes of GO, including its extensive surface area, excellent biocompatibility, and robust chemical properties, warrant further exploration across diverse biomedical applications. GO versatility extends to its adaptability for use in composite biomaterials and its suitability for specialized organ applications, such as in gastrointestinal tissues and spinal components. Extensive investigations into GO structure, production methods, drug loading capability, and potential as a gene delivery carrier have yielded promising results for future biomedical applications. However, challenges persist in comprehensively understanding GO behavior in living organisms, particularly in cancer therapy. Future research endeavors should focus on elucidating the molecular mechanisms underlying GO interactions with cells and its impact on cellular components. Additionally, addressing concerns related to GO long-term toxicity and effective clearance from body fluids is crucial for its successful clinical translation.

Keywords: Graphene oxide, Drug delivery, Gene delivery, Nanotechnology, Biomaterial, Cell interaction.

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1.0 Novel Biomaterials: Current gesture

In the early 21st century, there has been remarkable progress in nanotechnology, with a focus on characterizing, designing, synthesizing, delivering, and applying large-scale multifunctional materials measuring under 100 nm in one dimension. Nano-sized materials have shown promising advancements in therapeutic applications, capitalizing on their precise dimensions, optical properties, expansive surface area, and customizable configurations, which have spurred the development of inventive structures and formulations (1, 2). These materials' physicochemical attributes facilitate the creation of novel systems, plates, and devices with potential uses across diverse scientific disciplines. Recent clinical investigations have delved into various nano-materials, including quantum dots, carbon nanotubes, nano shells, pharmacogenetic nanoparticles, and others (3). Notably, GO has attracted considerable attention for biomedical purposes owing to its optimized functional groups and expansive specific surface area. GO stands out as a promising nanomaterial for therapeutic interventions, boasting remarkable chemical, mechanical, and physical characteristics. The field of graphene-based research achieved a significant milestone with the awarding of the Nobel Prize in Physics in 2010 (4).

2. GO as a biomaterial: An overview

In recent years, Research indicates that integrating graphene into drug formulations can improve therapeutic effectiveness without the need to increase the dosage of chemotherapy drugs in cancer treatment (5, 6). The unique traits of this material, such as its extensive surface area, excellent biocompatibility, and strong chemical properties, justify further exploration and instill considerable optimism. Due to its biocompatibility and mechanical resilience, GO shows promise for various applications in composite biomaterials, and its electrical conductivity makes it suitable for specialized organs such as gastrointestinal tissues and spinal components (5, 7, 8).

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2.1 Structural features of GO

GO is commonly produced by oxidizing and exfoliating graphite flakes. Its structure consists of layers of sp2 carbon atoms arranged in a honeycomb lattice, with sp3-conjugated oxygenated groups and electrically conductive carbon sites dispersed throughout. The interaction with aromatic compounds and π-π bonding enables the loading of hydrophobic drugs through non-covalent adsorption onto extensive hydrophobic regions (9-11). As a result, GO has been extensively explored for its potential in drug delivery. The presence of oxygenated groups and the large surface area of GO have spurred the development of various techniques for creating innovative drug delivery systems, such as polymer coatings, molecular conjugation, and chemical functionalization. GO's adaptable physicochemical, mechanical, and compositional properties render it suitable for a wide range of research applications (12-14).

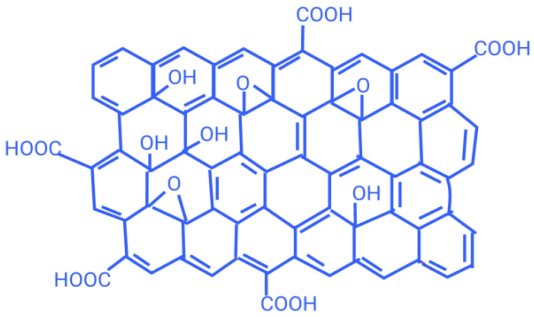

Fig. 1 Molecular Structure of GO. sp2 hybridization in GO with honeycomb structure.

2.2 Synthesis/exfoliation methods

In accordance with the Hummers method, GO was produced from graphite powder 17-20. The procedure commenced with the sonication of graphite powder in distilled water for a duration of 10 minutes. Subsequently, the resulting mixture underwent filtration using Whatman filter paper and was rinsed twice with distilled water prior to being air-dried at a temperature of 40°C for a

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period of 24 hours. The dried graphite powder was then combined with 98% sulfuric acid (H_2SO_4) in a beaker and stirred for 16 hours to achieve suspension. Potassium permanganate, acting as the oxidizing agent, was gradually introduced into the suspension under continuous stirring for 1 hour at 20°C. Following this step, the reaction mixture was maintained within a temperature range of 65-80°C for a duration of 60 minutes, after which deionized water was incorporated and the temperature was regulated between 98-105°C. The synthesis process concluded with the addition of distilled water and a 30% hydrogen peroxide solution. The resulting solutions underwent washing, successive centrifugation, and filtration employing a 5% aqueous solution of hydrochloric acid. Ultimately, the filtrated GO was dried and stored for subsequent utilization (14-17).

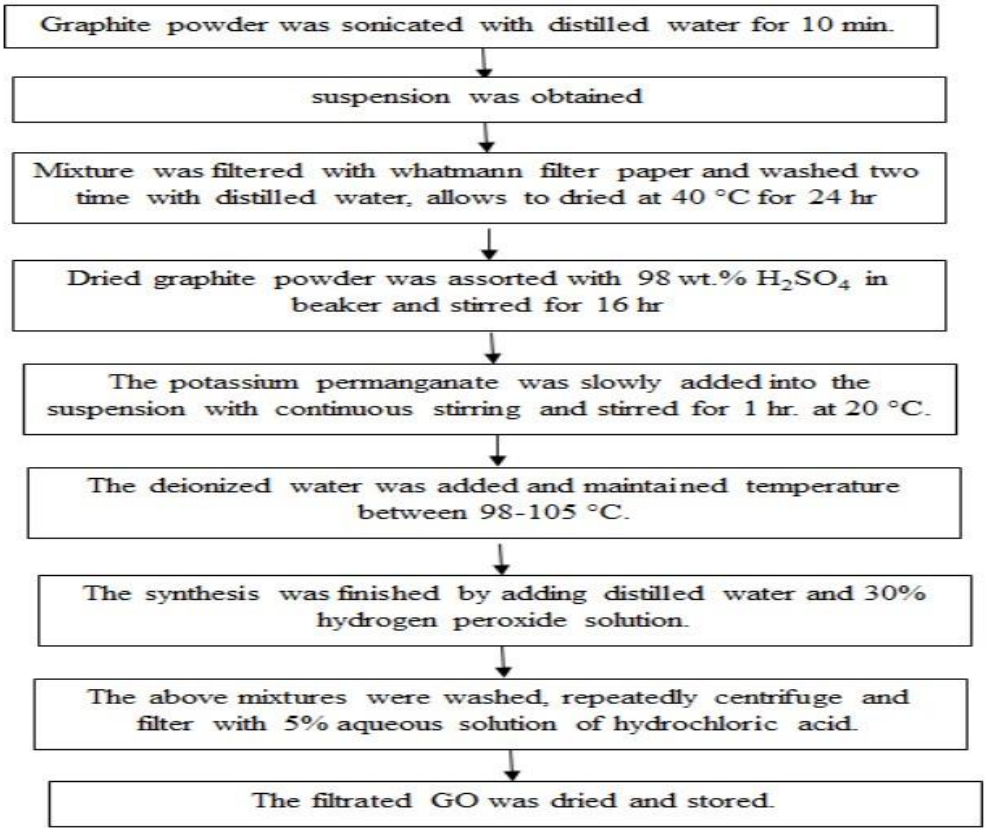

Fig. 2 Synthesis of GO by Hummers Method

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2.3 Properties

The therapeutic effectiveness of nano-scale GO is greatly influenced by its physicochemical properties, such as surface area, size, surface charge, and drug loading capability. Various studies have emphasized the use of nano-sized GO-drug complexes in different biological applications, including cellular imaging and targeted drug delivery. The unique structural features of nanoscale GO, characterized by a well-defined exterior and abundant oxygen-containing groups, contribute significantly to its capacity to efficiently load drug molecules, thereby improving physiological solubility and stability. Consequently, nano-scale GO demonstrates an impressive ability to absorb both hydrophilic drugs and aromatic compounds, making it a promising and versatile multifunctional drug delivery system with compelling potential (18-21).

2.4 Treatment in Cancer Therapy

As discussed earlier, the use of GO in drug delivery has shown promise in in vitro studies. However, further exploration of its behavior in living organisms, particularly in the context of cancer and other diseases, is necessary (22). Notably, Liu and his team (22) conducted ground breaking research on GO uptake by tumors and its photothermal effects using mouse models with xenograft tumors. Their findings demonstrated that PEG-modified GO could passively target tumors, facilitated by the enhanced permeability and retention (EPR) effect, resulting in the high uptake of the water-insoluble drug SN38 through non-covalent interactions. Subsequent low-power near-infrared (NIR) laser treatment efficiently destroyed tumors by leveraging GO strong absorbance in the NIR region. Similarly, Zhang et al. (23) explored the combined photothermal and chemotherapeutic effects of PEGylated GO in both lab and animal models. Their study revealed synergistic effects and improved anti-tumor activity when using DOX-loaded PEGylated GO. Additionally, Yang et al. (24) investigated the combined effects of

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chemotherapy and photothermal therapy using doxorubicin-loaded polyethylene glycol-modified GO in a mouse model of U87 xenografts, demonstrating enhanced local drug concentration and effective eradication of cancer cells. Furthermore, Huang and colleagues (25) developed a method for loading chlorine E6 onto GO through π-π and hydrophobic interactions, achieving an impressive 80% drug loading efficiency. Their research identified the optimal concentration of chlorine E6 in GO for photodynamic therapy, enhancing the accumulation of photosensitizers in cancer cells. The GO nano-materials used for excision of different anti tumor activity are shown in table 1.

Table 1: GO based formulation of drug against the tumor cell

S.No.	Drug loaded	Graphen derivaties	Cells
1.	DOX	rGO	MDA-MB_231 cells (26)
2	DOX & CPT	GO	MCF-7 Cells (27)
3	DOX	GO	Hela cells (28)
4	DOX	GO	HepG2 cells (29)
5	DOX	GQD	DU-145 cells, pc-3 cells (30)
6	DOX	GO	C6 glioma cells (31)
7	Monoclonal antibody	Go	MDA-MB-231 Cells (32)
8	RV	rGO	4T1 cells (33)
9	Methylene blue	GO	Hela cells (34)
10	DOX	rGO	U251 GLIOMA CELLS (35)
11	DOX	GO	Hela cells (36)
12	DOX	GQD	Hela cells (37)

2.4 Interaction with cell

This review delves into the molecular mechanisms governing the interactions between graphene-related nanomaterials and cells, offering a comprehensive understanding of how graphene engages with biomolecules, traverses the plasma membrane, translocates within the endosome/lysosome systems, and impacts crucial cellular components such as mitochondria, the nucleus, and the cytoskeleton (see Table 2). The process of internalizing (GO) into cells is

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influenced by the size of the particles. Previous studies have revealed that smaller GO particles are internalized through clathrin-dependent endocytosis, while larger particles are engulfed via phagocytosis as their size increases. The size of GO significantly influences its interaction with the plasma membrane, ultimately resulting in its sequestration within endosomes and lysosomes post-internalization. GO interaction with cellular organelles, including lysosomes and endosomes, elicits cellular responses and leads to the accumulation of autophagosomes. Mitochondria are identified as the primary source of reactive oxygen species (ROS) generation, impacting various cellular organelles (38). Research conducted by Jin and colleagues (39) has demonstrated that GO enters lung cancer cells (A549) and localizes within the nucleus and cytoplasm, a finding replicated in human dermal fibroblasts. GO is utilized as a drug carrier owing to its nucleus/cytoplasm shuttle effect.

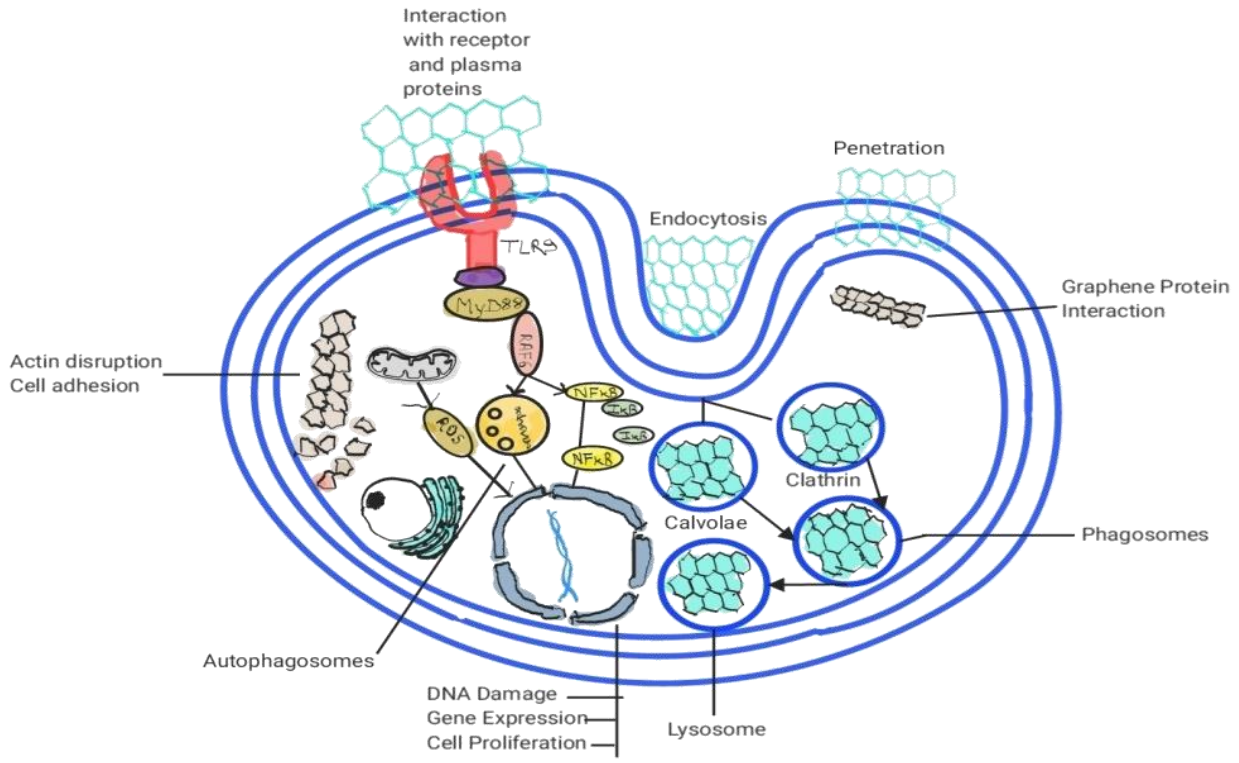

Fig. 3 Interaction of grapheme oxide with cells

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Table 2: Interaction of grapheme based materials with cells.

Compound	Cell Type	Interaction
GO	HepG2	NADPH oxidase dependent ROS formation; deregulation of antioxidant/DNA repair/apoptosis related genes (40).
GO	GLC-82	Alters the miRNA expression profile (41).
GO	HepG2	GO caused cytotoxicity in Hep G2 cells with plasma membrane damage and induction of oxidative stress (42).
GO functionalize with PEG	RAW-264.7; Saos-2; 3T3	Impact on cytoskeleton; alterations in cell cycle (43).
GO with Pluronic dispersed	Lung cells	Graphene-related nanomaterials increased the rate of mitochondrial respiration and the generation of reactive oxygen species, activating inflammatory and apoptotic pathways (44).
Graphene, GO	MDA-MB-231; B16F10; PC3	Graphene or GO inhibits the migration and invasion of various cancer cells by inhibiting the activities of ETC complexes (45).
GO and its nano assemblies	Mouse embryonic fibroblast (MEF)	Without induction of noticeable harmful effects (46).
GO	Red blood cells; Human skin fibroblasts	All the GO and GS show dose-dependent hemolytic activity on RBCs. Sonicated (smaller) GO exhibited higher hemolytic activity than untreated (larger) GO. Compared to individually dispersed GO sheets having higher surface oxygen content, the aggregated GS showed lower hemolytic activity (47).
GO	MEF	As the oxidation degree decreased, GO

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		derivatives led to a higher degree of cytotoxicity and apoptosis (48).
GO	Human fibroblast cell	GO could produce cytotoxicity in dose- and time-dependent means, and can enter into cytoplasm and nucleus, decreasing cell adhesion, inducing cell floating and apoptosis (49).
GO	Red blood cells	GO flakes have a very strong hemolytic activity increasing with the GO flakes size reduction. This activity was almost absent when the plasma protein corona was absorbed on the GO flakes surfaces (50).
GO	A549	The cytotoxicity of GO is largely attenuated when GO is incubated with FBS, which is due to the extremely high protein adsorption ability of GO (51).
GO	Peritoneal macrophage;J774 A.1; LLC;MCF-7; HepG2; Human umbilical vein endothelial cells (HUVEC)	The GO in micro-size induced much stronger inflammation responses while nano-sized graphene sheet showed better biocompatibility (52).
GO	RAW264.7	GO treatment provoked the toll-like receptor (TLR) signaling cascades and triggered ensuing cytokine responses (51).
GO	J774A.1; RAW 264.7	Interaction of GO with TLR4 results in activation of TLR4 signaling, which is the predominant molecular basis for GO-mediated macrophagic necrosis (53).
GO	Human monocyte	GO sheet size had a significant impact on

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	derived macrophages; Peritoneal macrophages	different cellular parameters (i.e. cellular viability, ROS generation, and cellular activation). The more the lateral dimensions of GO were reduced, the higher were the cellular internalization (54).
GO, PVP-GO	Dendritic cells	PVP-modified GO has a low immunogenicity than unadorned GO (55).
GO, sGO	PC-12	Inhibit Aβ peptide monomer fibrillation and clear mature amyloid fibrils (56).
GO Flake	Mesenchymal stem cells (MSC)	GO flakes effectively prevent a series of adverse cell-signaling cascades that result in the anoikis of MSCs in response to ROS (57).
GO with PEG	Saos-2; HepG2; RAW-264.7	Several processes are involved in FITC-PEG-GOs uptake, including micropinocytosis, microtubule-dependent mechanisms, clathrin-dependent mechanisms, and phagocytosis (58).
GO	C2C12	Small nanosheets enter cells mainly through clathrin-mediated endocytosis, and the increase of graphene size enhances phagocytotic uptake of the nanosheets (59).
GO	MDA-MB-231; MDA-MB-436; SK-BR-3	PEG-GO inhibited the migratory and invasive properties of human metastatic breast cancer cell lines by inhibiting ATP synthesis, leading to a disruption of F-actin cytoskeletal assembly (60).
NGO with PEG	HCT-116	No apparent toxicity as drug carrier (61).
NGO with PEG-Ce6 PEG-BPEI-Ce6	HeLa	No apparent toxicity as drug carrier (62).

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GO; rGO with blood proteins	A549	Protein-coated graphene resulted in a markedly less cytotoxicity than uncoated grapheme (63).
GO, rGO	HUVEC	GO is found to be more toxic than rGO of same size. GO and rGO induce significant increases in both intercellular ROS levels and mRNA levels of HO1 and TrxR. Moreover, a significant amount of DNA damage is observed in GO-treated cells, but not in rGO-treated cells. Oxidative stress-induced cytotoxicity reduces with a decreasing extent of oxygen functional group density on the rGO surface (64).
GO, rGO	A549; RAW 264.7	Cells treated with lower concentrations of GO/rGO did not lead to increases in ROS production. Cellular internalization of GO was observed in phagoendosomes without signs of any intracellular damage (65).
rGO/HArGO ICG-loaded	KB	No significant cell death observed in the absence of NIR irradiation (66).
rGO PNT-anchored	Ramos; CCRF-CEM	No apparent toxicity as drug carrier (67).
rGO	HepG2	hydrophobic rGO was found to mostly adsorbed at cell surface without internalization, ROS generation by physical interaction, poor gene regulation (40).
rGO biopolymer functionalized	Human blood cells; HUVEC	The biocompatible biopolymer functionalized rGO exhibited excellent biocompatibility (68).
rGO, GONP, rGONP,	MSC	The rGONPs exhibited a strong potential in destruction of the cells with the threshold

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		concentration of 1.0 mg/mL, while the cytotoxicity of the rGO sheets appeared at high concentration of 100 mg/mL after 1 h. The results indicated that interaction of graphene derivatives with stem cells strongly depends on the lateral size of the sheets (69).
GO, rGO	HUVEC	GO exhibits higher toxicity than rGO due to ROS generation. Small flake size graphene exhibit greater cytotoxicity compared to larger sheets due to intracellular accumulation of grapheme (64).
GO, rGO	Human platelets	GO can evoke strong aggregatory response in platelets comparable to that elicited by thrombin (70).
GO, rGO, G-NH_2 Amine Modified	Red blood cells	G-NH_2 is not endowed with thrombotoxic property (71).
GO, rGO	U87 U118	GO and rGO enter glioma cells and have different cytotoxicity. Both types of platelets reduced cell viability and proliferation with increasing doses, but rGO was more toxic than GO. Moreover, the level of apoptotic markers increased in rGO-treated tumors. rGO induces cell death mostly through apoptosis (72).
rGO with Arg, Pro	U87	Reduction in GBM tumor volume was observed. rGO + Arg shows anti-angiogenic and pro-apoptotic characteristics (73).

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3.0 Graphene: Diverse biomedical applications

3.1 Drug delivery carrier

In recent years, there has been significant progress in the development of drug delivery applications using GO nanomaterials (5). GO offers a promising platform for enhancing drug efficacy without the need for dosage escalation, owing to its distinctive properties. With carboxylic acid, epoxide, and hydroxyl groups on its surface, GO becomes hydrophilic and easily dispersible in aqueous media (74). These functional groups can be modified to attach bioactive molecules, potentially improving drug loading and delivery efficiency (75). The prolonged circulation half-life of GO, coupled with its excellent biocompatibility with target organs and red blood cells (RBCs), positions it favorably for biomedical applications (8). Notably, GO holds promise for targeted drug delivery to the lungs due to its high accumulation and prolonged retention time. Its reactive COOH and OH groups facilitate binding with various proteins, polymers, biotargeting ligands, biomolecules, DNA, quantum dots, and other entities (76).

The various authors worked on grapheme based drug delivery system and in order to Liu et al. (61) introduced innovative approaches for carbon nanotube-based drug delivery, presenting nanoscale GO (NGO) prepared through hydrophobic and π-π interactions involving the hydrophobic block of Nanographene oxide-7 ethyl 10 hydroxycamptothecin (NGO-SN38). NGO was subsequently conjugated with amine-ended six-armed polyethylene glycol (PEG) molecules to form PEG-NGO-SN38. In vitro cell cytotoxicity assays demonstrated that PEG-NGO-SN38 conjugation significantly enhanced cytotoxicity for HCT-116 cells, exhibiting potency 1000-fold greater than irinotecan (CPT-11). In a separate study by Wang et al. (77), doxorubicin (DOX) nanoparticles were prepared by loading DOX with galactosylated chitosan on a GO carrier (GC/GO/DOX) and chitosan/graphene oxide/doxorubicin (CS/GO/DOX) nanoparticles. In vivo

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anti-tumor experiments revealed that GC-GO-DOX nanoparticles exhibited superior tumor inhibition compared to CS-GO-DOX nanoparticles. Moreover, An et al. (78) developed gelatin-functionalized graphene nanosheets attached to methotrexate (MTX) to achieve pH-dependent release behavior. Cytotoxicity assessments on A459 cells indicated non-toxicity at specific concentrations for gelatin-graphene nanosheets, while MTX-gelatin-graphene nanosheets displayed biocompatibility. Similarly, Bai et al. (79) utilized polyvinyl alcohol (PVA) to fabricate a pH-sensitive GO composite hydrogel, which released approximately 84% of vitamin B12 (VB12) at pH 7.4. The GO/PVA hydrogel exhibited a pH-sensitive gel-sol transition, protecting acid-labile drugs from decomposition or causing stomach discomfort. In a recent study by Song et al. (80), a novel hyaluronic acid (HA)-GO-DOX (HA-GO-DOX) nanohybrid was developed, boasting excellent physiological stability, high drug loading capacity (42.9%), and entrapment efficiency (69.5%). The HA-GO-DOX nanohybrid exhibited superior cytotoxicity compared to free DOX and GO-DOX, with significantly lower DOX release (39.9%) at 24 hours compared to free DOX (87.7%) and GO-DOX (48.2%).These studies collectively demonstrate diverse strategies and promising outcomes in leveraging GO for advanced drug delivery applications.

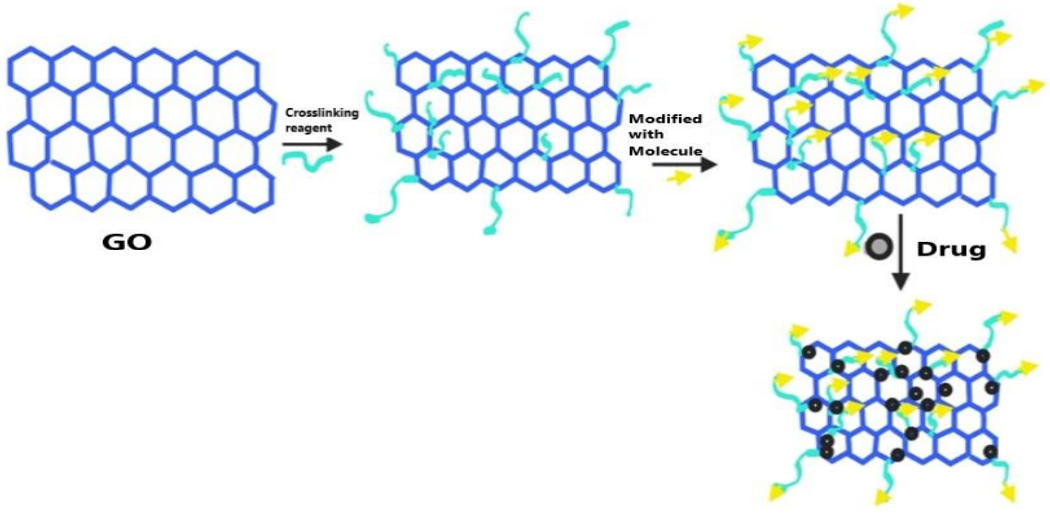

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Fig. 4 GO as a capable carrier with covalently attached drug molecules, and released by a native enzyme.

Table 3: Delivery of drug with graphene derivatives

S.No.	Drug loaded	Graphen derivaties	Characterization
1.	DOX	rGO	UV, AFM, TEM (29).
2	DOX & CPT	GO	UV, AFM, TEM, FTIR (30).
3	DOX	GO	FTIR, ZPD, TEM (31).
4	DOX	GO	UV, FTIR, AFM, NMR (32).
5	DOX	GQD	UV, LSD, FTIR, TEM (33).
6	DOX	GO	UV, LSD, AMF (34).
7	Monoclonal antibody	Go	AFM, TEM, DLS (35).
8	MTX	G	SEM, TEM, UV, AFM, XRD, Raman spectra,FTIR (81).
9	DOX	G	NMR, SEM, AFM, ATR- IR, TGA, UV (82).
10	VB12	GO	SEM, XRD (83).
11	Fluorescein sodium	rGO	UV, XRD, Raman spectra, XPS, AFM, TEM, SEM (84).
12	Methyl orange	rGO	SEM, UnC, ECC (85).
13	Dexamethasone	GO	EIS, AFM, SEM, FTIR,EA (86).
14	RS	rGO	AFM, UV, FTIR, Raman spectra (87).
15	Methylene blue	rGO	LSD, ZPD (88).
16	DOX	GO	TEM, AFM, FTIR, SQID, AASA, TGA (89).
17	DOX	GO	TEM, SEM, XRD, FTIR, XPS, Raman spectra, TGA, SQID, ZPS (90).
18	DOX	rGO	TEM, XRD (91).
19	DOX	GQD	UV, DLS, ZPD, XPS, Raman spectra, TEM (92).

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3.2 **Gene delivery carrier**

Developing a gene delivery system faces a significant challenge due to the lack of effective and secure gene vectors necessary for gene therapy. These vectors are crucial for facilitating the cellular regeneration of DNA with high transfection efficiency while preventing DNA nucleus degradation. GO has emerged as a promising gene delivery carrier, offering potential treatments for inherited diseases like Parkinson's disorder, cystic fibrosis, and cancer (93). In order to investigated by Feng et al. (94) explored the conjugation of polyethylenimine (PEI) with plasmid DNA condensation on GO sheet surface via electrostatic interaction. Their findings revealed that modifying PEI with GO enhanced the polymer's transfection potency while reducing its cytotoxicity. Conjugating PEI-GO/pGL-3 significantly boosted luciferase expression and enhanced DNA transfection efficiency in HeLa cells compared to PEI/pGL-3 conjugation. PEI is widely used in gene transfection due to its high transfection efficiency. Kim and colleagues (95) suggested that PEI-GO shows promise as an efficient gene delivery candidate. Bao et al. (96) developed camptothacin (CPT) loaded nanoparticles using chitosan (CS) modified GO (CS-GO) sheets. Their results indicated that CPT entrapped in CS modified GO efficiently killed cancer cells compared to pure CPT. CPT-CS-GO significantly enhanced DNA efficiency in HeLa cells at a fixed nitrogen/phosphate ratio by successfully condensing DNA plasmids into stable nanoparticles. The loading and delivery of combined drugs and genes through CS-GO nanocarriers hold immense potential for enhancing therapeutic efficacy. In another study, PEI-GO was utilized to deliver DOX and Bcl-2 targeted small interfering RNA (siRNA) to human cervical carcinoma (HeLa) cells. Initially, siRNA targeted GO-PEI loaded Bcl-2 was incubated with HeLa cells for 5 hours, followed by changing the medium and incubating for an additional 43 hours. Subsequently, DOX-PEI-GO complexes were treated with HeLa cells for 24 hours.

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The cytotoxicity results indicated that PEI-GO/Bcl-2 delivered siRNA complexes increased the cytotoxicity of PEI-GO/DOX compared to PEI-GO/scrambled siRNA complexes. These findings highlight the strong synergistic anticancer effects of PEI-GO/DOX and PEI-GO/Bcl-2 targeted siRNA (97).

Table 4: Targeted and non-targeted gene delivery of graphene based nanomaterials

Non-targeted	
Graphene nanomaterials	**Highlights of the study**
GO-BPEI/siRNA	LMW bPEI 1.8 kDa conjugated to the GO showed a stable polyelectrolyte complex with pDNA and highly positive surface charge with low toxicity and gene transfection efficiency increased with an increase in either the N/P ratio or conjugation ratio of bPEI to GO (95).
GO-LPEI/pDNA	Among all formulations, transfection efficiency reached up to 32.8 ± 3.2% for LPEI-GO-2/pDNA complex with ratio of lPEI:GO (2.5:1) (98).
GO-PEI/mRNA	GO-PEI/mRNA transfection efficiency was above 90%Whilelipofectamin/mRNA transfection efficiency was below 5% (99).
rGO-PEG-BPEI/pDNA	PEG–BPEI–rGO showed higher gene transfection efficiency without observable cytotoxicity compared to unmodified controls (100).
GO-PEI/DOX/siRNA	Strong fluorescence from GO-PEI/DOX/siRNA was observed in tested cells. However, when FITC–siRNA was incubated with HeLa cells, no intracellular fluorescence was seen (97).
GO-PAMAM/MMP9shRNA	It showed efficient gene transfer ability compared to PEI 25 kDa in the presence of serum, and it significantly inhibited the expression of MMP-9 protein in MCF-7 cells (101)
rGO-PL-PEGR8/siRNA	Internalization efficacy of FITC-siRNA was 82 ± 5.1% compared to HiPerFect® that is a commercial reagent for the transfection of

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	siRNA into mammalian cells (102).
Targeted	
Go-PEG-FA-pyNH2/siRNA	Both GO-PEG-FAPyNH2 and non-targeted one can deliver FAMlabeled DNA into HeLa cells effectively but FA-targeted complex can selectively target the surface of HeLa quickly. The folate targeted delivery of hTERT siRNA resulted in a more significant gene suppression compared to the non-specific delivery in both the hTERT mRNA and protein expression levels (103).
GO-LCO+/FAM-DNA	The confocal fluorescence microscopy images and flow cytometry data showed the fluorescence level of GO-LCO+ loaded with FAMDNA after incubation with positive cell lines were much higher than that of GO-CO+ loaded ones (104).
GO–PEI–PEG–FA/siStat3	The results indicated that 1:80 of GO:PEI had the highest transfection efficiency and expression of Stat3 was significantly reduced with GO–PEI–PEG–FA/si-Stat3 compared to other groups (105).

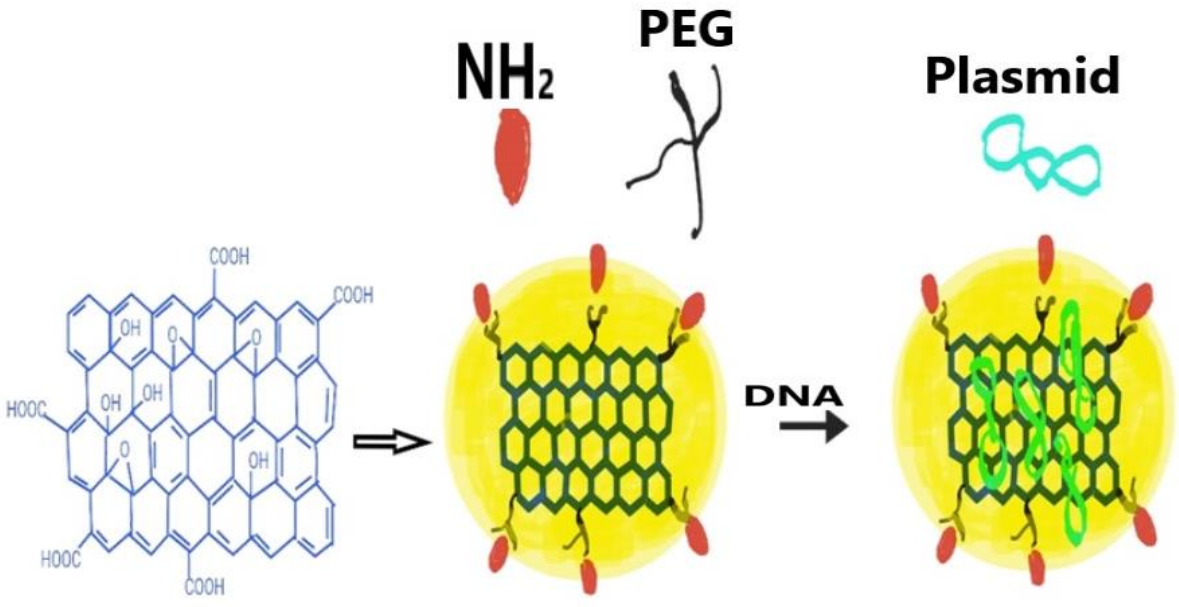

Fig. 5 Essential steps of gene delivery by GO

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3.3 Biosensing

GO has emerged as a versatile carrier for biosensors, with various biomolecules being attached to GO to create different biosensor platforms. These include chitosan-loaded GO nanocomposites, glucose oxidase-loaded GO, metal oxide-loaded GO, multi-nanomaterials, carbon nanotubes, quantum dots, DNA, and miRNA (106). In order to studied various authors, Kang et al. (107), developed a biosensor by coating graphene with glucose oxidase and chitosan, enhancing GO dispersion. The biosensor exhibited high sensitivity and enzyme loading capacity. Liu et al. (108), fabricated a biosensor using a GO-glucose composite through direct electrodeposition, resulting in increased current response and loading quantity over time. Qiu et al. (109) created a biosensor coated with CS-ferrocene-GO, showing a broad linear range, excellent sensitivity, reproducibility, and stability, facilitated by metal oxide for enzyme immobilization. Palanisamy et al. (110) developed a glassy carbon electrode loaded with zinc oxide microflowers on reduced GO, demonstrating good conductivity. In another study, Palanisamy et al. (111) designed a biosensor with glucose oxidase-loaded GO carbon nanotubes, showing enhanced direct electron transfer. A horseradish peroxidase (HRP)-based GO biosensor was developed for clinical diagnosis, catalyzing hydrogen peroxide oxidation for colorimetric detection. Liu et. al. (112) GO modified with N-aminobutyl-N-ethyl isoluminol (GO-ABI). Then solution of HRP can be mixed with GO-ABI to form GO-ABI-HRP. This hybrid suggested that excellent chemiluminescence (CL) properties for the detection of hydrogen peroxidase. In the work, Zhang et al. (113) enhanced electron transfer in a biosensor by coating a glassy carbon electrode with multi-wall carbon nanotubes and GO. To improve electrocatalytic response and stability, Zhang et al. (114) created a hybrid of nafion and GO, combined with HRP for enzyme immobilization. Wan et al. (115) developed a bi-protein electrode through layer-by-layer

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assembly, enabling effective detection of hydrogen peroxide. Palanisamy et al. (116) demonstrated high enzyme loading capacity and HRP concentration on a screen-printed carbon electrode mixed with GO-HRP solution. A laccase-based GO biosensor was fabricated by Zhou et al., (117) showing fast response time and stability. In nucleic acid-based GO biosensors, Hang et al. (118) designed a DNA biosensor with gold nanorods conjugated with GO sheets. Rayoo et al. (119) developed a biosensor for measuring microRNA expression levels in living cells, with peptide nucleic acid conjugated onto nano-GO surfaces.

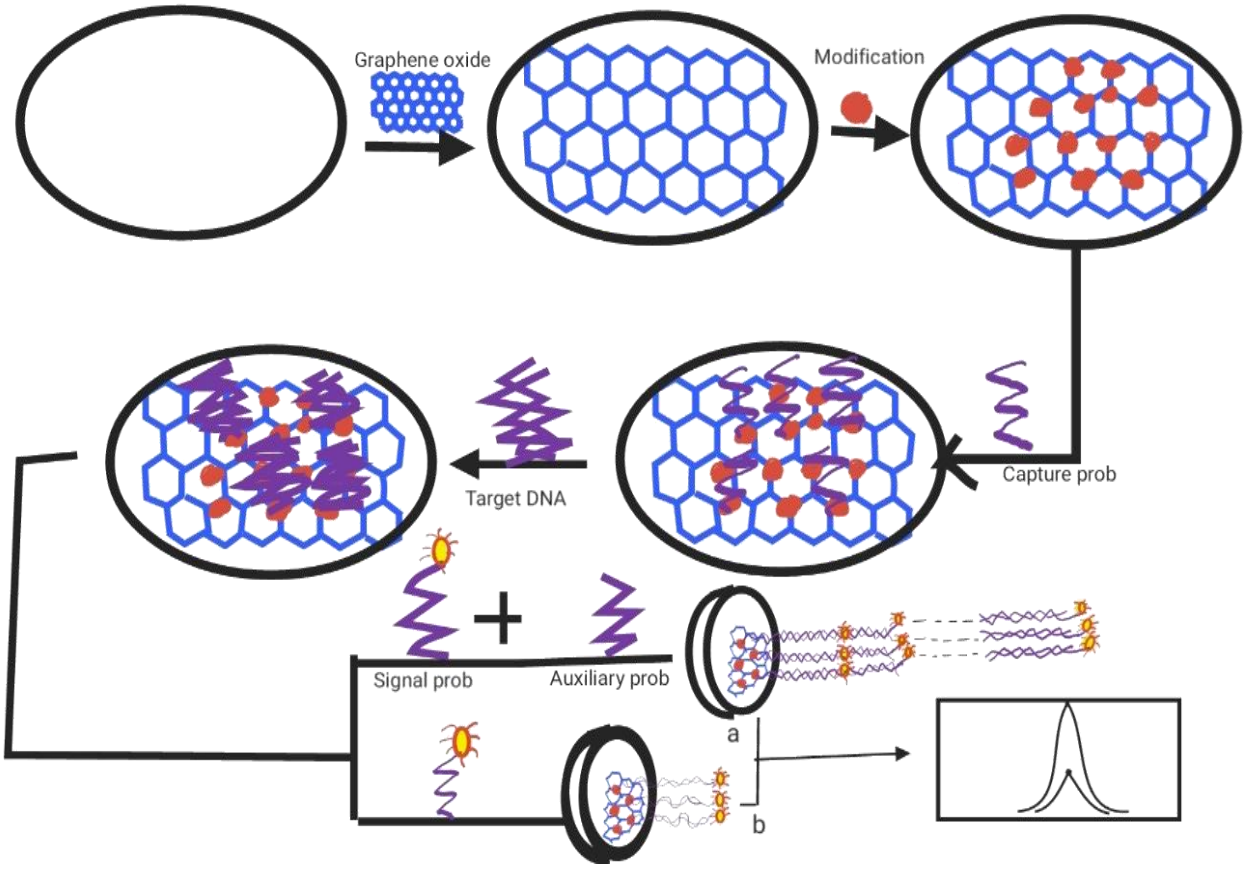

Fig. 6 The preparation scheme of GO based biosensor

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3.4 Bioimaging

Bioimaging encompasses techniques such as Raman spectroscopy, ultrasound, MRI, SERS, and X-ray technology to create images of biological cells. Recently, GO has gained attention in bioimaging, particularly with the introduction of the "ranostic" technique (120).

Authors focussed on the GO used in bioimaging, Min et al. (121) pioneered microplate sensing technology using GO nanosheets to study endonuclease activity. They attached colored dyes containing single and double-stranded DNA onto GO surfaces, enabling the examination of in vitro fluorescence plate images. By introducing a methylation group to the double-stranded DNA, they quantified endonuclease activity and inhibited it in the fluorescence resonance energy transfer region. Seo et al. (122) developed a luminescent GO array-based system to quantify rotavirus through antigen-antibody reactions, providing insights into antibody performance and virus antigenicity using in vitro biological imaging. In vitro cellular bioimaging employs GO as a cell-penetrating agent for drug delivery. Sun et al. (123) explored the cellular uptake of PEGylated GO loaded with compound drugs using GO luminescence in the near-infrared region. Researchers have developed reduced GO-gelatin complexes linked with coloring stains for drug delivery and bioimaging. Additionally, graphene quantum dots (GQDs), nano-sized GO, have shown potential in cellular imaging due to their internal luminescence (124). Pan et al. (125) synthesized GQDs with blue fluorescence from GO through hydrothermal laceration, enhancing fluorescence intensity through surface modification with allylamine and hydrazine vapor expulsion by Eda et al (126). Zhu et al. (127) demonstrated that GQDs are compatible with cells, exhibit low toxicity, and are soluble without surface modification. In vivo bioimaging harnesses GO's strong light absorption, particularly in the near-infrared (NIR) region. GO-PEG loaded with CySCOOH has demonstrated significant tumor volume reduction, while conjugation with

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sinoporphyrin sodium enhanced fluorescence intensity and facilitated repeated fluorescence imaging and photodynamic treatment in tumors (128). Moreover, the presence of oxygen groups on GO surfaces enables easy linkage with radioisotope chelators like 1,4,7-triazacyclononane-triacetic acid, facilitating quantitative and tomographic imaging for accurate biological and clinical information. Radionuclide imaging aids in understanding the localization and kinetics of GO in small animals, offering insights into its targeting ability for potential applications in humans (129).

3.5 GO-based antibacterial materials

In various environmental and clinical scenarios, this pioneering research has introduced novel applications of GO for antibacterial material. Fan et al. (130) innovated by crafting antibacterial paper using GO through a vacuum filtration technique. This GO-infused paper displayed remarkable effectiveness in killing germs. Additionally, Akhavan et al. (131) devised nanowalls comprising both GO and reduced graphene oxide (rGO) coated onto stainless steel surfaces to combat a spectrum of microorganisms, including both Gram-positive and Gram-negative strains. These nanowalls demonstrated notable resilience against Gram-negative Escherichia coli, primarily attributed to enhanced charge transfer between the rGO nanowalls and bacteria. Notably, the rGO nanowalls exhibited superior antibacterial efficacy upon contact compared to their GO counterparts. Furthermore, Liu et al. (132) pioneered the development of carbon nanotubes with proven antibacterial properties targeting oxidative stress and bacterial membranes. They proposed a comprehensive three-step mechanism to delineate the antibacterial mechanism of action exhibited by these carbon nanotubes.

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3.6 GO-based scaffold for cell culture.

In a separate investigation, researchers explored the suitability of GO films as scaffold materials in tissue engineering, employing a human mesenchymal stem cell model. These GO films were fabricated through a solution casting technique (134). The study revealed that instead of promoting cell proliferation, GO films facilitated targeted differentiation into muscle, cartilage, and bone tissues in a controlled manner, facilitated by the application of growth factors and osteogenic inducers. This suggests that GO films hold potential for applications in stem cell transplantation and proliferation (135). Additionally, a comparative analysis between GO substrates and silicon dioxide (SiO2) substrates prepared using chemical vapor deposition (CVD) was conducted. The results demonstrated that GO substrates exhibited excellent biocompatibility and promoted the growth and differentiation of human osteoblasts and mesenchymal stem cells, resulting in higher cell proliferation compared to SiO_2 substrates (136). Ryoo et al. (133), proposed utilizing GO as a surface coating material for implants due to its notable effectiveness in gene transfection. To assess the impact of a GO adhesion film on mammalian cell viability, they conducted experiments using fibroblast activities in NIH-3T3 cells as a model. Their results indicated that the presence of the GO layer did not significantly alter cell viability. Building upon these promising outcomes, Tang and Cheng et al. (137), developed a mouse hippocampal culture model utilizing CVD-deposited GO films as substrates for neurites. Their observations indicated enhanced neurite length on GO films compared to tissue culture polystyrene substrates one week after cell seeding. This early yet encouraging research suggests the potential utility of GO as scaffold materials for various cell culture applications.

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4.0 Toxicity aspect of GO: Big hurdle to biomedical implication

GO has attracted considerable attention in both life sciences and medicine. The toxicity of GO to cells can be influenced by factors such as its charge, quantity, structure, impurities, dimensions, and functionalization (138). Various mechanisms contribute to the toxicity of GO, including mitochondrial and DNA damage, swelling responses, necrosis, autophagy, ROS production, and apoptosis, all of which can induce oxidative stress, a critical step in carcinogenesis, aging, and mutagenesis. To leverage the unique properties of GO while minimizing its toxicity, researchers have explored different functionalization strategies (139). Early research focused on modifying the surface of GO using polymers like polyacrylic acid, PEG, dextran, poloxamer, gelatin, chitosan (CS), and their derivatives to mitigate its cytotoxic effects. PEG coating, for instance, proved effective in reducing tissue injuries caused by GO, preventing GO aggregation, and aiding its clearance from organs such as the lungs, liver, and spleen. Studies on PEGylated GO demonstrated high cell viability, with breast cancer cells maintaining over 95% viability at concentrations up to 100 μg/mL-1. Furthermore, PEG-loaded GO did not trigger pro-inflammatory cytokine secretion (140). Chitosan (CS) emerged as a promising polymer for counteracting the blood cell-destroying properties of GO by forming complexes between positively charged phosphatidylcholine lipids and negatively charged GO on the surface of red blood cells (RBCs). This interaction effectively reduced the binding of GO to RBCs, thus mitigating its blood cell-destroying effects (141). In vitro studies on GO–CS complexes showed no cytotoxicity in HepG2 and HeLa cells, while gelatin-functionalized GO exhibited no cytotoxicity in A549 cells at concentrations up to 300 mg/mL (142). Comparative studies on the cytotoxicity of dextran-loaded GO complexes and basic GO revealed no reduction in cell viability at concentrations up to 300 mg/mL for dextran-loaded GO, whereas basic GO exhibited

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high cytotoxicity at lower concentrations (143). Preliminary investigations highlight the importance of in vivo toxicological assessments using animal models to evaluate the clinical feasibility of GO-based medicinal products. Accumulation of unmodified GO in the lungs over time may lead to granuloma formation and pulmonary edema, while unmodified GO can also induce blood clot formation following intravenous administration. Therefore, modifying GO is crucial for reducing its toxicity in vivo (144).

5.0 Future scope

GO shows significant promise in delivering therapeutic agents, especially when combined with aptamers designed for specific targets. The interaction between medicinal agents and double-stranded oligonucleotides, facilitated by aptamer-specific GO, holds great potential for achieving impressive outcomes. These interactions involve non-covalent bonding and adsorption onto the GO surface, driven by hydrophobic interactions and π–π stacking. GO nanomaterials and their hybrids offer advantageous properties for surface modification, making them excellent candidates for developing innovative gene delivery vehicles in the future.

GO boasts high drug loading capability, enabling targeted therapeutic effects. In optimizing gene delivery with GO, complexing GO with polycations proves effective in overcoming limitations and creating highly efficient delivery systems. Enhancing GO's compatibility with polycations improves transfection efficiency, making the conjugation of GO with biocompatible polycations highly advisable. However, leveraging GO's unique properties necessitates careful consideration of its toxicity. Transforming GO with superior surface modifiers can generate more biocompatible surfaces. Early studies have explored the development of biodegradable and biocompatible GO-based release systems, with GO-modified gelatin systems showing particular promise due to their biocompatibility.

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Despite the promising applications of GO in biomedicine, particularly in cancer therapy through therapeutic cargo delivery, graphene-based nanomedicine has yet to advance to clinical trial phases. Successful integration of this technology into clinical practice relies on addressing issues related to long-term toxicity and effective clearance from body fluids, requiring further investigation.

6.0 Conclusion

The field of nanotechnology has witnessed remarkable progress in the early 21st century, leading to significant advancements in various scientific disciplines. Nano-sized materials have emerged as promising candidates for therapeutic applications, leveraging their precise dimensions, optical properties, and customizable configurations to develop innovative structures and formulations. Among these materials, GO has garnered considerable attention in biomedical research due to its unique attributes, including optimized functional groups and expansive specific surface area.

The versatility of GO in drug delivery has shown promise in enhancing therapeutic efficacy, particularly in cancer treatment, without the need for dosage escalation. Its adaptability for use in composite biomaterials and specialized organ applications further underscores its potential impact in biomedicine. Extensive research into GO structure, production methods, and drug loading capability has paved the way for future biomedical applications, including gene delivery, biosensing, bioimaging, and antibacterial materials.

However, challenges remain in fully understanding GO behavior in living organisms, especially in cancer therapy. Further exploration of the molecular mechanisms underlying GO interactions with cells and its impact on cellular components is essential for its successful clinical translation. Additionally, addressing concerns related to GO long-term toxicity and effective clearance from body fluids is crucial for advancing graphene-based nanomedicine to clinical trial phases.

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Despite these challenges, the promising applications of GO in biomedicine offer exciting opportunities to improve healthcare outcomes. Continued research efforts aimed at optimizing GO-based drug delivery systems and minimizing toxicity will contribute to the successful integration of graphene-based nanomedicine into clinical practice, ultimately benefiting patients worldwide.

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