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Review Article

Multifunctionalization of graphene and graphene oxide for controlled release and targeted delivery of anticancer drugs

Cui-Cui Liu[1], Jing-Jing Zhao[1], Rui Zhang[1], Hui Li[2], Bo Chen[1], Ling-Ling Zhang[1], Hao Yang[1]

[1]*Translational Medicine Center, Hong-Hui Hospital, Xi'an Jiaotong University College of Medicine, Xi'an 710054, China;* [2]*Joint surgery, Hong-Hui Hospital, Xi'an Jiaotong University College of Medicine, Xi'an 710054, China*

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Abstract: Among various nanomaterials, graphene and its derivatives have attracted considerable research interest in diverse application areas-including nanomedicine-because of their extraordinary physical, chemical, and optical properties. Intensive research is underway to investigate the biomedical application of graphene and graphene-based nanosystems as drug-delivery vehicles for cancer therapy, and this is considered as one of the novel therapeutic approaches for performing on-demand chemotherapy coupled with photothermal therapy or photodynamic therapy. Here, we systematically summarize recent progress in the synthesis and functionalization of graphene by using a vast range of materials, including small molecules, polymers, and biomolecules, in order to overcome the inherent drawbacks of graphene oxide (GO) nanocarriers and thereby make these nanocarriers suitable for delivering chemotherapeutic agents, genes, and short interfering RNAs. Moreover, we address the opportunities and challenges associated with future clinical application of GO for cancer therapy.

Keywords: Graphene, graphene oxide, functionalization, drug-delivery system, cancer therapy, nanomedicine

Introduction

Cancer is a leading cause of death that severely threatens human health worldwide, and cancer also constitutes a substantial burden for patients' family members, society, and medical institutions. Furthermore, cancer-related morbidity has been progressively rising yearly due to increases in the aging population, obesity/overweight, smoking, environmental pollution, and other risk factors [1]. One of the main clinical therapeutic modalities for cancer treatment is chemotherapy. Although major advances have been made and considerable success has been achieved in cancer treatment by using current therapeutic agents, several obstacles and challenges remain, including multidrug resistance, inaccurate drug delivery to normal tissues rather than tumor sites, and poor physiological solubility and rapid metabolism and clearance of the drugs, and these drawbacks could hinder further improvement of the curative effect of the therapeutic agents [2]. To address these concerns, numerous attempts have been made to develop novel anticancer drugs and/or optimize the selective delivery of existing anticancer agents.

Drug-delivery systems (DDS) have attracted considerable attention in cancer-therapy research because of their potential ability to not only deliver pharmacologically active agents at a controlled rate, site, and release time *in vivo*, but also increase the solubility and alter the pharmacokinetics of the agents, which helps avoid the aforementioned problems associated with drug delivery and thus enhance the therapeutic efficacy of antineoplastic agents [3, 4]. Furthermore, DDS can target tumors by either "passive" accumulation based on the endothelial permeability and retention effect [5] or "active" delivery through selective molecular recognition. Over the past few years, a wide range of DDS, including gold nanoparticles (AuNPs) [6], magnetic nanoparticles [7], polymer-based materials [8, 9], carbon nanotubes [10], and other delivery systems [11], have been investigated to develop a controlled and target-

ed delivery platform for therapeutic agents, with the aim being to increase the therapeutic efficacy of these agents and concomitantly reduce their toxicity and improve their pharmacokinetics. Accordingly, entrapping antitumor agents inside DDS represents one of the optimal strategies for developing therapies that effectively target cancers.

Among the most extensively studied delivery systems currently under investigation, graphene and its functionalized derivatives hold considerable potential for drug delivery by virtue of their unique physicochemical properties-such as high biocompatibility, cargo-loading capacity, and ability for chemical modification, and ultrahigh surface area-as well as low cost [12-14]. Here, we present an overview of various functionalized nanoscale graphene oxide (NGO) nanocarriers that are being used for the delivery of diverse drugs, including chemotherapeutic agents, genes, and short interfering RNAs (siRNAs). Furthermore, we review the specific methods that are used in the synthesis of these nanomaterials in order to render them biocompatible and stable, feature a high drug-loading capacity, and exhibit enhanced anticancer efficacy. We also highlight the effectiveness and the major challenges of using distinct graphene-based materials as drug carriers for cancer treatment, which could facilitate next-generation preclinical and clinical research.

Principal physicochemical properties of graphene

The discovery of graphene by Novoselov et al. in 2004 initiated a new era in the research on nanometer-sized materials [15]. Until 2008, graphene was used as a drug-delivery agent only for hydrophobic aromatic molecules [16, 17], but since then, research groups worldwide have investigated diverse graphene-based delivery systems and obtained results suggesting promising new possibilities with regard to the use of these systems for drug delivery [18]. Graphene is a carbon allotrope featuring an extraordinary structure: a one-atom-thick planar sheet of Sp^2-bonded carbon atoms. Because of the one-atom-thick structure, graphene possesses an extremely high surface area (~2600 m^2/g) [19], and because of the delocalized π electrons on its surface, graphene can interact with ultrahigh amounts of hydrophobic aromatic drugs that hold the potential to target cancer through π-π stacking. Graphene oxide (GO) is a water-soluble derivative of graphite oxidized with a strong acid and oxidants (Hummer's method), and GO contains large quantities of oxygen-containing functional groups on its surface and exhibits the property of near-infrared (NIR) photoluminescence, which makes GO suitable for use in photothermal therapy (PTT). This oxidized form of graphene serves effectively as a key precursor for further modification, which enables the use of the functionalized derivatives in pharmaceutical applications [20]. More importantly, accruing evidence indicates that the use of surface-functionalized NGO leads to substantial tumor-tissue distribution and retention of therapeutic drugs without any notable toxic effects *in vitro* and *in vivo*, which provides a foundation for further application of NGO in tumor therapy [21, 22]. Lastly, the strong NIR photoluminescence and high photothermal-conversion efficiency of GO should allow GO to be used as a photothermal agent for synergistic photothermal-chemotherapy.

GO has been reported to not only function as an effective drug carrier, but also exert inhibitory effects on tumor cells when used by itself [23]. For example, Gurunathan et al. found that resveratrol-functionalized GO induced membrane leakage and oxidative stress in ovarian cancer cells and reduced the viability of the cells by inducing apoptosis [24]. Chen et al. reported that GO elicits toll-like receptor responses and triggers autophagy in mouse CT26 cancer cells and inhibits tumor growth in a colon xenograft animal model, which suggests that GO could be used as a chemo-sensitizer for cancer treatment [25]. Furthermore, Zhou et al. found that polyethylene glycol (PEG)-modified GO not only functioned as a highly effective drug carrier, but also inhibited breast cancer cell metastasis by downregulating the expression of multiple mitochondrial OXPHOS-related proteins and ATP production in the cancer cells but not in non-cancerous cells [26]. Notably, the use of GO in combination with chemotherapy drugs such as cisplatin has been reported to synergistically enhance the antitumor effects of the drugs by inducing necrosis *in vitro* and *in vivo* [27]. In summary, the GO properties of high surface area, low cytotoxicity, and capacity for surface functionalization have made GO an unexpectedly valuable material for use in the treatment of cancer.

Synthesis of functionalized NGO

Although NGO presents several favorable characteristics (preceding section), native NGO is unstable and prone to aggregate in physiological solutions, such as cell-culture media or serum, and is toxic to cells, which precludes its use in biological applications [28-30]. For example, plain GO was found to exhibit strong pulmonary toxicity because it readily accumulated in the lungs following injection [31]. Thus, native NGO must be rationally and robustly functionalized with stabilizing agents before use in nanomedicine applications. In this regard, pristine graphene nanocarriers featuring precisely designed surface modifications have been widely demonstrated to possess favorable properties, such as very high biocompatibility, low cytotoxicity, high drug-loading capacity, and site-specificity [32-34] (**Table 1**).

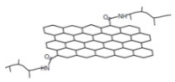
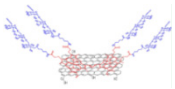
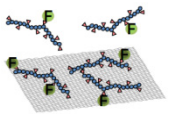
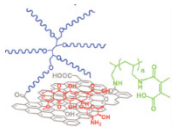
The past decade has witnessed a broadening of the purpose for which various synthesis and coating strategies are used: from being applied to influence only the biocompatibility and toxicity of NGO, to also being employed to increase the targeting ability of NGO and thus obtain novel high-performance graphene complexes [35-38]. Functionalization of graphene complexes with diverse additives, including small molecules, polymers, and/or biomolecules, not only leads to major enhancements of the existing GO properties, but also creates a comparatively more complex nanostructure presenting new features that are well-suited for antitumor applications. The two conventional approaches used for functionalization are the following: covalent attachment based on chemical bonding, and noncovalent wrapping be means of direct adsorption through π-π stacking, hydrophobic bonding, or van der Waals interactions [39-41].

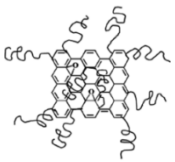
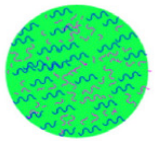
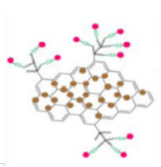
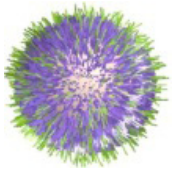
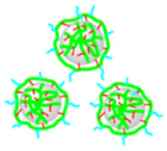
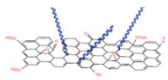
The use of covalent chemistry serves as an effective method of tailoring the properties of graphene. The presence of numerous functional groups on the surface of GO, such as epoxy, hydroxyl, and carboxy groups, allows GO to be readily linked covalently with other molecules. This chemical bonding can be mediated by direct coupling or through crosslinkers. One of the most widely used approaches involves wrapping the GO surface with water-soluble polymers such as PEG and polyethylenimine (PEI) [42]. For example, Miao et al. used conjugation chemistry, amide-bond formation, to prepare PEG-grafted NGO (pGO) as a codelivery vehicle for doxorubicin (DOX), a commonly used chemotherapy drug in the clinic and a model drug in nanomaterial research, and the photosensitizer chlorin e6 (Ce6); the obtained pGO nanosheets showed higher safety and improved anticancer efficacy relative to GO nanosheets [43]. Zhang et al. [44] covalently linked PEI to GO, and this was also through amide-bond formation; the resultant PEI-GO was well-dispersed in saline solution and was employed to sequentially deliver a Bcl-2-targeted siRNA and DOX into HeLa cells. Other covalent-chemistry methods, such as 1,3-dipolar cycloaddition, have also been employed to functionalize exfoliated graphene [45, 46].

In addition to chemical agents, numerous biological reducing agents have been used to modify graphene nanomaterials, including tea [47], proteins [48], bacteria [49], resveratrol [24], gelatin [50], and nicotinamide (NAM) [51]. Functionalization of graphene by using this facile and green chemical method also guarantees high dispersibility in physiological solvents. For instance, gelatin-functionalized NGO showed pH-dependent drug release and extremely high toxicity toward A549 cells when loaded with methotrexate [50], and reduced-GO (rGO) nanoconjugates synthesized by using NAM as a reducing and stabilizing agent showed high biocompatibility with mouse embryonic fibroblasts [51].

Covalent-conjugation strategies present the disadvantage of tending to alter the intrinsic structure and physical properties of the original graphene materials. Noncovalent functionalization is an alternative method that does not disrupt the chemical structure or electronic network of GO, and thus preserves the physical properties of GO. Moreover, noncovalent modification is a process that is simple and easy to implement. For example, Jin et al. generated an NGO-based nanocarrier that was noncovalently functionalized with hematin-dextran conjugate (HDex) through π-π interaction; the nanocarrier exhibited enhanced colloidal stability and cytocompatibility. Notably, Hdex-decorated NGO could be efficiently loaded with the chemotherapy drug DOX, and the DOX-loaded nanocarrier more efficiently killed drug-resistant MCF-7/ADR cells than did pure DOX, because the nanohybrid was specifically internalized by the

Table 1. Covalent and noncovalent functionalization of graphene and graphene oxide for biomedical applications

Nanoconjugates	Modified materials	Schematic illustration	Morphology	Thickness	Sizes	Dispersity	Zeta-potential	Loaded drugs	Interaction	Drug loading efficiency	Cancer cells	Results	References
GO-N=N-GO/PVA	Poly (vinyl alcohol) (PVA)		A lamellar structure	Increased	-	Showed uniform dispersion	-	Curcumin (CUR)	π-π stacking interactions	-	Colon cancer (*in vivo*)	Improved bioavailability of CUR and preferential accumulation in the colon	[37]
pGO	Polyethylene glycol (PEG)	-	A lamellar structure	1 nm	~170 nm	Showed dispersion with the polydispersity index (PDI) of 0.23 ± 0.01	-42.6 ± 0.2 mV	Ce6, DOX	π-π stacking and hydrophobic interactions	51.9 % for Ce6 and 61.7 % for Dox	Murin SCC7 squamous carcinoma cells (*in vitro* and *in vivo*)	Higher safety than GO nanosheets *in vivo* and improved photodynamic anti-cancer therapy efficiency	[43]
PEI-GO	Polyethylenimine (PEI)		A lamellar structure	3-4 nm	~200 nm	Showed dispersion in saline solution	+ 55.5 mV	DOX, siRNA	electrostatic and π-π interactions, respectively	-	Human cervical carcinoma (Hela) cells (*in vitro*)	Lower cytotoxicity, higher transfection efficacy of siRNA, and enhanced anticancer efficacy	[44]
NGO-HDex	Hematin-dextran conjugate (HDex)		A lamellar structure	3.6-4.3 nm	~220-240 nm	Remained homogeneous in PBS or the medium	-	DOX	π-π stacking and hydrophobic interactions	3.4 mg/mg	Drug-resistant MCF-7/ADR cells (*in vitro*)	Improved colloidal stability, cytocompatibility, and more efficient killing effect against drug-resistant MCF-7/ADR cells	[52]
LHT-rGO	Low-molecular-weight heparin (LHT7)		A lamellar structure	-	-	Showed great dispersion stability in buffers and serum	-	DOX	π-π stacking and hydrophobic interactions, electrostatic interactions	~90%	Human KB carcinoma cells (*in vitro* and *in vivo*)	Improved physicochemical properties and DOX-loading capacity, enhanced tumor tissue distribution and anti-tumor effect	[53]
PEO/CS/GO	Polyethylene oxide, chitosan	-	Nanofibers	-	~85 nm (Minimum diameter)	-	-	DOX	π-π stacking and hydrophobic interactions	98%	Human lung epithelial carcinoma A549 cells (*in vitro*)	pH dependence, and stronger cytotoxicity to the human lung epithelial carcinoma A549 cell lines	[54]
NGO-PEG-DA	PEG, poly (allylamine hydrochloride) (PAH), 2,3-dimethylmaleic anhydride (DA)		A lamellar structure	-	~70 nm	Showed stability in physiological solutions	-34.4 ± 0.19 mV	DOX	π-π stacking and hydrogen bonding interactions	50%	MCF-7/WT cells and drug-resistant MCF-7/ADR cells (*in vitro*)	pH-responsive cell-killing ability and improved cell killing for drug-resistant cancer cells	[55]

GO-PDEA	Poly (2-(diethylamino) ethylmethacrylate) (PDEA)		A lamellar structure	~29 nm	~100-200 nm	Showed high solubility and stability in physiological solutions	+13 mV	Camptothecin (CPT)	π-π stacking and hydrophobic interactions	0.156 g/g	Mouse neuroblastoma (N2a) cancer cells (*in vitro*)	pH-responsive property with high solubility and good stability, and high potency in killing N2a cancer cells *in vitro*	[56]
TPGs	Tea polyphenol	-	A lamellar structure	-	171.25 nm	Showed good hydrophilicity	-37.56 ± 0.15 mV	DOX	Electrostatic interactions, hydrogen-bonding interactions	95.41%	Human adenoid cystic carcinoma cells (ACC2) (*in vitro*)	A greater loading capacity and pH-sensitive drug release for enhanced cancer therapy	[57]
SMGO/P (NIPAM-co-AA) NGs	Salep, branched Nisopropylacrylamide (NIPAM), acrylic acid (AA) monomers		A lamellar structure	-	82 nm	Showed dispersion with the polydispersity index (PDI) of 0.375	-	DOX	π-π stacking and hydrophobic interactions	72%	Human cervical carcinoma (Hela) cells (*in vitro*)	Thermo/pH dependent releasing behavior and enhanced toxicity to HeLa cells	[58]
DOX-SS-NGO-Ag	PEI, Ag		A lamellar structure	-	100-400 nm	-	-	DOX	Disulfide bonds	2.535 mg/mg (3:1 ratio of DOX to NGO-Ag)	Human cervical carcinoma (Hela) cells (*in vitro*)	GSH regulated drug releasing behavior	[59]
$CPMAA_2$-GON-PEG	PEG, poly (methacrylic acid) (PMAA)		Spherical shaped nanoparticles	-	110 nm	Had outstanding dispersion stability in aqueous dispersion and PBS dispersion	-	DOX	Disulfide bonds	42.96%	Human cervical squamous cancer cells (SiHa) (*in vitro*)	Biocompatibility and reduction/pH stimulus-responsive properties toward the tumor microenvironments	[60]
GON-Cy-ALG-PEG	PEGylated alginate (ALG-PEG)		Spherical shaped nanoparticles	-	94.73 nm	Showed dispersion in PBS (pH 7.4) or water (pH 7.4)	-	DOX	Disulfide bonds	0.9764 mg/mg	Human hepatocarcinomaHepG2 cells (*in vitro*)	Biocompatible, reduction-responsive properties and high cytotoxicity to HepG2 cells	[63]
PAA-GO	Polyacrylic acid (PAA)		A lamellar structure	1.9 nm	36 nm	-	-	1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU)	Covalent binding (amide bond formation)	198 μg BCNU/mg PAA-GO	Mouse GL261 brain cancer cells (*in vitro*)	Prolonged half-life of BCNU, increased *in vitro* anticancer efficacy	[112]
UA-rGO	Uric acid (UA)	-	A lamellar structure	-	~1780 nm	-	-	-	-	-	Human A2780 ovarian cancer cells (*in vitro*)	Significant dose-dependent cytotoxicity in A2780 ovarian cancer cells	[113]

cells and DOX accumulation in the cells was prolonged [52]. For the delivery of anticancer drugs, a similar study was conducted to develop a nanoscale-rGO carrier, which was tagged with low-molecular-weight heparin (LHT7), an antiangiogenic derivative, through physical adsorption; here, LHT7 served as not only the surface-coating material but also the anticancer agent. LHT7-coated rGO (LHT-rGO) sheets were physiologically stable for at least 24 h and exhibited higher DOX-loading capacity relative to plain rGO, and *in vivo* studies demonstrated that intravenously administered LHT-rGO/DOX accumulated in tumor cells to a 7-fold higher level and exerted a stronger antitumor effect as compared to rGO/DOX [53]. However, one disadvantage of noncovalent functionalization is nonspecific attachment, which must be overcome by using blocking agents or linker molecules. Moreover, graphene binds weakly to biomolecules, particularly molecules that lack conjugated structures.

Thus, covalent and noncovalent modification can be used to improve the biocompatibility of GO and enable its use as an efficient drug-loading platform for numerous anticancer drugs. When modifying GO with various molecules, several parameters must be considered, including charge density, hydrophobicity, solubility, size, and the binding affinity for cancer cells.

GO nanocomposites responsive to tumor microenvironments

The intracellular microenvironments of tumor tissues present several unique physical and biological properties as compared with those of normal tissues, such as weak acidity, increased temperature (by 2-5°C), and elevated levels of tumor-specific proteins and enzymes. For example, the average pH levels inside cancerous tissues (6.5-7.2), endosomes (5.0-6.5), and lysosomes (4.5-5.0) are lower than the extracellular pH in normal tissues (7.4), and the glutathione (GSH) concentration also differs substantially between normal and tumor tissues: 10 μM and 10 mM, respectively. These unique properties can be exploited to design microenvironment-responsive NGO delivery systems. Assembling additional molecules on the GO surface to obtain tumor environment-responsive GO nanocomposites would be extremely useful, because this could enable the controlled release of loaded drugs at tumor sites based on external stimuli.

GO nanocomposites exhibiting pH sensitivity

Because the average pH is lower in cancer environments than in normal tissues, pH-responsive GO nanocomposites could serve as nanocargo carriers for site-specific drug delivery. Ardeshirzadeh et al. successfully designed and synthesized a polyethylene oxide/chitosan/GO (PEO/CS/GO) nanofibrous complex by using the electrospinning process and subsequently, encapsulated DOX in these nanofibrous scaffolds through π-π stacking and hydrophobic interactions. The results of drug-release experiments showed that PEO/CS/GO functionalization endowed the GO sheets with pH-dependent drug-release property, with a greater amount of drug being released at lower (acidic) pH (5.3) than at basic or neutral pH, and thus DOX loaded into this nanocarrier exhibited stronger cytotoxicity toward cancer cells as compared with free DOX [54]. Another NGO conjugate was obtained by co-conjugating NGO with a PEG and poly(allylamine hydrochloride) (PAH) polymer, with PAH being further modified using 2,3-dimethylmaleic anhydride (DA) to confer pH-responsiveness to the carrier system. Atomic force microscopy imaging revealed that NGO-PEG-PAH acquired increased thickness and decreased size, and DOX-loaded NGO-PEG-DA nanocomposites showed enhanced cellular uptake and accelerated drug release, because the nanocomposites become positively charged in an acidic solution. Notably, in cytotoxicity assays, the nanocomposites exhibited enhanced inhibition efficacy toward both wild-type MCF-7/WT cells and drug-resistant MCF-7/ADR cells [55]. Kavitha et al. fabricated a GO-PDEA nanohybrid through chemical conjugation of GO with pH-sensitive poly (2-(diethylamino) ethylmethacrylate) (PDEA). The PDEA-grafted GO was highly soluble and stable in acidic and neutral aqueous solutions, and the camptothecin (CPT) loading and release of the GO-PDEA nanohybrid showed strong pH dependence: negligible CPT release occurred at physiological pH, but ~60% of the drug was released at reduced pH. This pH-responsive GO-PDEA system potently killed cancer cells *in vitro* [56]. Wang et al. modified the GO surface with a tea polyphenol, which resulted in pH-sensitive drug-release property, improved biocompatibility, and enhanced anticancer efficacy toward ACC2 tumor cells; the obtained tea polyphenol-functionalized GO provided a multifunctional DDS exhibiting highly favorable capabilities for enhanced cancer

therapy [57]. For the delivery of anticancer drugs, Bardajee et al. synthesized a novel dual thermo/pH-responsive biodegradable carrier through the chemical interaction of salep-modified GO with branched N-isopropylacrylamide (NIPAM) and acrylic acid (AA) monomers. NIPAM is a thermosensitive material in that it changes from hydrophilic to hydrophobic in water upon heating, and incorporation of NIPAM and AA guaranteed the dual thermo/pH-sensitive property of the nanographenes (NGs). Conversely, salep was used as a reducing and capping agent to obtain highly biocompatible salep-functionalized GO. As expected, the composite NGs loaded with DOX showed higher cytotoxicity toward human cervical cancer cells than did free DOX [58].

Redox-responsive GO nanocomposites

Differences in GSH levels between tumor cells and normal cells have formed the basis for investigations into redox-responsive GO nanocomposites. The expectation here is that if anticancer drugs are attached to GO through a redox-sensitive bond, the drugs would be released specifically at tumor sites.

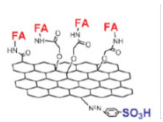
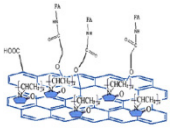
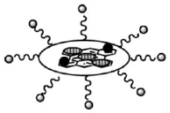
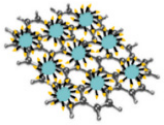
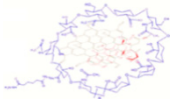
Chen et al. synthesized a unique redox-responsive NGO-based nanohybrid whose drug loading and release could be controlled inside tumor cells by the GSH level, because GSH concentration is higher in tumor cells than in normal cells. Moreover, the intrinsic SERS (surface-enhanced Raman scattering) signals of NGO enabled improved tracking of the distribution of the drug carrier during the entire process. Thus, this unique carrier exhibited stimulus-triggered drug release and concurrently allowed optical tracking, and when loaded with anticancer drugs, the carrier demonstrated enhanced therapeutic efficiency toward tumors and was considered potentially suitable for clinical application in cancer treatment [59]. Zhao et al. employed a similar strategy and successfully developed a reduction-triggered NGO composite, named CPMAA-GON-PEG (GON: GO nanoparticle), through the PEGylation and redox-radical polymerization of PMAA (poly(methacrylic acid)) brushes. The introduction of disulfide-bond-crosslinked PMAA (CPMAA) brushes endowed the resultant CPMAA-GON-PEG with a reduction-triggered switching characteristic; thus, premature DOX release in normal tissues could be avoided, and the release could be maximized under conditions of high cysteine or GSH levels in tumor tissues [60]. In another study, Zhao et al. presented a facile design of surface modification on NGO to develop a smart DDS by conjugating PEGylated alginate (ALG-PEG) brushes onto GO nanoparticles through S-S bonds; the GON-Cy-ALG-PEG hybrid was spherical and featured an average diameter of 94.73 nm. These small-sized nanoparticles showed increased ability to cross reticuloendothelial-system barriers and were exuded from the bloodstream into tumors, and the nanoparticles concomitantly exhibited diminished toxicity and enhanced cellular uptake [61, 62]. Furthermore, the nanocarriers demonstrated high loading capability and encapsulation efficiency for DOX, and also exhibited a distinctive redox-responsive property coupled with high stability under normal biological conditions; this was because the disulfide bonds between the grafted Cy-ALG-PEG moieties and the GO nanoparticles were cleaved in response to the reducing stimulus provided by tumor cells [63].

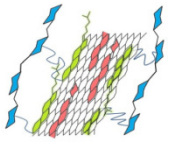
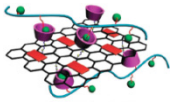
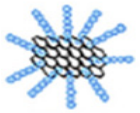
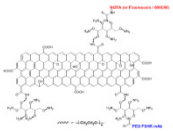
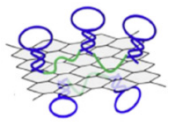
GO nanocomposites presenting positive targeting effects

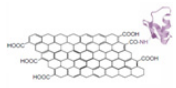
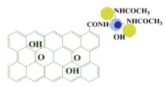
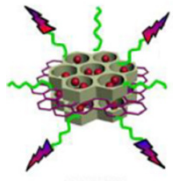
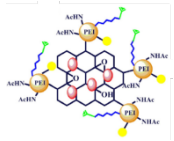
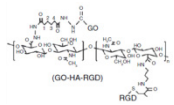
Attachment of biorecognition ligands, such as tumor-specific antibodies, peptides, specific DNA sequences, or other potential targeting molecules, to GO can confer an active targeting effect to GO-based drug carriers; this enables the delivery of therapeutics drugs specifically to neoplastic cells, and thus improves antitumor effects and therapeutic capabilities. To date, numerous covalent and noncovalent attachment methods have been intensively investigated for grafting biological moieties onto the GO surface to assemble tumor site-directed therapeutic platforms that selectivity target tumor cells (**Table 2**).

Folic acid (FA) is a molecule commonly used for targeting cancer cells that overexpress folate receptors: Conjugating FA to NGO through covalent amide bonds could enable targeted delivery of anticancer drugs to folate receptor-expressing cancer cells, and thus increase the cytotoxic and apoptotic effects of the anticancer drugs. Accordingly, Zhang et al. fabricated an FA-NGO nanohybrid, which was functionalized with FA molecules through covalent bonding. In this strategy, sulfonic acid groups were used to stabilize NGO in physiological solution

Table 2. GO nanocomposites presenting positive targeting effects

Nanoconjugates	Modified materials	Schematic illustration	Morphology	Thickness	Sizes	Dispersity	Zeta-potential	Loaded drugs	Interaction	Drug loading efficiency	Cancer cells	Results	References
FA-NGO	Folic acid (FA)		A lamellar structure	1-2 nm	-	Showed physiological stability	-116 mV	DOX, CPT	π-π stacking and hydrophobic interactions	400% for DOX, ~4.5% for CPT	Human breast cancer (MCF-7) cells (*in vitro*)	Specific targeting and remarkably high cytotoxicity to MCF-7 cells	[64]
FA-NGO-PVP	Folic acid, polyvinylpyrrolidone (PVP)		A lamellar structure	-	<100 nm	Showed solubility and stability in water and physiological media	-	DOX	π-π stacking and hydrophobic interactions	107.5 wt%	Human cervical carcinoma (Hela) cells (*in vitro*)	Utrahigh loading ratio of anticancer drug and selective chemo-photothermal-therapy	[65]
Fe_3O_4/GO-CHI-FA	Folic acid, Fe_3O_4		A lamellar structure	-	-	Showed homogeneous dispersion without magnetic field, aggregation When an magnetic field was applied	-	DOX	π-π stacking and hydrophobic interactions	0.98 mg/mg	-(*in vitro*)	Strong pH dependence and a dual-biological and magnetical targeting capabilities	[66]
FA-BSA/GO	Folic acid, bovine serum albumin (BSA)		A lamellar structure	~2.29 ± 0.32 nm	~73.7 nm	Showed an enhanced stability and dispersibility in physiological fluids	-	DOX	π-π stacking and hydrophobic interactions	437.43 μg DOX/mg FA-BSA/GO	Human breast cancer cells (MCF-7) and human non-small-cell lung cancer cells (A549) (*in vitro*)	pH responsive and sustained drug release, and the targeted drug delivery towards folate receptor rich cells (MCF-7 cells)	[67]
FA-GO	Folic acid		A lamellar structure	<1 nm	180 nm	Exhibited excellent stability in physiological fluids	-	Sorafenib	π-π stacking and hydrophobic interactions	125 μg/100 μg (SF/GO)	Human oral epidermoid (KB) cancer cells and non-small-cell lung cancer cells (A549) (*in vitro*)	Increased cellular uptake in folate receptor-expressing cancer cells, enhanced cytotoxicity and apoptotic effects of SF	[68]
HA-GO-DOX	Hyaluronic acid (HA)		A lamellar structure	<2.0 nm	~500 nm	Possessed excellent physiological stability	-	DOX	π-π stacking and hydrophobic interactions	42.9%	Human HepG2 cancer cells, H22 hepatic cancer cells (*in vitro* and *in vivo*)	High drug loading capacity for DOX, the ability of pH response, and sustained release of anticancer drugs	[69]

CHA-rGO	Cholesteryl hyaluronic acid (CHA)		A lamellar structure	3.0 nm	~180 nm	Showed dispersion stability in physiological conditions	-	DOX	π-π stacking and hydrophobic interactions, electrostatic interactions	90.9%	Human oral epidermoid (KB) cancer cells (*in vitro* and *in vivo*)	Greater Dox loading capacity, higher cellular uptake of Dox by CD44 positive tumor cells, and enhanced tumor cell-targeting ability	[70]
GO-CD-HA-ADA	β-cyclodextrin (CD), hyaluronated adamantane (HA-ADA)		A lamellar structure	2.5 nm	80 nm	Exhibited an excellent stability in both saline and serum environments	-	CPT	π-π stacking and hydrophobic interactions	3.3%	Human MDA-MB-231 breast cancer cells (*in vitro*)	Excellent stability and a higher inhibition effect toward malignant cells	[71]
HA-GO	Hyaluronic acid (HA)		A lamellar structure	-	170 nm	Formed a stable dispersion in both PBS and cell culture medium	-30.5 mV	miR-21	Electrostatic interactions	-	Human breast cancer cells (MBA-MB231) (*in vitro* and *in vivo*)	Specifically targeting and apoptosis effect of CD44-positive MBA-MB231 cells	[72]
GPMQNs	Monoclonal P-glycoprotein (P-gp) antibodies, quantum dots	-	A lamellar structure	-	300 nm	-	-	miR-122	Electrostatic interactions	-	Human HepG2/ADM hepatoma cells (*in vitro* and *in vivo*)	Low toxicity, selective targeting, apoptosis promotion of drug-resistant liver cancer cell, the near-infrared imaging	[73]
NOTA-GO-FSHR-mAb	A monoclonal antibody (mAb) against follicle stimulating hormone receptor (FSHR)		A lamellar structure	-	~120 nm	-	3.99 mV	Dox	π–π stacking and hydrophobic interactions	756 mg/g	Human MDA-MB-231 breast cancer cells (*in vitro* and *in vivo*)	Enhanced drug delivery efficiency in MDA-MB-231 metastatic sites	[74]
GO-DEX-Apt	Dextran (DEX), AS1411 aptamer	-	A lamellar structure	1.8-2 nm	< 200 nm	Exhibited an improved stability in PBS and 20% BSA solution	-	Curcumin	π-π stacking interactions	~29 %	4T1 murine breast carcinoma and MCF-7/CHO cells (*in vitro*)	Enhanced nucleolin positive cellular uptake and *in vitro* pH responsive drug release behavior	[76]
PNTrGO	Protein tyrosine kinase 7 receptor (PTK7), 22-mer oligoT bridges (PNT)		A lamellar structure	-	~150-180 nm	Exhibited an enhanced dispersion stability in phosphate-buffered saline (PBS)	-35 mV	DOX	π-π stacking and hydrophobic interactions	99.9%	Human T-cell acute lymphoblastic leukemia (CCRF-CEM) cells, human Burkitt's lymphoma (Ramos) cells (*in vitro* and *in vivo*)	Specific targeting effect to PTK7 overexpressed CCRF-CEM leukemia cells and inhibiting the tumor growth *in vitro* and *in vivo*	[75]

CTX-GO	Chlorotoxin		A lamellar structure	~3.0 nm	200 nm	Well dispersed in PBS	-	DOX	π-π stacking and hydrophobic interactions	570 mg/g	Rat C6 glioma cells (*in vitro*)	pH-dependent, glioma-specific targeted property	[78]
GO-CMC-FI-LA-Ac	Carboxymethyl chitosan (CMC), fluorescein isothiocyanate (FI), lactobionic acid (Ac)		A lamellar structure	-	-	-	-40.1 ± 1.2 mV	Dox	π-π stacking and hydrophobic interactions	>96%	Human SMMC-7721 hepatoma cells (*in vitro*)	High drug loading content, pH-sensitive release, and selectively inducion of the death of hepatoma cells	[79]
GSPI	Residues of interleukin 13, mesoporous silica		Hexagonal arrays of pores on the single-layer GO nanosheet structure	-	-	Exhibited an enhanced dispersion stability	-22.4 mV	DOX	π-π stacking and pore adsorption	1.27 μg DOX/μg GS	Human glioma U251 cells (*in vitro*)	Heat-stimulative, pH-responsive, and the advanced chemo-photothermal synergistic targeted therapy	[80]
GO/PEI.Ac-FI-PEG-LA	Polyethyleneimine (PEI), fluorescein isothiocyanate (FI), polyethylene glycol (PEG), lactobionic acid (LA)		A lamellar structure	-	-	Showed solubility and stability between pH 5.0 and 9.0	-11.4 ± 0.82 mV	DOX	π-π stacking and hydrophobic interactions	85.0%	Human SMMC-7721 hepatoma cells (*in vitro*)	Specifically targeting of SMMC-7721 cells overexpressing ASGPR receptors, and specific inhibition effect to the cancer cells	[114]
GO-HA-RGD	Hyaluronic acid (HA), Arg-gly-asp peptide (RGD)		A lamellar structure	13 nm	70-490 nm	Exhibited an excellent solubility and long-term physiological stability	-	DOX	π-π stacking and hydrophobic interactions	72.9%	SKOV-3 and HOSEpiC cells (*in vitro*)	A high loading rate, pH-response and sustained drug release behavior, the specificity and selectivity of anticancer drug delivery via a synergic effect of CD44-HA and integrin-RGD mediated endocytosis	[115]

and FA molecules were attached to the NGO to make it target MCF-7 cells differentially and efficiently. Furthermore, these graphene-based nanocarriers showed controlled loading and targeted delivery of two cargo drug molecules, DOX and CPT [64]. Applying the same approach, Qin et al. developed an FA-NGO-PVP (polyvinylpyrrolidone) nanocarrier, with which targeted chemotherapy-PTT was achieved after the delivery of anticancer drugs [65]. Wang et al. developed a dual-targeting system by using FA and Fe_3O_4 bifunctionalized GO to transport anticancer drugs. The combination of biological (active) and magnetic (passive) targeting on the GO nanocarrier maximized the efficiency of drug delivery to tumor sites [66]. In another study, Ma et al. constructed an FA-BSA/GO nanocomposite; the as-prepared nanohybrids exhibited the favorable properties of targeting, stability, and pH responsiveness. Thus, the developed FA-BSA/GO/DOX system was demonstrated to specifically deliver DOX to MCF-7 cells (FA-receptor-positive) but not A549 cells (FA-receptor-negative) [67]. Thapa et al. synthesized an FA-GO/SF delivery system for targeted delivery of sorafenib (SF); the conjugation of FA led to higher cellular uptake in KB cancer cells (folate-receptor-positive) than in A549 cells (which express comparatively fewer folate receptors) [68].

Hyaluronic acid (HA) is a linear glycosaminoglycan that can specifically recognize cancer cells expressing the transmembrane glycoprotein CD44. Besides featuring this specific targeting ability, HA is biocompatible, water-stable, biodegradable, and inexpensive. Because HA is regarded as the ligand for CD44 receptors, it can also be exploited as an anchoring molecule for active targeted drug delivery. For instance, Song et al. functionalized GO with HA through H-bonding interactions between the N-terminus of HA and epoxy groups of GO, which endowed the nanocarrier with targeting ability and allowed the controlled release of the anticancer drug DOX. In this process, the added HA provided both stable hydrophilic groups and a targeting moiety, and the entire preparation process was simple and easy because it avoided complex covalent modification. The nanohybrid demonstrated specific targeting and high dispersibility, accompanied with high DOX-loading efficiency and sustained drug-release capability, and DOX was released from HA-GO-DOX more rapidly in acidic solution (pH 5.3) than in neutral solution. Furthermore, examination of the *in vitro* and *in vivo* antitumor effect of HA-GO-DOX revealed selective inhibition of hepatic cancer cells [69]. In another study, Miao et al. synthesized a tumor-targeting delivery system (CHA-rGO) by coating cholesteryl HA (CHA) onto rGO nanosheets through hydrophobic interactions. As compared with plain rGO nanosheets, the CHA-rGO nanosheets demonstrated increased stability, greater DOX-loading capacity, and higher DOX uptake by CD44-positive tumor cells, together with enhanced tumor cell-targeting ability. Moreover, *in vivo* analysis revealed that intravenous administration of CHA-rGO/DOX nanosheets resulted in higher tumor accumulation and prolonged retention in tumor sites expressing CD44 receptors, accompanied with improved *in vivo* safety, relative to what was observed with free DOX or rGO/DOX. Given these favorable properties, CHA-rGO demonstrated CD44-mediated delivery of antitumor agents and thus could be used for effective anticancer therapy [70]. By using the same method, Zhang et al. obtained a small-sized GO supramolecular assembly (CPT@GO-CD-HA-ADA), exhibiting very high stability and targeting ability, through a two-step noncovalent interaction: first, the β-cyclodextrin (CD) cavity complexed with the adamantyl group of hyaluronated adamantane (HA-ADA); and second, the aromatic drug CPT bound onto the planar GO surface through π-π stacking. The small-sized composition displayed an enhanced curative effect toward MDA-MB-231 breast cancer cells, which overexpress HA receptors on their surface [71]. Moreover, Hwang et al. recently prepared HA-coated GO nanocarriers loaded with a specific fluorophore-conjugated antisense peptide nucleic acid (PNA); these nanocarriers can sense oncogenic miR-21 and concurrently target and inhibit CD44-positive MBA-MB231 cancer cells [72].

As compared with other ligands, antibodies exhibit superior target-recognition ability and higher affinity for tumors; thus, antibodies can serve as favorable targeting candidates for conjugation to GO. Zeng et al. coupled NGO with a monoclonal P-glycoprotein (P-gp) antibody and quantum dots loaded with miR-122 to generate the nanocomposites GPMQNs; the tumor cell-recognizing moieties of the P-gp antibody conferred GPMQNs with the ability to target the HepG2/ADM cell membrane. The multifunctional GPMQNs effectively induced apopto-

sis and inhibited tumor growth *in vitro* and *in vivo*, while also presenting the characteristics of low cytotoxicity, controlled release, and selective targeting of hepatoma cells; moreover, the NIR absorbance of GPMQNs, which showed increased tissue penetration, could be applied for tumor imaging [73]. More recently, Yang et al. developed GO-FSHR-mAb complexes, in which a monoclonal antibody (mAb) against follicle-stimulating hormone receptor (FSHR) was used as an FSHR-targeting ligand to functionalize GO nanosheets; this FSHR-targeting nanoplatform loaded with DOX demonstrated satisfactory *in vitro* and *in vivo* treatment efficacy for breast-cancer lung metastasis [74].

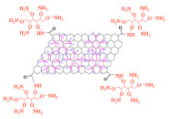
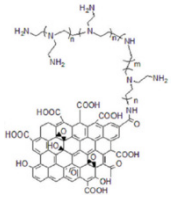
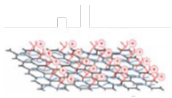
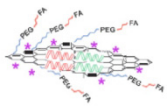
Aptamers are a type of synthetic single-stranded oligonucleotides whose affinity and specificity for tumors equal or exceed those of antibodies. Kim et al. coated the surface of rGO nanosheets with protein tyrosine kinase 7 receptor (PTK7) aptamers, which show tumor affinity and specificity, to confer tumor-targeting ability to the rGO nanosheets; moreover, a 22-mer oligonucleotide bridge domain (PNT) was introduced between each aptamer sequence to strengthen the anchoring of the polyaptamers onto rGO. The functionalized nanosheets efficiently and specifically killed PTK7-overexpressing CCRF-CEM cells but not PTK7-negative Ramos cells, and in accord with the *in vitro* anticancer effects, systemic administration of DOX/PNTrGO nanosheets strongly retarded the growth of PTK7-positive tumors in mice [75]. Alibolandi et al. fabricated dextran (DEX)-coated NGO composites and then covalently attached the AS1411 aptamer in order to increase uptake by nucleolin-positive cells and enhance drug-delivery efficiency. The AS1411 aptamer-functionalized GO-DEX loaded with curcumin (CUR) efficiently entered nucleolin-overexpressing 4T1 and MCF-7 cells and induced the death of these cancer cells at a higher rate than did non-targeting GO-DEX or free CUR [76]. Bahreyni et al. designed two nanocomplexes, MUC1 aptamer-NAS-24 aptamer-GO and MUC1 aptamer-cytochrome C aptamer-GO, which efficiently induced apoptosis in MUC1-positive cancer cells [77].

Chlorotoxin (CTX) is a toxic peptide consisting of 36 amino acids that is derived from the giant Israeli scorpion (*Leiurus quinquestriatus*). Because CTX specifically binds to gliomas or other tumors originating from the neuroectoderm, it can be used as a targeting ligand to improve efficacy of glioma treatment. For example, Wang et al. successfully synthesized a glioma-targeting CTX-conjugated GO composite (CTX-GO) as a nanocarrier and loaded it with DOX; the nanocarrier showed pH-dependent and sustained DOX release, as well as high specificity for gliomas [78]. Other targeting ligands have also been conjugated to graphene nanocarriers for therapeutic-delivery purposes. Pan et al. prepared multifunctionalized GO decorated with carboxymethyl chitosan, fluorescein isothiocyanate, and lactobionic acid (LA) for targeted delivery of DOX to liver cancers. The addition of LA enabled the GO system to selectively induce the death of cancerous hepatic cells, because LA can be specifically recognized by the asialoglycoprotein receptors that are overexpressed on hepatoma cells [79]. Wang et al. used the residues of interleukin-13 as a glioma-targeting ligand with which to modify mesoporous silica-coated graphene nanosheets; these nanocarriers demonstrated targeted accumulation within glioma cells but not normal cells and enabled a chemo-photothermal synergistic targeted therapy [80]. In summary, the introduction of specific ligands onto the GO surface endows targeting capability to GO nanocomposites, which anchor at the sites of cancer cell localization through high-affinity interactions mediated by the attached biological probes for cancers, and are thus selectively internalized by the cancer cells.

Graphene nanomaterials for gene-delivery applications

Besides the use of regular chemical drugs, gene therapy is regarded as an effective treatment modality that could cure cancer by altering or modifying the expression of specific genes or proteins [81-83]. With improvements in its synthesis and processing, graphene has now been shown to act as an effective nucleic acid carrier that can deliver genes and related molecules efficiently and safely into cancer cells [84, 85] (**Table 3**). Accumulating evidence has confirmed that GO modified with cationic polymers, such as PEI, polypropylenimine (PPI), polyamidoamine (PAMAM), or their derivatives, can facilitate the fabrication of gene-delivery systems [86-88]. Chemical modification of its surface has made GO suitable for the delivery of molecules such as DNA and RNA, because the negatively charged nucleotide chains can

Table 3. Graphene nanomaterials for gene-delivery applications

Nanoconjugates	Modified materials	Schematic illustration	Morphology	Thickness	Sizes	Dispersity	Zeta-potential	Loaded drugs	Interaction	Drug loading efficiency	Cancer cells	Results	References
PEI-GO	Polyethylenimine (PEI)	-	A lamellar structure	-	~188 nm	-	+27.4 ± 1.25 mV	siRNA	Electrostatic interactions	-	Human MDA-MB-231 breast cancer cells (*in vitro*)	Effectiveness in the delivery of siRNA	[89]
GO-PEI-PEG	Polyethylenimine (PEI), polyethylene glycol (PEG)	-	A lamellar structure	10 nm	100-300 nm	Showed significant stability in physiological solutions	+21.02 mV	SAT3	Electrostatic interactions	-	Mouse malignant B16 cells (*in vitro*)	Significant regression in tumor growth and tumor weight, and apoptosis in mouse malignant melanoma	[90]
NGO-PEG-dendrimer	Polyamidoamine (PAMAM) dendrimer, polyethylene glycol (PEG)		A lamellar structure	-	-	Exhibited outstanding stability in PBS and cell medium containing serum	+28.9 ± 0.7 mV	Anti-miR-21	Electrostatic interactions	-	Human non-small-cell lung cancer A549 cells (*in vitro* and *in vivo*)	Lower cytotoxicity, higher transfection efficiency, effectively inhibit lung cancer cell migration and Invasion	[92]
BPEI-GO	Branched polyethylenimine (BPEI)		A lamellar structure	6-8 nm	100-200 nm	Showed excellent colloidal stability under physiological conditions	-	-	-	-	Human cervical HeLa cancer cells, prostate PC-3 cancer cells (*in vitro*)	High gene delivery efficiency and excellent photoluminescence activities	[116]
PPG	Polyethylenimine (PEI), poly(sodium 4-styrenesulfonates) (PSS)		A lamellar structure	-	~500 nm	-	+26.6 ± 0.4 mV	Anti-miR-21, adriamycin (ADR)	Static interaction (anti-miR-21), physical mixing (ADR)	a volume ratio of 1.0 for anti-miR-21, 0.7 mg/ml for ADR	Human multidrug resistance (MDR) resistant breast cancer cells (MCF-7/ADR) (*in vitro*)	Much higher cytotoxicity, enhanced therapeutic efficacy, and reverse ADR resistance of MCF-7/ADR	[117]
GO/FA/PEG/PAH	Polyallylamine hydrochloride (PAH), polyethylene glycol (PEG), folic acid (FA)		A lamellar structure	-	~400 nm	-	+50 mV	HDAC1 and K-Ras siRNAs	Electrostatic interaction	-	MIA PaCa-2 pancreatic cancer cells (*in vitro* and *in vivo*)	The synergistic combination of gene silencing and NIR light thermotherapy, significant anticancer efficacy	[118]

adsorb onto the surface of the positively charged GO complex through electrostatic interactions.

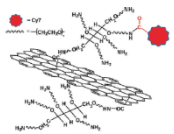
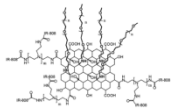
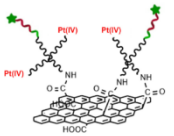
PEI is most commonly used for modifying GO for gene delivery because PEI can attract nucleic acids through electrostatic interaction. PEI-modified GO systems show high solubility, low cytotoxicity, and high transfection efficiency, and have been used for DNA and RNA delivery applications. For example, Huang et al. functionalized GO with PEI to transfect siRNAs against C-X-C chemokine receptor type 4 (CXCR4) expressed by breast cancer cells; CXCR4 serves as a biomarker for cancer metastasis. The results suggested that PEI-GO effectively delivered the siRNAs and led to the suppression of CXCR4 expression and cancer metastasis [89]. An efficient gene-vector platform functionalized with both PEI and PEG (GO-PEI-PEG) linked through amide bonds was prepared for the delivery of siRNAs targeting STAT3 (signal transducer and activator of transcription 3); the platform demonstrated a synergistic effect: it delivered the STAT3-siRNA plasmid into mouse malignant melanoma cells and also supported PTT. The siRNA plasmid could be delivered into cells in a light-controllable manner because of the photothermal effect of NGO; the cationic PEI polymers guaranteed high condensation of the negatively charged STAT3-siRNA plasmid; and PEG provided the biocompatibility and stability of the GO-PEI complex. Treatment with the synthetic GO-PEI-PEG carrier led to marked regression of tumor growth and to apoptosis in mouse malignant melanoma cells without producing any overt side effects [90, 91]. Wang et al. integrated PAMAM dendrimers and PEG with NGO to develop an NGO-PEG-dendrimer conjugate, which was used for efficient delivery of anti-miR-21. The NGO-PEG-dendrimer exhibited substantially diminished cytotoxicity and increased transfection efficiency toward non-small-cell lung cancer cells and furthermore, the delivery process could be monitored because of an activatable luciferase reporter that was constructed into this conjugate. Treatment with NGO-PEG-dendrimer/anti-miR-21 strongly inhibited lung cancer cell migration and invasion [92]. Rezaei et al. used ethidium bromide (EtBr) as an intercalating agent to fabricate, for the first time, a stable amphiphilic graphene-EtBr composite, which could serve as a new vehicle for gene-delivery applications [93]. These studies have shown that graphene-based materials act as effective nanovectors for the delivery of genetic materials into specific cells of interest.

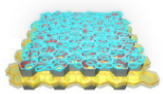
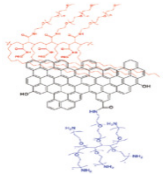
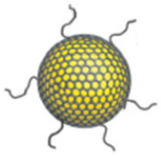
In addition to genetic materials, proteins have been delivered into cells by using functionalized NGO nanocarriers, which effectively overcomes the problem of their short half-life and instability in physiological environments [94]. Jana et al. functionalized GO with Tris-[nitrilotris (acetic acid)] and biotin for cellular delivery of oligohistidine- and biotin-tagged biomolecules [95]. Shen et al. showed that PEI-grafted GO can efficiently deliver proteins into cells, while also protecting the proteins against enzymatic hydrolysis and retaining their biological functions [96]. These findings have demonstrated the possibility of using GO for therapeutic protein delivery.

Phototherapy with graphene nanomaterials

Cancer phototherapy involving photodynamic therapy (PDT) and PTT is a promising alternative to traditional therapies for curative and palliative treatment of various types of cancer; this therapy enables spatiotemporal selectivity and causes minimal injury to normal tissues. The successful application of PDT and PTT involves the uptake of a photosensitizing agent by a tumor tissue and then treatment with light of a specific wavelength. Upon illumination, the photosensitizer triggers the generation of molecular oxygen in the malignant tissue and thus induces oxidative cell damage and apoptosis or necrosis [97-99]. Over the past few years, numerous studies have indicated that graphene nanoparticles can strongly absorb NIR radiation and generate sufficient heat for killing cancer cells, which suggests that GO holds considerable potential for use in PTT of cancer [100-102] (**Table 4**). Markovic et al. synthesized PVP-coated graphene nanoparticles, which displayed higher NIR-absorbing capacity and photothermal anticancer efficiency as compared to carbon nanotubes; the photothermal killing of cancer cells was mediated by the induction of oxidative stress and mitochondrial damage, which eventually led to mixed apoptotic and necrotic death of the cancer cells [103]. Yang et al. intravenously injected PEGylated NG sheets for *in vivo* PTT and achieved extremely high tumor destruction without marked side effects, and this was comparable to what was observed with an extensively studied photothermal

Table 4. Phototherapy with nanosized graphene and graphene oxide

Nanoconjugates	Modified materials	Schematic illustration	Morphology	Thickness	Sizes	Dispersity	Zeta-potential	Loaded drugs	Interaction	Laser	Cancer cells	Results	References
GON-PVP	PVP	-	-	2 nm	~70 nm	-	-	-	-	808 nm NIR laser	Human U251 glioma cells (*in vitro*)	Good NIR-absorbing capacity, and excellent photothermal anticancer efficiency	[103]
NGS-PEG-Cy7	PEG		A lamellar structure	-	10-50 nm	Showed high stability in physiological solutions	-	-	-	808 nm NIR laser	Murine 4T1 breast cancer, KB human epidermoid carcinoma tumors, U87MG human glioblastoma tumors (*in vivo*)	Excellent *in vivo* tumor near-infrared (NIR) photothermal therapy agent without exhibiting noticeable toxicity to the treated mice	[104]
SPI/rGO	Soy protein isolate (SPI)	-	A lamellar structure	10-15 nm	-	Showed stability without any aggregation	-	-	-	808 nm NIR laser	Human cervical carcinoma (Hela) cells (*in vitro*)	Much better photothermal effects and killing effect on HeLa cells	[105]
NGO-PEG-BPEI	PEG, branched polyethylenimine (BPEI)		A lamellar structure	~2 nm	20-40 nm	-	-	-	Covalent conjugation	808 nm NIR laser	Human non-small-cell lung cancer cells (A549), Lewis lung cancer cells (*in vitro* and *in vivo*)	Combining PDT and PTT treatment and improved cancer targeted accumulation, highly efficient cancer phototherapy with minimal side effects	[106]
rGO/AE/AuNPs	Amaranth extract (AE), gold nanoparticles (AuNPs)		A lamellar structure	-	-	-	-	Amaranth extract	π−π stacking and hydrophobic interaction, electrostatic attraction	808 nm NIR laser	Human cervical cancer HeLa cells or Chinese hamster ovary cells (CHO cells) (*in vitro*)	Remarkably improved and synergistic antitumor effect	[108]
PEG-NGO-Pt	PEG, caspase-3 recognition sequence (DEVD)		A lamellar structure	-	10-70 nm	Exhibited good dispersibility and stability	-	Pt	Covalent attachment via amide linkages	808 nm NIR laser	Human cervical cancer HeLa cells (*in vitro* and *in vivo*)	Efficient Pt drug loading; dual stimuli responsiveness towards both GSH and NIR irradiation	[110]

pRGO@MS-HA	Polydopamine, mesoporous silica (MS), hyaluronic acid (HA)		A lamellar structure	-	~200 nm	Exhibited good dispersibility	-34.7 mV	DOX	π-π stacking and hydrophobic interactions	808 nm NIR laser	Human cervical cancer HeLa cells (*in vitro* and *in vivo*)	Efficient synergistic targeted chemo-photothermal therapy, have minimal cytotoxicity, good specificity to target tumor cells	[111]
nano-rGO-RGD	PEG, Arg-Gly-Asp (RGD) -based peptides		A lamellar structure	-	~20 nm	Regained stability as a homogeneous suspension in buffers and other biological solutions	-	-	-	808 nm NIR laser	U87MG cancer cells (*in vitro*)	Small size, high photothermal efficiency	[119]
PEG-Au@GON	PEG, gold nanoparticles		Spherical shape	2-3 nm	~60 nm	-	-	zinc phthalocyanine	π-π interaction	808 nm NIR laser	Human cervical cancer HeLa cells (*in vitro*)	Excellent multifunctional properties for combinational treatment of photothermal and photodynamic therapy	[120]

agent: PEGylated gold nanorods [104]. Another nanocomposite, soy protein isolate/rGO, prepared by Jiang et al., also showed PTT properties and killed HeLa cells efficiently following treatment with 808-nm (NIR) laser [105].

Synergistic PDT/PTT treatment facilitates concurrent cancer phototherapy and thus considerably improves therapeutic efficacy. For example, Luo et al. conjugated a PDT photosensitizer (IR-808) to PEG and PEI dual-functionalized NGO; the as-prepared NGO-808 achieved high phototherapeutic efficacy and preferential accumulation in tumors. The modified-PEG/PEI dual-functionalized NGO not only facilitated the covalent attachment of IR-808, but also enhanced collaborative PDT/PTT cancer treatment [106]. To control drug release in cancer treatment, Lee et al. developed an endogenous microRNA (miRNA)-responsive drug-activation system, which consisted of the photosensitizer Ce6, a PNA that was complementary in sequence to cancer-specific miR-21, and dextran-coated rGO. The obtained Ce6-PNA/Dex-RGON complex inhibited tumor growth selectively, and its application resulted in reduced potential risk as compared with conventional PDT because of the use of the internal miRNA as a cancer-specific stimulus to tightly control the activation of the photosensitizer [107]. For use in antitumor therapy, Chang et al. fabricated another highly integrated multifunctional composite hydrogel that contained rGO, amaranth extract (AE), and AuNPs; the rGO/AE/AuNP nanocomposites demonstrated extremely high photodynamic sensitization and photothermal capacity in the NIR range, and could thus serve as a PDT/PTT integrated platform for enhanced cancer therapy [108].

Considerable research effort has also been devoted toward combining PTT with chemotherapy (chemophototherapy) to generate synergistic effects to combat cancer; this combined therapy has been demonstrated to increase the sensitivity of chemotherapy, enhance antitumor efficiency, and overcome multidrug resistance [109]. In this strategy, the chemotherapy agent and the photosensitizer (which presents strong NIR-absorbance and photothermal effects) are combined in a single platform to achieve favorable antitumor responses. For example, to establish a targeted DDS, Li et al. designed a multifunctional nanocomposite, PEG-NGO-Pt, in which PEGylated NGO (PEG-NGO) served as the multifunctional platform and NIR-light-absorbing agent, the Pt(IV) complex was included as the antitumor drug, and a caspase-3 recognition sequence (DEVD) was used as an apoptosis sensor; this system enabled not only combined thermal-chemotherapy, but also real-time evaluation of the therapeutic efficacy of cancer treatment. In addition to possessing the favorable properties of high dispersibility and stability, the PEG-NGO-Pt nanocomposites were responsive to both GSH and NIR irradiation. Furthermore, *in vivo* studies revealed that PEG-NGO-Pt markedly enhanced the anticancer effect of cisplatin and produced minimal side effects as compared with any single treatment that was tested [110]. Lastly, Shao et al. also constructed a versatile nanoplatform and multimodal therapy system that was based on pRGO@MS(DOX)-HA nanocomposites; the as-prepared pRGO@MS(DOX)-HA exhibited very high synergistic chemo-photothermal antitumor efficacy, which resulted in an extremely strong suppression of tumor growth that was clearly distinct from what was observed with any monotherapy [111].

Summary and perspectives

The *in vitro* and *in vivo* studies reviewed here have yielded encouraging results demonstrating the successful use of GO and its functionalized derivatives as effective drug carriers for anticancer therapy. Recent advances in surface engineering in the design of smart nanosystems have facilitated the development of GO nanocomposites possessing highly favorable properties in terms of appropriate size, stability in physiological fluids, and controlled drug release, and these nanocomposites have served as effective multifunctional platforms for the delivery of various drugs, genes, and proteins. The nanosystems have been comprehensively designed to enable the carriers to release their payload inside tumors in response to specific triggers, such as the tumor pH or redox state, or irradiation. The nanocarriers possess targeting capability, which allows them to anchor at sites where cancer cells are localized; consequently, anticancer drugs can be effectively internalized by cancer cells due to stimulation by the tumor microenvironment or through endocytosis mediated by ligand-receptor interactions, and this enhances drug accumulation inside the cancer cells. Graphene-based drug-delivery platforms have improved

the pharmacological and therapeutic index of administered drugs while concurrently limiting their toxicity, and have thus provided additional advantages over traditional treatments. Notably, the use of these nanocarriers can also be integrated with other novel therapies, such as PTT and gene therapy, for synergistic treatment of various cancers to further improve therapeutic efficacy.

Despite notable achievements in the development of NGO-based carriers as drug-delivery vehicles, certain limitations remain, such as the potential long-term toxicity of the carriers. Current investigations are typically short-term *in vitro* studies that do not adequately reflect the long-term safety of materials. Moreover, recent reports on biofunctionalized graphene have been restricted to those describing studies conducted by using nanocarriers at specific doses with cell lines or rodent models; further research is therefore necessary to ascertain the impact of NGO on the immune system, reproductive system, and nervous system of primates and humans. Furthermore, some of the materials used to modify graphene, such as PEG, are expensive and thus increase the total cost of synthesizing graphene bioconjugates. Overall, the development of various surface-modification methods has led to the fabrication of numerous high-quality graphene-based materials for drug delivery; however, before these nanocarriers can be freely applied to the clinic, additional investigations will be necessary, such as analyses of the *in vivo* behaviors of NG, including its effects on metabolism.

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Disclosure of conflict of interest

None.

Address correspondence to: Hao Yang, Hong-Hui Hospital, Xi'an Jiaotong University College of Medicine, Xi'an 710054, China. E-mail: yanghao.71_99@yahoo.com

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