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REVIEW

Applications and toxicity of graphene family nanomaterials and their composites

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Abstract: Graphene has attracted much attention of scientific community due to its enormous potential in different fields, including medical sciences, agriculture, food safety, cancer research, and tissue engineering. The potential for widespread human exposure raises safety concerns about graphene and its derivatives, referred to as graphene family nanomaterials (GFNs). Due to their unique chemical and physical properties, graphene and its derivatives have found important places in their respective application fields, yet they are being found to have cytotoxic and genotoxic effects too. Since the discovery of graphene, a number of researches are being conducted to find out the toxic potential of GFNs to different cell and animal models, finding their suitability for being used in new and varied innovative fields. This paper presents a systematic review of the research done on GFNs and gives an insight into the mode and action of these nanosized moieties. The paper also emphasizes on the recent and up-to-date developments in research on GFNs and their nanocomposites for their toxic effects.

Keywords: graphene, quantum dots, desalination, drug delivery, antibacterial, cytotoxicity, genotoxicity

Video abstract

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Introduction

Graphene has emerged as a sensational nanocarbon with unusual properties. Graphene is a two-dimensional planar and hexagonal array of carbon atoms. Each of these carbons is sp^2-hybridized and has four bonds, one σ bond with each of its three neighbors and one π-bond that is oriented out of plane. It is a gapless material with ballistic conduction at room temperature and high carrier mobility. The complete planar exposure of the carbon atoms renders graphene a theoretical surface area >2,500 m^2/g. The new properties of graphene and its hybrid structures have been widely explored for advanced technological applications in electronics, optics, and various other fields. Graphene has attracted attention among the scientific community since it was developed as a single layer of material by Novoselov et al[1] by using the scotch tape method. It consists of carbon atoms arranged in a honeycomb lattice and has a thickness of a single atom. It has the property of extremely high intrinsic mobility of charge carriers. It has a zero band gap and high chemical stability. Related materials include few-layer graphene, ultrathin graphite, graphene oxide (GO), reduced graphene oxide (rGO), and graphene nanosheets. Graphene materials vary in layer number, lateral dimension, surface chemistry, defect density, or quality of the individual graphene sheets, and composition or purity. In this way, GFNs are analogous to carbon nanotubes (CNTs), which vary in wall number, diameter, length, surface chemistry and the amount, composition, and

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physical form of metal impurities.[2] Graphene quantum dots (GQDs) constitute a zero-dimensional photoluminescence carbon-based nanomaterial consisting of very thin graphene sheets (3–20 nm). The band gap in GQDs is nonzero and can be set by altering the size and the surface chemistry of the dots.[3] Graphene and its derivatives, referred to as graphene family nanomaterials (GFNs) have been evaluated for their applications and toxicity.

Recent developments in applications of GFNs

In recent years, various novel nanomaterials have received much attention due to their great potential for applications in agriculture,[4–7] food safety, and food packaging.[8–16] Among them, GFNs are emerging as promising nanomaterials that will have a major role to play in different application fields of medical and physical sciences.

Electronics

GFNs have been extensively used in the field of electronics.[17–22] Graphene has been used for broadband and ultrafast photodetection and optical modulation. These optoelectronic capabilities can augment complementary metal oxide semiconductor (CMOS) devices for high-speed and low-power optical interconnects.[23] Recent studies have shown that GFNs have been used for their applications in organic electronics,[19] making ultra high-rate supercapacitors for wearable electronics,[20] metal–graphene contacts,[21] integrated circuits and multifunctional electronics,[22] CNT network for monolithic all-carbon electronics,[24] low-voltage organic electronics,[25] and flexible electronics.[17,22,26–31] Graphene also has attracted much interest in radio frequency electronics because of its superior electrical properties.[32] Graphene circuits exhibited outstanding thermal stability with little reduction in performance when integrated circuits operates as a broadband radio frequency mixer at frequencies up to 10 GHz.[33] Composite multiwalled CNTs-GO electrochemical capacitor electrodes with superior performance to solely GO electrodes were reported. The measured capacitance improved threefold and reached a maximum specific capacitance of 231 F/g.[34]

Desalination

Graphene, with its microporous structure, has become a center of attraction in the field of water filtration and desalination.[35–41] Graphene was found to effectively filter NaCl salt from water.[41] Nanoporous graphene (NPG) shows tremendous promise as an ultrapermeable membrane for water desalination, which is due to its atomic thickness and precise sieving properties.[37] Graphene can be modified by creating nanopores on the surface[35] and well-structured channels of pores facilitate the flow of water making the flow fast as compared with reverse osmosis (RO) membranes. By modifying the size of the pores, specific materials other than salt, based on their molecular size, can also be filtered out. There is less understanding as to whether NPG is strong enough to maintain its mechanical integrity under the high hydraulic pressures inherent to the RO desalination process.[36] An NPG membrane can maintain its mechanical integrity in RO, but the choice of substrate for graphene is critical to this performance. Nicolai et al[38] assessed GO framework membranes for water desalination using classical molecular dynamics simulations. For a given pore size (n=16 or 32), water permeability of GO framework membranes increases when the pore spacing decreases, whereas for a given pore spacing (n=32 or 64), water permeability increases by up to 2 orders of magnitude when the pore size increases. Carboxyl functional groups can enhance ion exclusion for all pores considered, but the effect becomes less pronounced as both the ion concentration and the pore diameter increase.[39] When compared with a CNT of similar pore diameter, graphene sheet pores functionalized with COO^- groups were found to be more effective in excluding Cl^- ions from passing through the membrane.

Tissue engineering

Graphene has been found useful in the field of bone tissue applications.[42–46] GO has a beneficial effect on cell proliferation and differentiation, thus holding promise for bone tissue engineering approaches.[47] A study proposed the combination of a three-dimensional (3D) graphene foam scaffold loaded with bone marrow-derived mesenchymal stem cells to improve skin wound healing.[48] The development of materials and strategies that can influence stem cell attachment, proliferation, and differentiation toward osteoblasts is of high interest to promote faster healing and reconstructions of large bone defects. Graphene finds its biomedical applications as they present remarkable properties such as high surface area, high mechanical strength, and ease of functionalization.[49] Graphene was tested as a biocompatible inert nanomaterial, for its effect on in vitro growth and differentiation of goat adult mesenchymal stem cells. Cell proliferation and differentiation were compared between polystyrene-coated tissue culture plates and graphene-coated plates. Graphitic materials were found to be cytocompatible, which supported cell adhesion and proliferation.[50] Soft graphene nanofibers designed for the acceleration of nerve growth and development are also reported.[51] New and recent developments are being done

by various researchers by taking GFNs in the field of tissue engineering, like Lopez-Dolado et al[52] investigated neural regeneration with subacute responses of the rat with injured spinal cord to three-dimensional graphene oxide (3DGO) scaffolds; Zhang et al[53] incorporated GO into poly(lactic acid) (PLA) as a reinforcing nanofiller to produce composite nanofibrous scaffolds using the electrospinning technique for potential tissue engineering applications; Liao et al[54] prepared a hybrid scaffold composed of methacrylated chondroitin sulfate, poly(ethylene glycol) methyl ether-ε-caprolactone-acryloyl chloride, and GO revealing that methacrylated chondroitin sulfate/poly(ethylenetglycol) methyl ether-ε-caprolactone-acryloyl chloride/GO hybrid porous scaffold can be applied in articular cartilage tissue engineering. Osteoblast proliferation and differentiation was found to be significantly higher in the poly(ε-caprolactone) (PCL) scaffolds containing the strontium-decorated rGO particles in contrast to neat PCL and PCL/rGO scaffolds.[55]

Cancer treatments

Graphene and its nanocomposites have gained much attention in recent times in cancer therapy as nanotheranostics. They have a low production cost, ease in synthesis, and different physicochemical properties including ultralarge surface area with planar structure and p-p conjugation with the unsaturated and aromatic biomolecules that are favorable for drug targeting.[56] Limited studies are available on the use of graphene family nanoparticles in cancer therapy, yet they point out a new possibility of using these key compounds in this field. Different recent studies have revealed the potential of graphene family nanoparticles in the detection of different cancer types.[57–62] Rhodamine-functionalized GQDs have been used for detection of Fe^{3+} in cancer stem cells.[63] A label-free, suspended single crystalline graphene sensor has been used for multiplex lung cancer tumor marker detection.[64] Peroxidase-active nanohybrid of gold nanoparticle-loaded mesoporous silica-coated graphene has also been used for cancer cell detection.[65] Yim et al[66] used GO-encoded silver nanoshells with single-particle detection sensitivity toward cancer cell imaging. The ultrasensitive nanoprobe successfully demonstrated its potential for bioimaging of cancer cells using Raman spectroscopy. Circulating tumor cells are a group of rare cancer cells that have detached from a primary tumor and circulate in the bloodstream. Circulating tumor cells were detected in prostate cancer based on carboxylated GO-modified light addressable potentiometric sensor.[59]

Cancer biomarker is a substance that is indicative of the presence of cancer in the body. A biomarker may be a molecule secreted by a tumor or a specific response of the body to the presence of cancer. Detection of cancer biomarkers has always been the field of concern for researchers. GFNs have been extensively exploited for the detection of various cancer biomarkers.[62,67–72] Ovarian cancer biomarker (CA-125) was detected using chemiluminescence resonance energy transfer to GQDs.[62] Similarly, electrochemical immunosensor with N-doped graphene-modified electrode was used for label-free detection of the breast cancer biomarker (CA 15-3).[71] A paper-based microfluidic electrochemical immuno-device integrated with nanobioprobes onto graphene film was used for ultrasensitive multiplexed detection of cancer biomarkers.[72] Highly sensitive luminol electrochemiluminescence immunosensor based on zinc oxide nanoparticles and glucose oxidase decorated graphene has been used for cancer biomarker detection.[73] Similarly, different other methods have been proposed for the detection of cancer biomarkers including magnetic graphene nanosheets-based electrochemiluminescence immunoassay using cadmium telluride QD-coated silica nanospheres as labels[74]; graphene-encapsulated nanoparticle-based biosensor[75]; cathodic electro-generated chemiluminescence immunosensor based on luminol and graphene[76]; and sensitive immunosensor based on dual signal amplification strategy of graphene sheets and multienzyme functionalized carbon nanospheres.[77] Graphene was found useful in delivering gambogic acid (GA) to breast and pancreatic cancer cells in vitro with no shown toxicity. Antiproliferative effects of GA were found to be significantly enhanced by its nanodelivery.[78]

Metal detection and removal

Graphene has been found to have high metal adsorption tendencies.[79–81] Nafion–graphene nanocomposite solution in combination with an in situ plated mercury film electrode was used as a highly sensitive electrochemical platform for the determination of Zn(II), Cd(II), Pb(II), and Cu(II) in 0.1 M acetate buffer (pH 4.6) by square-wave anodic stripping voltammetry.[82] Viraka Nellore et al[83] reported the development of PGLa antimicrobial peptide and glutathione-conjugated CNT-bridged 3DGO membrane, which can be used for removal of As(III), As(V), and Pb(II) from water. GO sheets were used in aqueous samples for a fast and efficient adsorption of Pb(II), Cd(II), Bi(III), and Sb(III) owing to its hydrophilic character and the electrostatic repulsion among the GO sheets.[84] The effectiveness of GO/carboxymethyl cellulose (GO/CMC) monoliths was tested for

their adsorbing capabilities. The porous GO/CMC monoliths were found to exhibit a strong ability to adsorb metal ions. As CMC is biodegradable and nontoxic, the porous GO/CMC monoliths were found to be potential environmental adsorbents.[79] An et al[85] described the fabrication and characterization of ionic liquid-gated field-effect transistor (FET)-type flexible graphene aptasensor with high sensitivity and selectivity for mercury in mussels. This aptasensor has potential for detecting Hg exposure in human and in the environment. Henriques et al[86] explored the preparation of 3DGO macroscopic structures, shaped by self-assembling single GO sheets with control of its surface chemistry by combining with nitrogen functional groups or with nitrogen and sulfur functional groups and their application in the removal of Hg(II) from aqueous solutions.

Drug delivery systems

Local delivery of drug molecules to target tissues provides a means for effective drug dosing, while reducing the adverse effects of systemic drug delivery.[87] Chowdhury et al[88] reported the use of poly(ethylene glycol)-distearoylphosphatidylethanolamine-coated oxidized graphene nanoribbons as agent for delivery of antitumor drug lucanthone into glioblastoma multiforme cells targeting base excision repair enzyme apurinic endonuclease-1. Weaver et al[87] explored an electrically controlled drug delivery nanocomposite composed of GO deposited inside a conducting polymer scaffold. The nanocomposite was loaded with dexamethasone and exhibited favorable electrical properties. In response to voltage stimulation, the nanocomposite releases drug with a linear release profile and a dosage that can be adjusted by altering the magnitude of stimulation. No toxic byproducts were found to leach from the film during electrical stimulation. Antiproliferative effects of GA on breast and pancreatic cancer cells were found to be significantly enhanced by its nanodelivery using graphene with no shown toxicity.[78] Many recent studies have also used graphene or its composites for delivering or monitoring the delivery systems of various compounds.[89–94] Angelopoulou et al[89] investigated the application of water-dispersible poly(lactide)–poly(ethylene glycol) (PLA-PEG) copolymers for the stabilization of GO aqueous dispersions and using the PLA-PEG-stabilized GO as a delivery system for the potent anticancer agent paclitaxel. PLA-PEG was found to stabilize GO for the controlled delivery of paclitaxel into A549 cancer cells. GFNs and their nanocomposites as starch functionalized graphene,[95] Pt(IV) conjugated nano-GO,[96] PEGylated GO,[97–100] GO stabilized in electrolyte solutions using hydroxyethyl cellulose,[101] DNA–graphene hybrid nanoaggregates,[102] and GO-wrapped mesoporous silica nanoparticles[103] were used for various drug delivery systems. Table 1 shows the systematic compilation of the studies involving graphene and its composites in drug delivery systems. Chen et al[90] demonstrated a GQD-based fluorescence resonance energy transfer system for nuclear-targeted drug delivery that allows a real-time monitoring of the drug release. A multifunctional nanocomposite of PLA-PEG-grafted GQDs was also proposed for simultaneous intracellular microRNAs imaging analysis and combined gene delivery for enhanced therapeutic efficiency.[91] A chemically tuned GO for its oxidation state was used to construct a GO-based nanoplatform combined with a pH-sensitive fluorescence tracer designed for both pH sensing and pH-responsive drug delivery.[92]

Nuclear waste treatment

There is a vast application potential of GO-based materials in nuclear waste processing. Wu et al[116] investigated the interaction mechanisms between actinide cations such as Np(V) and Pu(IV, VI) ions and four types of GOs modified by hydroxyl, carboxyl, and carbonyl groups at the edge and epoxy group on the surface. The binding energies in aqueous solution revealed that the adsorption abilities of all GOs for actinide ions follow the order of Pu(IV) > Pu(VI) > Np(V) and this finding is expected to provide useful information for developing more efficient GO-based materials for radioactive wastewater treatment. Polyacrylamide-grafted GO was applied as an adsorbent for the removal of radionuclides from radioactive wastewater. Maximum sorption capacities of U(VI), Eu(III), and Co(II) on polyacrylamide-grafted GO were found to be 0.698, 1.245, and 1.621 mmol/g, respectively at pH 5.0±0.1 and T=295 K, which were much higher than those of radionuclides on nascent GO.[117] Wu et al[118] also studied the bonding nature of uranyl ion and GO for effective removal of uranium from radioactive wastewater using GO-based materials. Romanchuk et al[119] studied the interaction of GO with actinides, including Am(III), Th(IV), Pu(IV), Np(V), U(VI), and typical fission products Sr(II), Eu(III), and Tc(VII). Cation/GO coagulation was expected to facilitate their removal.

Toxicity of graphene family nanoparticles

The dose, shape, surface chemistry, exposure route, and purity play important roles in differential toxicity of GFNs.[120] Different authors have used various toxicity tests to evaluate the toxicity of GFNs.[121–124] Studies have been conducted to

Table 1 Studies using GFNs or composites with respect to drug delivery systems

Sr no	Authors	GFN or composite used	Purpose of use
1.	Li et al[96]	Pt(IV) conjugated nano-GO	To enhance the therapeutic efficacy of Pt drug
2.	Wang et al[104]	GQDs	Simultaneous targeted cellular imaging and drug delivery
3.	Wang et al[105]	Chlorotoxin-conjugated GO	Targeted delivery of an anticancer drug doxorubicin
4.	Wang et al[106]	Reduced GO-supported gold nanostars	Improved surface-enhanced Raman scattering sensing and drug delivery
5.	Wang et al[107]	Cyclic RGD-modified chitosan/GO polymers	Drug delivery and cellular imaging
6.	Song et al[108]	Hyaluronic acid-decorated GO nanohybrids	Targeted and pH-responsive anticancer drug delivery
7.	Ou et al[109]	Fe_3O_4/SiO_2/graphene-CdTe QDs/chitosan nanocomposites	Targeted drug delivery
8.	Liu et al[110]	Polyamidoamine dendrimer and oleic acid-functionalized graphene	Biocompatible and efficient gene delivery vectors
9.	Kim and Kim[111]	rGO-polyethylenimine nanocomposite	Photothermally controlled gene delivery
10.	You et al[112]	Nano-GO	Cancer imaging and drug delivery
11.	Rahmanian et al[113]	Nano-GO	Oral delivery of flavonoids
12.	Liu et al[95]	Starch-functionalized graphene	pH-sensitive and starch-mediated drug delivery
13.	Yang et al[114]	GO/manganese ferrite nanohybrids	Magnetic resonance imaging, photothermal therapy, and drug delivery
14.	Wu et al[115]	Peptide–GO hybrid hydrogel	Drug delivery and pulsatile triggered release in vivo
15.	Tang et al[103]	GO-wrapped mesoporous silica nanoparticles	An aptamer-targeting photoresponsive drug delivery system
16.	Mo et al[102]	DNA–graphene hybrid nanoaggregates	Anticancer drug delivery doxorubicin
17.	Chen et al[100]	PEGylated GO	Photothermally controlled drug delivery
18.	Song et al[97]	PEGylated GO	Sequential delivery of lidocaine and thalidomide drugs
19.	Xu et al[99]	PEGylated GO	Delivery of paclitaxel
20.	Mianehrow et al[101]	GO stabilized in electrolyte solutions using hydroxyethyl cellulose	Drug delivery

Abbreviations: CdTe, cadmium telluride; GFNs, graphene family nanomaterials; GO, graphene oxide; QD, quantum dot; GQDs, graphene quantum dots; rGO, reduced graphene oxide; Sr no, serial number.

find out the toxicity of GFNs on different cellular and animal models, including stem cells,[121,125–127] HeLa cells,[128,129] HepG2 cells,[130,131] bacteria,[132,133] *Drosophila melanogaster*,[134,135] Zebrafish,[122,136] marine organisms,[137] rats,[138] mice,[123,128,139] and mammalian cells.[140] Cytotoxicity tests indicated that the rGO can damage cells with direct contact.[141] In this part of the paper, an attempt has been made to compile the recent and up-to-date studies related to toxicological aspects of GFNs to different models.

Toxicity toward bacteria

Graphene, its derivatives, and composites have been widely reported to possess antibacterial properties.[142] Different studies involved graphene in bacterial detection methods.[143–146] rGO has been used for the detection of bacteria.[147] Bioactivity of *Escherichia coli* and their interaction with the environment was controlled by their capture within aggregated graphene nanosheets. Aggregation of the sheets in the melatonin-bacterial suspension was found to trap the bacteria within the aggregated sheets. This trapping results in isolation of the bacteria from their environment, leading to bacterial inactivation.[148] Bacterial toxicity of graphene nanosheets in the form of graphene nanowalls deposited on stainless steel substrates was investigated for both Gram-positive and Gram-negative models of bacteria. By measuring the efflux of cytoplasmic materials of the bacteria, it was found that the cell membrane damage of the bacteria was due to direct contact of the bacteria with the extremely sharp edges of the nanowalls. It proved to be an effective mechanism in bacterial inactivation.[132] Gram-negative *E. coli* with an outer membrane were found to be more resistant to this cell membrane damage than the Gram-positive *Staphylococcus aureus*, which lack the outer membrane. Polyvinyl-*N*-carbazole-GO (PVK-GO) nanocomposite containing 3 wt% of GO well dispersed in a 97 wt% PVK matrix show excellent antibacterial properties without significant cytotoxicity to mammalian cells. Toxicity of PVK-GO was studied with planktonic microbial cells, biofilms, and NIH 3T3 fibroblast cells against *E. coli*, *Cupriavidus metallidurans*, *Bacillus subtilis*, and *Rhodococcus opacus*. PVK-GO in solution was found to encapsulate the bacterial cells resulting in their reduced metabolic activity and death.[149] Hydrazine reduction of the nanowalls was also found to be effective in increasing the magnitude of the cell membrane damage. Graphene oxide nanowalls (GONW) reduced by hydrazine were found to be more toxic to the bacteria than the unreduced

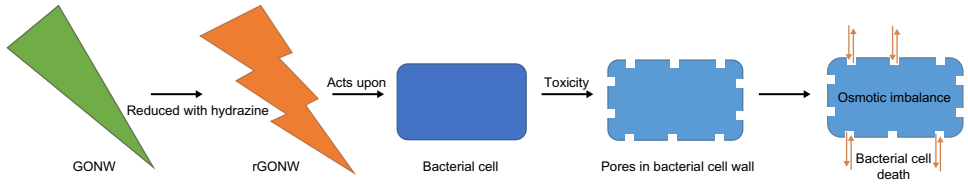

Figure 1 Possible mechanism of bacterial cell death with reduced GONW.
Abbreviations: GONW, graphene oxide nanowall; rGONW, reduced graphene oxide nanowall.

GONW. Figure 1 shows the possible mechanism for the death of the bacterial cell by GONWs reduced by hydrazine. Better antibacterial activity of the reduced nanowalls was found to be due to better charge transfer between the bacteria and more sharpened edges of the reduced nanowalls.[132]

The density of the edges of the graphene was one of the principal parameters that contributed to the antibacterial behavior of the graphene nanosheet films. Antibacterial mechanism involved the possible formation of pores in the bacterial cell wall, causing a subsequent osmotic imbalance leading to cell death.[142] Nguyen et al[150] investigated the antibacterial properties of GO against human intestinal bacteria and in vitro cytotoxicity using the Caco-2 cell line derived from a colon carcinoma, but found no toxicity of GO at different concentrations (10–500 µg/mL) against the selected bacteria. Only a mild cytotoxic action on Caco-2 cells after 24 hours of exposure was observed suggesting its biocompatibility. Nanda et al[151] determined the antibacterial property of cystamine-conjugated GO against four types of pathogenic bacteria. Minimum inhibitory concentration values were found to be 1 µg/mL against *E. coli* and *Salmonella typhimurium*, 6 µg/mL against *Enterococcus faecalis*, and 4 µg/mL against *B. subtilis*, suggesting the possible use of cystamine-conjugated GO nanohybrid in the treatment of dermatological disorders.

Peptide-conjugated GO membrane has been found to be efficient in the removal and effective killing of multiple drug-resistant bacteria.[152] Similarly, Viraka Nellore et al[83] reported the development of PGLa antimicrobial peptide and glutathione-conjugated CNT-bridged 3DGO membrane, which can be used for efficient disinfection of *E. coli* O157:H7 bacteria. Disinfection data indicated that the PGLa-attached membrane enhances the possibility of destroying pathogenic *E. coli* via synergistic mechanism. Studies revealing the interaction between bacterial cell membranes and the surface of graphene have proposed that the graphene-induced bacterial cell death is caused either by the insertion of blade-like graphene-based nanosheets or the destructive extraction of lipid molecules by the presence of the lipophilic graphene.[142] Magnetic Fe_3O_4–graphene composite (G-Fe_3O_4) were also found efficient in removing a wide range of bacteria, including *S. aureus*, *E. coli*, *Salmonella*, *E. faecium*, *E. faecalis*, and *Shigella*. The removal efficiency of *E. coli* for was found to reach 93.09% as compared with 54.97% for pure Fe_3O_4 nanoparticles.[153] The synergistic effects of GO and zinc oxide nanoparticles were found to give a superior antibacterial activity of the composites.[154]

Toxicity to aquatic environments

Systematic investigation of any potential toxic effects of GO in wastewater microbial communities is essential to determine the potential adverse effects and the fate of these nanomaterials in the environment.[155] GFNs including pristine graphene, rGO, and GO offer great application potential, leading to the possibility of their release into aquatic environments. Upon exposure, graphene/rGO and GO exhibit different adsorption properties toward environmental adsorbates.[80] Ahmed and Rodrigues[155] investigated the toxicity of GO on the microbial functions related to the biological wastewater treatment process and showed that toxic effects of GO on microbial communities were dose dependent, especially in concentrations between 50 and 300 mg/L.

Cytotoxicity and genotoxicity

Studies are being conducted on the toxicity of graphene on different cell types and genetic material. Various recent studies include cytotoxicity factor of GFNs.[140,156–158] In biological microenvironment, biomolecules bind onto nanoparticles forming corona and endow nanoparticles a new biological identity.[157] Duan et al[156] showed that the so-called "protein corona" formed in serum medium decreased the cellular uptake of GO, thus significantly mitigating its potential cytotoxicity. Molecular dynamic simulations also revealed that the adsorbed bovine serum albumin in effect weakened the interaction between the phospholipids and graphene surface due to reduction of the available surface area and an unfavorable steric effect, thus significantly reducing the graphene penetration and lipid bilayer damaging. Protein-coated GO were found to be markedly less cytotoxic than pristine and protein-coated single-walled carbon nanotubes (SWCNTs).[157]

GO nanosheets were suggested to intercalate efficiently into DNA molecules and GO sheets combining with copper ions were illustrated to cause scission of DNA.[159] The scission of DNA by the GO/Cu^{2+} system is critically dependent on the concentrations of GO and Cu^{2+} and their ratio. Similarly, Wu et al[158] studied the potential cytotoxicity of GO nanosheets on human breast cancer MDA-MB-231 cell line and suggested that higher concentrations of GO (≥100 μg/mL) exhibited time- and dose-dependent cytotoxicity against MDA-MB-231 cells. The exposure suppressed the colony-forming capacity and cellular proliferation. Even higher concentrations of GO increased the proportion of G0/G1 phase cells and resulted in higher generation of intracellular reactive oxygen species (ROS), which may be directly related to cytotoxicity. The paclitaxel-loaded composites were found to enter the A549 cancer cells and exert cytotoxicity.[89] PEGylation of GO has shown to reduce or change its cytotoxicity.[160–162] Lymphoma cells were treated with different concentrations (10–100 μg/mL) of PEGylated GO at different time points (6, 12, and 24 hours), but a low toxicity to lymphoma cells was found suggesting a fair chance for the application of PEGylated GO in medicines.[160] GO has been found to present an attenuation effect on X-ray-induced genotoxicity in cultured lymphocytes.[163] The effect of surface coatings on cytotoxicity of GFNs was studied.[161] Naked GO could induce a significant toxicity to macrophages, while the coated GO with biocompatible macromolecules such as PEG or bovine serum albumin could greatly attenuate their toxicity. Qu et al[164] found that QDs posed great damage to macrophages through intracellular accumulation of QDs coupled with ROS, particularly for QDs coated with PEG-NH_2. QDs modified with PEG-conjugated amine particles were found to exert robust inhibition on cell proliferation of J744A.1 macrophages. Graphene nanoplatelets (GNPs) synthesized using potassium permanganate-based oxidation and exfoliation followed by reduction with hydroiodic acid (rGNP-HI) were found to show excellent potential as biomodal contrast agents for magnetic resonance imaging and computed tomography. In vitro cytotoxicity analysis performed on NIH 3T3 mouse fibroblasts and A498 human kidney epithelial cells showed CD_{50} values of rGNP-HI to lie between 179 and 301 μg/mL.[165] The cell viability experiments revealed that the presence of the nanocomposites of GO with gold nanoparticles can significantly reduce the cytotoxicity of the amyloid peptides.[166] GOs were also found to induce apoptosis of erythroid cells through oxidative stress in E14.5 fetal liver erythroid cells.[164] Waiwijit et al[167] assessed cytotoxicities of MDA-MB-231 breast cancer cells (MDA cells) on carbon paste and graphene–carbon paste substrates. Cell viability on graphene–carbon paste substrate was found to initially increase as graphene content increased from 0 to 2.5 wt%, but then decreased as the content increased further. Similarly, Wu et al[168] evaluated the cytotoxicity on human multiple myeloma cells (RPMI-8226) treated with GO, doxorubicin (DOX), and GO loaded with DOX (GO/DOX) and revealed that cells treated with GO, DOX, and GO/DOX for 24 hours showed a decrease in proliferation. GO/DOX was found to significantly inhibit cell proliferation as compared with pure DOX ($P<0.01$). But purified GO as prepared and characterized in the study[169] did not induce significant cytotoxic responses in vitro, or inflammation and granuloma formation in vivo. GO and carboxyl GNPs were found to cause dose- and time-dependent cytotoxicity in HepG2 cells with plasma membrane damage. But no toxicity was found when applied at very low concentrations (<4 μg/mL).[170] Cell division of *Chlorella vulgaris* was found to be promoted at 24 hours and then inhibited at 96 hours after GO and carboxyl single-walled carbon nanotube (C-SWCNT) exposure. At 96 hours, both GO and C-SWCNT inhibited the rates of cell division by 0.08%–15% and 0.8%–28.3%, respectively.[171] Table 2 shows different cytotoxic studies on graphene and its nanocomposites.

GQDs with their unique morphology and exceptional properties, hold great promise for many applications, especially in the biomedical field.[178] Cytotoxicity of GQDs have been assessed by various researchers on erythroid cells and macrophages,[164] human gastric cancer MGC-803 and breast cancer MCF-7 cells,[178] and human A549 lung carcinoma cells and human neural glioma C6 cells.[179] Cytotoxicity of three kinds of GQDs with different modified groups (NH_2, COOH, and CO–N $(CH_3)_2$, respectively) in human A549 lung carcinoma cells and human neural glioma C6 cells was investigated using thiazoyl blue colorimetric (MTT) assay and trypan blue assay. GQDs were found to randomly disperse in the cytoplasm, but not get diffused into nucleus. The study suggested the three modified GQDs to have good biocompatibility even at the concentration of 200 μg/mL.[179] GQDs were demonstrated to get internalized primarily through caveolae-mediated endocytosis and have lower toxicities as compared with micrometer-sized GO on the cell viability, internal cellular ROS level, mitochondrial membranes potential, and cell cycles.[178]

Genetic toxicity in animal models represents a potential health hazard that may lead to different health-related ailments including cancer. Oxidative stress is also known to cause toxicity.[140] Different authors have shown that ROS formation

Table 2 Comparative analysis of cytotoxicity of GFNs

Sr no	Authors	Study material	Results
1.	Ali-Boucetta et al[169]	Purified GO dispersions	Purified GO did not induce significant cytotoxic responses
2.	Cai et al[172]	Sodium 1-naphthalenesulfonate-functionalized rGO	Low cytotoxicity and long-term antibacterial activity
3.	Du et al[160]	PEGylated GO on lymphoma cells	PEG-GO had excellent dispersion and low toxicity on lymphoma cells
4.	Lammel et al[170]	GO and carboxyl graphene nanoplatelets in the human hepatocellular carcinoma cell line HepG2	Dose- and time-dependent cytotoxicity in HepG2 cells; plasma membrane damage and induction of oxidative stress. No toxicity at low concentrations (<4 μg/mL)
5.	Li et al[166]	GO/gold nanocomposites	Nanocomposites can significantly reduce the cytotoxicity of the amyloid peptides
6.	Liao et al[173]	GO and graphene in human erythrocytes and skin fibroblasts	Graphene sheets are more damaging to mammalian fibroblasts than GO. GO showed higher hemolytic activity than graphene sheets. Coating GO with chitosan nearly eliminated hemolytic activity
7.	Qu et al[164]	QDs and GO to erythroid cells and macrophages	QDs coupled with ROS are highly damaging to macrophages, particularly QDs coated with PEG-NH_2. GO could provoke apoptosis of erythroid cells
8.	Wang et al[174]	GO on human lung fibroblast cells	Surface charge on GO plays an important role in its toxicity to HLF cells
9.	Wu et al[158]	GO on human MDA-MB-231 cells	Higher GO concentrations increased G_0/G_1 phase cell proportion; induced LDH release and intracellular ROS production
10.	Wu et al[168]	GO and GO loaded with doxorubicin	GO caused low cytotoxicity and did not induce cell apoptosis or change the cell cycle in multiple myeloma cells
11.	Yuan et al[175]	Oxidized SWCNTs and GO on human hepatoma HepG2 cells	Oxidized SWCNTs induced oxidative stress; interfered with intracellular metabolic routes, protein synthesis and cytoskeletal systems; perturbed the cell cycle and significant increase in the proportion of apoptotic cells
12.	Zhang et al[176]	Uniform ultrasmall GO nanosheets	Excellent biocompatibility; lower cytotoxicity, and higher cellular uptake
13.	Zhang et al[177]	GO	GO reduced Vpr13-33-induced cytotoxicity to neuroblastoma cells and T-cells

Abbreviations: GFNs, graphene family nanomaterials; GO, graphene oxide; HLF, human lung fibroblast; LDH, lactate dehydrogenase; QD, quantum dot; rGO, reduced graphene oxide; ROS, reactive oxygen species; SWCNTs, single-walled carbon nanotubes; Sr no, serial number.

may be the indirect way of GFN cytotoxicity on different models.[141,164,167,170,173–175,178,180] The authors have reported genotoxicity in lymphocyte cells,[181] human stem cells,[125] and human lung fibroblast cells.[174] De et al[182] studied genotoxicity of GO by varying its concentration and flake sizes. A 24-hour cytotoxicity test showed loss in the viability for A549. Comet assay showed a marked genotoxicity, which was found to be positively correlated with the concentration in case of micrometer-sized GO flakes while for nanometer-sized GO flakes, a high degree of genotoxicity was found at the lowest concentration tested. Genotoxicity of GO to human lung fibroblast cells was also reported to be concentration dependent.[174] Genotoxicity was predicted to be a result of GO-mediated oxidative stress. PVK-GO nanocomposites were found to have no significant cytotoxicity to mammalian cells yet have good antibacterial properties.[149] Different factors, especially the oxidation degree of GOs, affect their toxicity. Zhang et al[140] evaluated the cytotoxicity of three GO samples with varied oxidation degrees on mouse embryo fibroblasts and found that as the oxidation degree decreased, GO derivatives led to a higher degree of cytotoxicity and apoptosis. On the contrary, Na et al[183] evaluated the cytoprotective effect of GO and showed that GO can protect cells from internalization of toxic hydrophobic molecules, nanoparticles, and nucleic acids such as small interfering RNA and plasmid DNA by interacting with cell surface lipid bilayers.

Oxidative stress

Oxidative stress can damage proteins, DNA, and lipids, and is involved in the progression of many diseases. Damage to infected cells caused by oxidative stress is related to increased levels of ROS. During oxidative stress, hydrogen peroxide levels are often increased and catalase levels are decreased inside cells.[151] Induction of oxidative stress is a known mode of cellular toxicity. Higher oxidative stress levels may induce cytotoxicity as well as genotoxicity and be involved in formation of incipient tumor and carcinomatous cells.[184] Many recent studies have revealed the potential of

various nanoparticles to induce oxidative stress[185–192] and some studies also investigated the alleviating effect of different nanoparticles on oxidative stress induced by ethanol and D-galactosamine and lipopolysaccharide.[193,194] Similarly, many researchers have also conducted studies on the oxidative stress aspect of GFNs.[140,164,174,175,180] GO and carboxyl graphene accumulation in the cytosol was found to increase intracellular ROS levels in HepG2 cells.[170] GO treatment has also been found to reduce the production of X-ray-induced ROS among fibroblasts.[163]

Liu et al[195] proposed a three-step antimicrobial mechanism for graphene-based materials, including initial cell deposition on graphene-based materials, membrane stress caused by direct contact with sharp nanosheets, and the ensuing superoxide anion-independent oxidation. In a recent study,[140] GOs with three different oxidation degrees stimulated a dramatic increase in the production of ROS in mouse embryo fibroblasts. The less oxidized GO produced a higher level of ROS, suggesting the major role of oxidative stress in the oxidation-dependent toxicity of GOs. Electron spin resonance spectrometry showed a strong association of the lower oxidation degree of GOs with their stronger indirect oxidative damage through H_2O_2 decomposition into OH and higher direct oxidative abilities on cells. Similarly, C-SWCNT-exposed cells exhibited higher ROS levels than GO-exposed cells. The metabolism of alkanes, lysine, octadecadienoic acid, and valine was found to be associated with ROS production and were regarded as new biomarkers of ROS.[171] Nanda et al[151] produced a cystamine-conjugated GO, which resulted in low cytotoxicity but a strong ROS effect. The electronic charge on the surface of GO was suggested to play a very important role in oxidative stress-mediated toxicity of GO to human lung fibroblast cells.[174] Similarly, exposure of *Pseudomonas aeruginosa* to GO and rGO was found to induce a significant production of superoxide radical anion.[196]

Conclusion

Graphene is emerging as a dynamic nanocarbon. Despite a wide scope and numerous advantages of graphene and its nanocomposites in different fields of scientific world, it also poses toxic effects on different biological models as described in the paper. After reviewing the literature in relation to the possible toxic effects posed by the graphene and its composite forms, it can be summarized that using graphene as a modular transport material among biological systems including humans, it is highly suggestive of checking their toxicity to varied cellular types. Even mild toxicity factors should not be ignored. Research in the field of applications and toxicity of graphene is going on, but the author is of the view that further research in coming times will surely open up new gateways for use of graphene-based nanomaterials in newer fields of biological, physical, and chemical sciences.

Disclosure

The author reports no conflicts of interest in this work.

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