Graphene-Based Nanomaterials for Theranostic Applications

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Graphene and graphene-based nanomaterials such as graphene oxide (GO), reduced graphene oxide (rGO) and graphene quantum dots (GQDs) have gained a lot of attention from diverse scientific fields for applications in sensing, catalysis, nanoelectronics, material engineering, energy storage and biomedicine due to its unique structural, optical, electrical and mechanical properties. Graphene-based nanomaterials emerge as a novel class of nanomedicine for cancer therapy for several reasons. Firstly, its structural properties like high surface area and aromaticity enables easy loading of hydrophobic drugs. Secondly, presence of oxygen containing functional groups improve its physiological stability and also act as site for biofunctionalization. Thirdly, its optical absorption in the NIR region enable them to act as photoagents for photothermal and photodynamic therapies of cancer, both *in vitro* and *in vivo*. Finally, its intrinsic fluorescence property helps in bioimaging of cancer cells. Overall, graphene-based nanomaterials can act as agents for developing multifunctional theranostic platforms for carrying out more efficient detection and treatment of cancers. This review provides a detailed summary of the different applications of graphene-based nanomaterials in drug delivery, nucleic acid delivery, phototherapy, bioimaging and theranostics.

Keywords: Graphene; graphene oxide; reduced graphene oxide, drug delivery; nucleic acid delivery; phototherapy; bioimaging; theranostics.

1. Introduction

With its discovery in the year 2002 followed by a Nobel prize in Physics in 2010, graphene has attracted a lot of attention from diverse scientific fields for applications in sensing, catalysis, nanoelectronics, material engineering, energy storage and biomedicine. The reason behind this diverse set of applications is its unique structural,

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optical, electrical, and mechanical properties that have been found to be superior than other widely explored carbon and plasmonic nanomaterials.^{1–4} Graphene is a two-dimensional (2D) nanomaterial having a monoatomic layer of sp^2 hybridized carbon atoms organized in the form of a honeycomb lattice.⁵ Synthesis of graphene oxide (GO) and reduced graphene oxide (rGO) have been made possible with the development of new synthesis methods that have further enhanced interest in this material specifically in the biomedical field due to the incorporation of new features and novel properties in these derivatives (Fig. 1).^{5–7} The ultra-thin nanosheets of graphene and its derivatives like GO and rGO provide very high surface area for efficient loading of therapeutic cargoes like drugs, nucleic acids, fluorescent probes, biomolecules and cells. Additionally, these materials also provide a distribution of delocalized π electrons on the surface giving it an aromatic character, that help in increasing the loading and binding efficiency of graphene-based nanomaterials with other molecules of therapeutic interest through hydrophobic interactions and π - π stacking.⁵ The synthesis procedures of GO introduce wide variety of oxygen containing functional groups like carboxyl, hydroxyl, carbonyl, ethoxy, epoxy, etc., on both planes of GO sheets, thereby increasing its stability in aqueous solutions. This also provides easy functionalization and derivatization of these materials through both covalent and non-covalent modifications with different biocompatible polymers like PEG, chitosan and conjugation with targeting moieties like peptides and antibodies, to develop biofunctionalized nanocomposite systems with improved biological properties.^{5,8,9} Apart from these surface properties, GO and rGO also possess exciting intrinsic optical properties like photoluminescence and increased absorbance in the near-infrared region (NIR) of electromagnetic spectrum, that make them excellent photo-responsive agents for cancer therapies like photothermal therapy (PTT) and photodynamic therapy (PDT).⁵ Similar to plasmonic nanomaterials, GO and rGO show photothermal conversion properties in response to NIR irradiation that has been widely explored as a therapeutic strategy for treating cancers. In this context, rGO has been found to be much more efficient than GO because of the

Fig. 1. Structures of graphene-based materials show (a) the pristine graphene (pure-arranged carbon atoms) with sp^2 -hybridized carbon atoms, and the chemically modified graphene, including (b) GO; (c) rGO and (d) graphene quantum dot (GQD). Reproduced with permission from Ref. 7.

presence of more defects and greater aromaticity in its structure that are introduced into it during reduction of GO.⁵ Owing to these excellent physicochemical and biocompatible properties, graphene-based nanomaterials have found a wide variety of applications in the field of biomedicine, mostly in cancer research, ranging from serving as nanocarriers and photoagents for drug/gene delivery to cancer cells and for photothermal and photodynamic therapies of cancer, both *in vitro* and *in vivo*, till its use as bioimaging agents for developing multifunctional theranostic platforms for carrying out more efficient detection and treatment of cancers.¹⁰

In this review, we provide a detailed and systematic description of the journey that graphene-based nanomaterials have undertaken in the field of biomedical sciences in the last decade by discussing the different applications in drug delivery, gene delivery, phototherapy, bioimaging and theranostics. The main objective of this review is to make the readers understand the evolution of graphene as a nanomedicine over the years and appreciate the immense potential that this nanomaterial holds for the future.

2. Graphene-Based Nanomaterials for Drug Delivery

In the recent years, graphene-based nanomaterials such as GO and rGO have emerged as excellent nanocarriers for drug delivery owing to their structural and surface properties that make them very suitable for biomedical applications.^{10,11} The monolayer 2D structure of GO and rGO provide ultrahigh specific surface area and delocalized π electrons on the surface that allow highly efficient loading of hydrophobic anti-cancer drugs through hydrophobic interactions and π - π stacking, and also provide different oxygen containing functional groups for easy biofunctionalization.^{12–15} In this section, we discuss the different drug delivery applications of graphene-based nanomaterials under two broad categories — (A) Direct drug delivery systems and (B) Stimulus responsive drug delivery systems, to understand the different strategies by which graphene and its derivatives have been exploited for improving cancer chemotherapy. Direct drug delivery systems involve delivery of drugs to a cell or tissue either in a targeted or non-targeted manner without any control on the delivery mechanism. On the other hand, stimulus responsive drug delivery systems provide a controlled and systematic release of drugs from the nanocarrier in response to a specific stimulus, which can be both internal such as pH, redox, etc., as well as external such as temperature, light, magnetic and electrical field, etc. Table 1 summarizes the different graphene-based drug delivery systems developed so far.

2.1. Direct drug delivery systems

2.1.1. Non-targeted drug delivery

Delivery of anti-cancer drugs using GO as a nanocarrier was reported for the first time by Liu *et al.* in 2008.¹⁶ They used PEGylated GO to load a hydrophobic

Table 1. Summary of graphene-based nanomaterials for drug delivery.

Nanocomposite	Drug	Target	Reference
PEG-GO	SN38	HCT-116 cells	16
	DOX		17
	DOX & CPT	MCF-7 cells	18
nGr-PLA-PEG	PTX	U-138 glioblastoma cells	19
PEG-GO	PTX	A549 & MCF-7 cells	20
PLA-PEG-GO	PTX	A549 cells	21
PDA-GO-FA	DOX		22
HA-GO	DOX	KB epidermal carcinoma cells and tumors	23
HA-GO	DOX	HeLa tumors	24
Tf-PEG-GO	DOX	Glioma	25
CTX-GO	DOX	Glioma	26
GONR-PEG-DSPE	Lucanthone	EGFR positive glioblastoma multiforme cells	29
GO/PVA hydrogel	Vitamin B12		30
GO-CS-FA	DOX		31
Pluronic F38, Tween80 and maltodextrin functionalized GO	Ellagic acid	MCF-7 & HT29 cells	32
GO	ADR	MCF-7/ADR	33
Lipid monolayer membrane functionalized graphene sheets	DOX		34
GN-CNT-Fe ₃ O ₄	5-FU	HepG2 cells	35
HA-GO	DOX	H22 hepatic carcinoma tumors	36
Albumin-GO	PTX		37
rGO-C6H4-CO-NH-PEI-NH-CO-CD-Biotin	DOX	HepG2 cells	38
GO-Abs/PEI/PAH-Cit	DOX	U87 MG & MCF-7 cells	39
NGO-SS-mPEG	DOX	HeLa cells	40
PEG-GO	DOX	A549 cells	41
PEG-BPEI-rGO	DOX	PC-3 cells	42
Graphene/Fe ₃ O ₄ composites	DOX		43
Fe ₃ O ₄ /GNS	Rhodamine B		44
rGO/PVA hydrogel	Lidocaine hydrochloride		45

anti-cancer drug SN38 onto the surfaces of GO through π - π stacking and hydrophobic interactions. This SN38 loaded GO-PEG nanocarrier was then used to deliver the drug to HCT-116 colon cancer cells *in vitro*. It was observed that the nanocarriers could efficiently deliver the drug into the cancer cells and demonstrated 2–3 fold improvement in the therapeutic efficiency as compared to the water soluble prodrug of SN38, irinotecan. In the same year, Yang *et al.* also showed highly efficient loading and delivery of another very commonly used hydrophobic cancer drug, doxorubicin using GO as a nanocarrier.¹⁷ To further improve the therapeutic efficiency, Zhang *et al.* loaded both doxorubicin and camptothecin onto GO sheets and demonstrated efficient killing of MCF-7 breast cancer cells by combining the efficacies of both drugs into a single platform.¹⁸ Moore *et al.* demonstrated drug delivery to glioblastomas

using biodegradable polylactic acid (PLA) coated nanographene (nGr) functionalized with PEG (nGr-PLA-PEG) as a biocompatible drug delivery system.¹⁹ nGr-PLA-PEGs could encapsulate the anticancer drug Paclitaxel (PTX) at a high loading efficiency and showed controlled release of the drug over a period of 19 days. PTX loaded nGr-PLA-PEGs exhibited 20% killing of U-138 glioblastoma cells *in vitro* and *in vivo* biodistribution studies revealed accumulation of nGr in intracranial U-138 glioblastoma xenografts and organs of the reticuloendothelial system. In a recent study, PEGylated GO was used as a nanocarrier for the delivery of PTX to A549 human lung cancer cells and MCF-7 breast cancer cells *in vitro*.²⁰ PTX was first attached to a 6-armed PEG which was then covalently conjugated to GO sheets. The GO-PEG-PTX nanocomposites showed efficient cellular uptake and greater cytotoxicity against the cancer cells as compared to free PTX (Fig. 2(i)). PLA-PEG stabilized GO nanocomposites were developed and used as a drug delivery vehicle by Angelopoulou *et al.* for delivering PTX to A549 human lung cancer cells in culture.²¹ The nanocomposites showed efficient loading and sustained drug release properties along with significant cellular uptake and cytotoxicity against the cancer cells.

2.1.2. Targeted drug delivery

Cancer cell specific targeted drug delivery systems have also been developed using graphene-based nanomaterials for further increasing the therapeutic efficiency by selectively delivering the drugs into cancer cells, thereby minimizing the dose of the therapeutic load and also avoiding unnecessary side-effects to normal cells. Lin *et al.* reported the synthesis of folic acid decorated, polydopamine capped GO nanocomposites by a one-pot method and demonstrated targeted delivery of doxorubicin to cancer cells *in vitro* with high therapeutic efficiency.²² Cholesteryl hyaluronic acid (HA) conjugated GO loaded with doxorubicin was developed by Miao *et al.* for targeted delivery of doxorubicin to KB epidermal carcinoma cells both in *in vitro* and *in vivo*.²³ *In vitro* studies showed that the targeted nanocomposite induced 40.3% increase in cell death as compared to the non-targeted one. Similarly, subcutaneous injection of the targeted nanocomposites in tumor bearing athymic mice resulted in 14.1% decrease in the tumor volume within 24 days. In another study, HA was linked to GO via an adipic acid dihydrazide (ADH) linkage to form HA tagged GO-based targeted drug delivery system that was used for delivering doxorubicin to mice with subcutaneous HeLa tumors.²⁴ Within 16 days, the targeted nanocomposites showed 12% and 17% greater tumor inhibition rates as compared to free doxorubicin and non-targeted nanocomposites, respectively. In addition to FA and HA, transferrin (Tf) protein is also used as a targeting agent. Liu *et al.* synthesized Tf conjugated PEGylated nanographene oxide composites loaded with doxorubicin (Tf-PEG-GO-Dox) for targeted delivery of drug to glioma cells.²⁵ The nanocomposites showed a drug loading ratio of up to 115.4% and demonstrated significantly higher cytotoxicity against gliomas as compared to free drug and PEG-GO-Dox, both in *in vitro*

Fig. 2. Graphene-based nanomaterials for drug delivery. (i) Non-targeted drug delivery system (a) Preparation of GO-PEG-PTX Nanoscale Drug Delivery System. Differential interference contrast (DIC), FITC, DAPI, and merge (FITC + DAPI) images of A549, (b) and MCF-7, (c) cells incubated with GO-PEG-FITC for 3 h; scale bar 25 μm , (d) Relative cell viability of MCF-7 cells after treatment with different concentrations of PTX and GO-PEG-PTX for 72 h. Reproduced with permission from Ref. 20. Copyright 2014 American Chemical Society. (ii) Targeted drug delivery system (a) Schematic representation of targeted delivery of Tf-PEG-GO-Dox to cells *in vitro* and *in vivo*. (b) C6 viability-concentration curves for Dox, PEG-GO-Dox, and Tf-PEG-GO-Dox. (c) Glioma volume in different therapeutic groups on the 14th day. Reproduced with permission from Ref. 25. Copyright 2013 American Chemical Society. (iii) Internal-stimulus responsive drug delivery system. Schematic diagram showing antitumor activity of redox-sensitive DXR-loaded NGO-SS-mPEG Reproduced with permission from Ref. 40. Copyright 2012 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (iv) External-stimulus responsive drug delivery system. Schematic representation of photothermally triggered cytosolic drug delivery via endosomal disruption using PEG-BPEI-rGO. Reproduced with permission from Ref. 42. Copyright 2013 American Chemical Society.

(iii) Internal Stimuli Responsive DDS **(iv) External Stimuli Responsive DDS**

Fig. 2. (Continued)

and *in vivo* (Fig. 2(ii)). Another glioma targeted drug delivery system was developed by Wang *et al.*, where they conjugated GO with chlorotoxin (CTX-GO) as the targeting agent for selective delivery of doxorubicin to glioma cells.²⁶ Chlorotoxin is a toxin derived from *Leiurus quinquestratus* having high affinity for matrix-metalloprotease-2 proteins that are known to be overexpressed in gliomas. CTX-GO loaded with doxorubicin showed higher accumulation of drug inside gliomas and caused greater cytotoxicity against glioma cells as compared to free drug and GO-Dox. A new type of graphene derivative referred to as graphene oxide nanoribbons (GONR) was synthesized by Chowdhury *et al.* by using carbon nanotubes (CNT) as the precursor material.²⁷ These GONRs upon functionalization with PEG-DSPE (1, 2-distearoyl-sn-glycero-3 phosphoethanolamine) (GONR-PEG-DSPE) showed cancer cell targeted cytotoxicity without the use of a targeting agent. The targeting specificity was found towards cells overexpressing EGFR and this property of GONRs was exploited by the group to selectively deliver lucanthone

(apurinic endonuclease-1 (APE-1) inhibitor) to EGFR over-expressing glioblastoma multiforme cells in culture.^{28,29} Lucathone-GONR nanocomposites showed significant cytotoxicity against glioblastoma multiforme cells but demonstrated no toxicity to EGFR negative glial precursor cells, thereby showing the specificity of GONRs as a novel drug delivery system.

2.2. Stimuli-responsive drug delivery systems

Stimulus-responsive drug delivery systems allow for a controlled release of drugs at a particular site only in the presence of a specific stimulus, that directs the nanocarrier system to release its therapeutic payload. Controlled release of drugs improves the therapeutic efficiency of the system by minimizing the chances of premature and burst release of drugs in the circulation. Stimulus can be either internal or external. Internal stimulus refers to the changes in the intracellular environment such as pH, redox, etc., that can trigger release of drugs from nanocarriers specifically designed to respond to such stimuli, or it can respond to external stimuli such as light, temperature, magnetic and electrical field.

2.2.1. Internal stimuli responsive systems

Several graphene-based drug delivery systems have been developed in the recent years that are responsive to internal stimulus like pH and redox. Cancer cells and tumor microenvironments are known to have an acidic pH and high concentrations of redox enzymes like GSH as compared to surrounding healthy cells and tissues. So, these two intracellular stimuli have been exploited to efficiently modulate and control the release of drugs from nanocarriers specifically inside the tumor cells and tissues. Bai *et al.* reported a pH-sensitive composite hydrogel consisting of GO and poly (vinyl alcohol) (GO/PVA) that showed pH-responsive gel-sol transition properties, thereby allowing efficient loading and release of drugs at different pH conditions.³⁰ Depan *et al.* developed a doxorubicin loaded GO-chitosan-FA nanocomposite (DOX-GO-CH-FA) and studied its drug release properties in response to different pH values.³¹ It was observed that the nanocomposites showed higher and faster release of DOX at an acidic pH than physiological pH, and also showed morphological transition from non-porous to porous structure after drug release. Similarly, a biocompatible polymer pluronic F38, Tween80 and maltodextrin functionalized GO nanocomposite was prepared by Kakran *et al.* which showed good loading of a poorly water soluble anti-cancer drug ellagic acid and increased release profiles at acidic pH.³² In an interesting study by Wu *et al.*, GO was used as carrier for adriamycin (ADR) to overcome ADR resistance in MCF-7/ADR breast cancer cells *in vitro*.³³ GO showed a high ADR loading efficiency of around 93.6% and demonstrated pH-dependent release of ADR. The *in vitro* studies revealed that GO could efficiently deliver the drug into MCF-7/ADR cells and have significantly higher cytotoxicity against the drug resistant cancer cells in comparison to free ADR. Lipid monolayer membrane functionalized graphene sheets were developed by

Liu *et al.* as highly efficient physiologically stable pH responsive drug delivery system, that showed excellent DOX loading capacity and acidic pH stimulated controlled release of the drug.³⁴ Fan *et al.* reported the synthesis of a graphene-CNT-Fe₃O₄ nanoparticle hybrid (GN-CNT-Fe₃O₄) and showed its pH-dependent drug release properties.³⁵ 5-FU loaded GN-CNT-Fe₃O₄ nanohybrids showed high toxicity against HepG2 cells *in vitro*. Another targeted pH responsive drug delivery system was reported by Song *et al.* comprising of HA linked GO nanosheets that showed an acidic pH responsive DOX release property, which caused 17% higher tumor inhibition in H22 hepatic carcinoma cell tumors in mice as compared to only GO loaded DOX groups.³⁶ In addition to these studies, there are several other reports on the development of pH responsive graphene-based drug delivery systems that further highlight the potential and importance of graphene-based nanomaterials in drug delivery.^{37–39}

Wen *et al.* exploited the high levels of glutathione (GSH) in cancer cells and tumors as a stimulus to induce drug release and developed a NGO-based drug delivery system consisting of a redox-responsive PEG shell attached to NGO sheets via disulfide linkages.⁴⁰ In presence of GSH in the environment, the PEG shells got detached from the system due to disulfide bond breakage and caused 1.55 times faster drug release than in the absence of GSH (Fig. 2(iii)). Another study reported GSH-triggered anti-cancer drug release from PEGylated graphene oxide (PEG-GO) nanocarriers, both in *in vitro* and *in vivo*.⁴¹

2.2.2. External stimuli responsive systems

A NIR responsive photothermally triggered cytosolic drug delivery system was developed by Kim *et al.* using PEG and BPEI dual polymer functionalized rGO nanosheets (PEG-BPEI-rGO).⁴² The PEG-BPEI-rGO nanocomposites showed high drug loading efficiency with absorbance in the NIR region. Drug release was mediated by both GSH and photothermal effect of NIR light irradiation. It was observed that after cellular uptake, the nanocomposites escaped the endosomes via photothermally induced endosome disruption and proton sponge effect and subsequently released the drug into the cytosol in response to high GSH concentrations inside cancer cells (Fig. 2(iv)). This photothermal effect on the drug release was found to cause more cytotoxicity against cancer cells in comparison to those not irradiated with NIR light.

Graphene-based nanocomposites containing magnetite or iron oxide nanoparticles can be effectively used as a drug delivery system responsive to an external magnetic field. This was shown by Zhou *et al.* who synthesized graphene/Fe₃O₄ composites through a simple one-pot solvothermal process.⁴³ These graphene/Fe₃O₄ composites showed good water dispersibility alongwith excellent magnetic properties ideal for a magnetically guided drug delivery system. Doxorubicin was loaded onto these composites with a high loading efficiency of about 65%. The release of drugs from these composites could be controlled and guided by an external magnetic field.

In another study, Li *et al.* reported the synthesis of Fe_3O_4 nanoflowers encapsulated in graphene nanosheets ($\text{Fe}_3\text{O}_4/\text{GNS}$) and demonstrated the feasibility of these nanocomposites to be used as drug delivery systems under the influence of an external magnetic field by loading Rhodamine B onto them with a high loading efficiency.⁴⁴

Apart from magnetic field, electric field can also be used as a stimulus to control drug delivery. Liu *et al.* reported the development of an electrically modulated drug delivery system comprising of rGO sheets embedded in a matrix of PVA, forming a hydrogel.⁴⁵ Lidocaine hydrochloride was loaded into this hydrogel as a model drug and its release properties were studied under the influence of an external electric field. In the absence of an electrical stimulus, drug release was not observed. Drug release occurred only in the presence of electric field, thereby demonstrating the electrical stimulus responsive properties of this hydrogel.

3. Graphene-Based Nanomaterials for Nucleic Acid Delivery

The most sought after vectors for carrying out efficient gene delivery to cells, both *in vitro* and *in vivo* have been viral vectors because of their very high transfection efficiency.⁴⁶ However, concerns related to the safety and biocompatibility of these viral vectors such as chances of random genomic integration, activation of oncogenes, stimulation of unwanted immune response in the host and re-activation of the inactivated viruses have limited the large scale application and clinical translation of these vectors for gene therapy.⁴⁶ So, for the development of safer vectors, researchers have moved towards non-viral approaches of gene delivery.⁴⁷ Different types of materials such as cationic polymers, lipids, polysaccharides and dendrimers have been explored for the development of non-viral vectors, which demonstrated their abilities to carry and deliver genes into cells and tissues, however could not reach the high level of transfection efficiencies as shown by viral vectors.⁴⁷ In addition to this, cytotoxicity and biocompatibility issues were also observed with cationic lipids and polymers due to their high density of surface positive charges that are required for efficient nucleic acid complexation. Thus, the search for an efficient and safe vector which can provide high level of transfection efficiency without inducing any toxicity or undesirable side-effects in the host is still on.

The structural, optical and biocompatible properties of graphene and its derivatives such as GO and rGO have raised a lot of interest in this material in the recent years, that have led to studies related to the synthesis and development of non-viral vectors using graphene-based materials as a novel carrier system for gene delivery.⁴⁸ Small size, high surface area to mass ratio, easy surface functionalization, ability to load and condense nucleic acids even in the absence of cationic groups through π - π interactions, protection of nucleic acids from endonuclease digestion, high cellular uptake and low toxicity has made graphene and its derivatives a suitable candidate for gene delivery.⁵ The ease of functionalization of these materials with cationic polymers and targeting moieties to improve their biocompatibility, nucleic acid

loading efficiency and overall therapeutic efficacy is due to the presence of numerous oxygen containing functional groups on the surface of these materials that allow for easy conjugation and derivatization.^{5,8} Covalent and non-covalent functionalization of GO and rGO nanosheets with cationic polymers like poly-ethylenimine (PEI), polysaccharides like chitosan and dendrimers such as poly-amidoamine (PAMAM) have been carried out to develop nanoconstructs with improved nucleic acid binding efficiency and physiological stability for achieving better transfection and therapeutic outcomes.^{8,48} The cationic molecules provide a net positive charge on the surface of the nanoconstructs that help in improving the nucleic acid condensation efficiency through electrostatic interactions. Incorporation of PEG alongwith these groups have also shown to improve the aqueous stability and biocompatibility of these nanoconstructs. In addition to this, these grafting strategies of cationic polymers and dendrimers onto the surface of GO and rGO also helped to minimize their cytotoxicity by decreasing the concentration of the positive charges in the nanoconstructs without affecting the efficient nucleic acid binding capacities.

The first attempt towards successful delivery of a nucleic acid using graphene-based nanomaterial as a vector was made by Lu *et al.*⁴⁹ The group used NGO sheets to deliver a molecular beacon (MB) to HeLa cells in culture for intracellular sensing of the expression level of survivin gene at the mRNA level (Fig. 3(i)). A molecular beacon is basically a single stranded DNA folded into a hairpin structure having a self-complementary stem alongwith a single stranded loop that is complementary to a specific target. In the closed conformation, the stem brings together a fluorophore and a quencher attached to them in close proximity resulting in fluorescence quenching. However, in the presence of the target sequence, the MB undergoes a conformational change and opens up to hybridize with the target, thereby taking the fluorophore-quencher pair away from each other, which ultimately leads to restoration of the fluorescence. This fluorescence off/on mechanism is used as a probe to detect the presence of a target sequence. In this study, the authors observed that the NGO sheets not only showed efficient loading of the MB on its surface, but also protected it from endonuclease digestion. In addition to this, the MB retained its target sequence binding ability even after loading onto NGO which was evidenced by the appearance of fluorescence inside the HeLa cells, thereby also demonstrating successful intracellular delivery of the nucleic acid payload. Simultaneously in the same year, Wang *et al.* also demonstrated real-time biosensing of ATP in JB6 cells using an aptamer/GO nanocomplex.⁵⁰ Apart from mRNA and biomolecule sensing, PEI grafted graphene nanoribbons (PEI-GNR) loaded with MB were also used as molecular probes by Dong *et al.* for sensing of miR-21 in cells.⁵¹ The PEI-GNR showed more efficient delivery of MB as compared to PEI-MWCNTs and PEI alone. In a follow up study by the same group, the authors developed a multifunctional poly (L-lactide)-Polyethylene glycol-grafted graphene quantum dot-based platform for intracellular sensing of miR-21 combined with fluorescent tracking of the internalization of the complexes using GQDs.⁵²

Fig. 3. Graphene-based nanomaterials for nucleic acid delivery. (i) Molecular probing. Schematic representation of NGO delivery of MB into HeLa cells to detect survivin mRNA. Reproduced with permission from Ref. 49. Copyright 2010 Royal Society of Chemistry. (ii) Gene delivery. (a) Schematic illustration of photothermal transfection mechanism. (b) Transfection of the BPEI (1.8 and 25 K), PEG-BPEI-GO and PEG-BPEI-rGO with/without NIR irradiation in PC-3 and NIH/3T3 cell lines. Reproduced with permission from Ref. 60. Copyright 2013 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim (iii) mRNA delivery. Efficient reprogramming from fibroblasts into iNs by GO-PEI-mRNA complexes; (a) Representative images and (b) percentages of Tuj1-positive iNs induced by lentiviral ABM, GO-PEI-ABM, and Lipofectamine ABM; (c) qRT-PCR analysis of the neuronal genes Tuj1, Map2, NeuroD1, and Syp in Lentiviral ABM and GO-PEI-ABM transduced fibroblasts; Three independent experiments were performed in triplicate. Data are presented as means $\pm$ standard errors of the mean (SEM); Student's *t*-test; **p* < 0.05, ***p* < 0.01. (d) Immunofluorescence of Map2, NeuN, PSD97, and vGlut1 in GO-PEI-ABM-induced neurons; Scale bars, 100 μ m. (e) Flow cytometry analysis of the induction of Tau-eGFP+ cells by GO-PEI-ABM; proportions of Tau-eGFP+ cells among treated cells are indicated as percentages. (f) GO-PEI-ABM complexes induced iNs expressed Syp, vGlut1, and Glutamate; Scale bars, 100 μ m. (g) Number of Syp, vGlut1, and Glutamate positive iNs induced by Go-PEI-ABM complexes. (h) Confirmation of transgene integration using genomic-DNA PCR; Lane 1, Lipofectamine-ABM transfected fibroblasts; Lane 2, lentiviral-ABM infected fibroblasts; Lane 3, GO-PEI-ABM transfected fibroblasts. Reproduced with permission from Ref. 61. Copyright 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

(iii) mRNA Delivery

Fig. 3. (Continued)

In addition to molecular sensing, graphene-based nanovectors have been extensively used for the delivery of foreign genes encoded in plasmid DNAs for expression in cells. However, most of these works have focussed mainly on the delivery of plasmid DNA encoding a fluorescent protein like EGFP to demonstrate the loading and transfection efficiencies of different surface functionalized graphene-based nanovectors, without any emphasis on therapeutic application. For example, Chen *et al.* used GO sheets covalently functionalized with BPEI as a gene delivery tool for delivering plasmid DNA encoding EGFP to HeLa cells.⁵³ Similarly, in another study, Kim *et al.* used PEI functionalized GO sheets for delivering luciferase gene into cells for bioimaging applications.⁵⁴ Several other studies utilized this PEI-GO

nanoconstruct as a vector and demonstrated highly efficient delivery of EGFP plasmid DNA into cells *in vitro*, either in a non-targeted or targeted manner.^{55–58} Recently, the concept of photothermally enhanced gene delivery was introduced for the first time by Feng *et al.* followed by a subsequent study by Kim *et al.*, who independently showed that the transfection efficiency of graphene-based nanovectors could be improved significantly by exploiting the NIR responsive photothermal properties of GO and rGO at the time of transfection.^{59,60} Feng and co-workers developed a PEG-PEI dual polymer functionalized nGO-based composite (NGO-PEG-PEI) and demonstrated highly efficient and greater EGFP transfection efficiency as compared to GO-PEI and PEI alone, in presence of low power NIR irradiation due to the localized photothermal heating that enhanced the cellular uptake of the nanocarriers as a result of increased cell membrane permeability, without inducing any cytotoxicity.⁵⁹ They further utilized this nanocarrier for siRNA delivery into cells and demonstrated enhanced downregulation of the target gene (Plk1) in presence of NIR laser mediated transfection. In a subsequent work, Kim *et al.* reported the application of PEG-PEI-rGO nanocomposites for photothermally enhanced gene delivery and further demonstrated that the enhancement in transfection efficiency upon NIR irradiation was caused due to accelerated endosomal escape of polyplexes augmented by locally induced heat (Fig. 3(ii)).⁶⁰ Li *et al.* reported a new method of gene delivery using patterned nGO matrixes as cell culture substrates onto which PEI/pDNA complexes were adsorbed. These substrates showed gradual release of the PEI/pDNA complexes which resulted in transfection of the cells being cultured on those substrates.⁶¹ In a remarkable study conducted by Khademhosseini and group, PEI functionalized GO nanosheets (fGO) incorporated in methacrylated gelatin (GelMA) hydrogel was used as a carrier (fGO_{VEGF}/GelMA) for vascular endothelial growth factor-165 (VEGF) gene delivery for myocardial therapy.⁶² In a rat model of myocardial infarction, the fGO_{VEGF}/GelMA hydrogels were injected intramyocardially in the peri-infarcted regions, which showed a significant increase in the microcapillary density in the infarcted region alongwith a reduction in scar area and improvement in cardiac function as compared to controls, without invoking any inflammatory and toxic responses in the host. This study demonstrated the immense potential of graphene-based materials to serve as non-viral vectors in gene therapy. In another equally interesting study published recently by Choi *et al.*, GO-PEI nanocomposites were exploited for the first time as mRNA delivery vectors for the generation of “footprint-free” induced pluripotent stem cells (iPSCs).⁶³ Footprint-free iPSCs are very important from a clinical point of view because their creation do not involve genomic integration of the reprogramming factor genes, thereby minimizing the risk of insertional mutagenesis in humans. GO-PEI nanocomplexes showed highly efficient loading of mRNA of reprogramming transcription factors (Oct3/4, So2, Klf4, cMyc) and also provided protection to the mRNAs from RNase degradation. In addition to this, the GO-PEI/RNA complexes could transfect adult adipose tissue-derived fibroblasts in suspension cultures with a high efficiency and successfully generated both rat and human iPSCs from them. The

generated iPSCs showed all markers of pluripotency like expression of pluripotency genes, epigenetic reprogramming, and differentiation into the three germ layers. This study laid the framework for the application of graphene-based materials as non-viral mRNA delivery vectors for generation of iPSCs and for potential applications in direct cell-reprogramming-based therapies for the treatment of a wide variety of diseases. Two very recent works demonstrated the application of GO-PEI complexes as mRNA and gene delivery vectors for direct reprogramming of human somatic cells into cells of neural and mesodermal lineage.^{64,65} Baek *et al.* demonstrated the reprogramming of fibroblasts into integration-free induced neurons (iNs) through the transfer of mRNA of neural lineage specific reprogramming factors using GO-PEI complexes, that alleviated Parkinson’s disease symptoms in a mouse model (Fig. 3 (iii)).⁶⁴ In another study, Chiang *et al.* demonstrated the direct reprogramming of human peripheral blood mononuclear cells into cells of the mesodermal lineage using

Table 2. Summary of graphene-based nanomaterials for nucleic acid delivery.

Nanocomposite	Nucleic acid cargo	Target	Reference
NGO	Molecular beacon for surviving gene sensing	HeLa cells	49
GO	Aptamer for ATP sensing	JB6 cells	50
PEI-GNR	Molecular beacon for miR-21 sensing	HeLa cells	51
PLA-PEG-GQD	Molecular beacon for miR-21 sensing, miR-21 and surviving targeting antisense ODNs	HeLa cells	52
BPEI-GO	pEGFP	HeLa cells	53
PEI-GO	Luciferase gene	HeLa & PC-3 cells	54
PEI-GO	pEGFP	HeLa & 293 T cells	55
PEI-GO	pEGFP	H293T, U20 cells, zebrafish embryos	56
PEI-GO	pEGFP	HeLa cells	57
PEI-GO	pEGFP	HeLa cells	58
NGO-PEG-PEI	pEGFP, Plk1 siRNA	HeLa cells	59
PEG-PEI-rGO	Luciferase gene	NIH3T3 and PC-3	60
nGO matrix	pGFP, luciferase	HEK293T, HeLa & hMSC	61
PEI-GO/GelMA hydrogel	VEGF-165 gene	Mouse model of myocardial infarction	62
PEI-GO	mRNA of Oct3/4, So2, Klf4, cMyc transcription factors	Adipose tissue derived fibroblasts	63
GO-PEI	mRNA of neural lineage specific transcription factors	Fibroblasts	64
GO-Fe ₃ O ₄ -PEI	Episomal plasmids encoding pluripotent related factors	PBMC	65
GO-CS	Luciferase gene	HeLa cells	66
GO-PAMAM	Luciferase gene	HeLa & MG63 fibroblasts	67
GO-PEI	pGFP & anti-pGFP siRNA	HEK293 & HeLa cells	68
GO-PEI	anti-CXCR4 siRNA	MDA-MB-231 cells	69
rGO-PL-PEG-Octarginine	Cell death siRNA	MCF-7 cells	70

GO-Fe₃O₄-PEI complexes carrying three episomal plasmids encoding pluripotent related factors via a process of combined magnetic stirring and photothermal stimulation to enhance the transfection efficiencies of the complexes in suspension culture.⁶⁵ The transfected PBMCs were reprogrammed into iPSCs, which rapidly transdifferentiated into mesodermal lineages without full reprogramming.

Chitosan functionalized GO nanovector was also explored as a gene delivery vector by Bao *et al.* for delivering luciferase gene into HeLa cells.⁶⁶ However, the transfection efficiency of such vectors was not found to be as high as PEI. Cationic dendrimers like PAMAM have also been explored for gene delivery. Liu *et al.* developed a PAMAM and oleic acid functionalized GO nanocomposite and demonstrated transfection of HeLa and MG63 fibroblast cells with luciferase gene at a high transfection efficiency.⁶⁷ These composites also showed good physiological stability.

Graphene-based nanomaterials have also been explored for delivering siRNAs to cells *in vitro* for downregulating the expression of specific genes that are found to be overexpressed in pathological conditions such as cancer. Tripathi *et al.* used GO-PEI nanocomplex for delivering GFP expressing pDNA to cells in culture and using the same vector delivered a anti-GFP siRNA 3 h later.⁶⁸ The authors reported 70% knockdown in the fluorescence levels. Using a similar GO-PEI nanocomplex, Huang *et al.* recently demonstrated suppression in cancer cell migration through efficient knockdown of CXCR4 gene using anti-CXCR4 siRNA, which is strongly upregulated in cancer metastasis.⁶⁹ In another recent study, Imani *et al.* reported the synthesis of a rGO-based nanocarrier functionalized with phospholipid-PEG and conjugated with a cell penetrating peptide octarginine.⁷⁰ The nanocarrier was used to selectively deliver a cell death siRNA to MCF-7 cells *in vitro*. The nanocarriers showed good nucleic acid binding, endonuclease protection, high transfection efficiency, superior buffering capacity in the acidic pH range of 4–6 and induced 51% cell death in treated cells as compared to non-treated ones. Table 2 summarizes the different graphene-based nucleic acid delivery systems developed so far.

4. Graphene-Based Nanomaterials for Phototherapy

Graphene and graphene-based materials such as GO and rGO have emerged as excellent nanomaterials for carrying out photothermal and photodynamic therapies of cancer because of their surface chemistry and intrinsic optical properties. Photothermal therapy is a widely exploited non-invasive cancer therapy procedure that involves the absorption of light of a particular wavelength especially NIR by nanomaterials or dyes or a combination of both, which are capable of converting this light energy into heat for photothermal killing or ablation of cancer cells and tumors, both *in vitro* and *in vivo*.⁷¹ Large surface area, presence of *sp*² clusters on the crystal lattice, strong absorption in the NIR region and small size have made both GO and

rGO to act as excellent photothermal agents in response to NIR irradiation and generate heat through plasmonic photothermal conversion.⁷² The NIR absorbance of rGO has been found to be six times more as compared to GO because of the highly intact aromatic structure of rGO created during reduction process. On the other hand, photodynamic therapy that involves the use of light responsive photosensitizers as therapeutic agents for generation of highly cytotoxic reactive oxygen species (ROS) upon irradiation with light, have also attracted a great deal of attention in cancer therapy. GO and rGO have been used as scaffolds or carriers for loading and delivering these photosensitizers into cancer cells and tissues for photodynamic cancer therapy.⁷³ The presence of oxygen containing functional groups on both surfaces of GO nanosheets make them ideal platforms for highly efficient loading of hydrophobic as well as hydrophilic photosensitizer molecules through several non-covalent interactions such as π - π interactions, hydrophobic interactions, hydrogen bonding and electrostatic interactions. The following section describes the progress that has been made in the development of different kinds of graphene-based nanomaterials as efficient photoagents for successful photothermal and photodynamic therapy of cancers. Table 3 summarizes the different phototherapeutic applications of graphene-based nanomaterials.

4.1. Photothermal therapy

The Liu group was the first to report the photothermal properties of PEGylated nano-GO in a tumor model of mice.⁷⁴ The group demonstrated that nGO-PEG exhibited enhanced absorption in the NIR region and caused complete photothermal ablation of tumors in mice, intravenously injected with the nanocomposites in response to NIR irradiation. Robinson *et al.* reported the use of rGO as a photothermal agent for the first time and showed that non-covalently PEGylated nanosized rGO sheets were more efficient in the photothermal conversion as compared to covalently PEGylated GO sheets.⁷⁵ The group demonstrated targeted photothermal ablation of U87MG cancer cells *in vitro* by using RGD peptide tagged PEGylated nano-rGO in response to NIR irradiation. The nanocomposites showed negligible toxicity in the absence of NIR irradiation, thereby establishing itself as novel biocompatible photothermal agents for cancer therapy. The Liu group carried out a detailed study to understand the effects of size and surface chemistry of graphene sheets on the photothermal properties and further validated the findings of Robinson *et al.* that non-covalently PEGylated nanosized reduced graphene oxide sheets were more efficient in the photothermal conversion as compared to covalently PEGylated GO sheets.⁷⁶ In their study, Liu and co-workers demonstrated 100% photothermal ablation of tumors in mice injected with nRGO-PEG and irradiated with a low-power NIR laser (0.15 W/cm^2), which was one order of magnitude lower than that normally used for *in vivo* photothermal ablation by other nanomaterials. Following a different approach, Akhavan *et al.* reported a novel strategy for the synthesis of reduced graphene oxide by using glucose as both, reducing and functionalizing agent.⁷⁷

Table 3. Summary of graphene-based nanomaterials for phototherapies.

Nanocomposite	Therapeutic modality	Target	Reference
nGO-PEG	PTT	4T1 tumor bearing Balb/c mice, KB, and U87MG tumor bearing nude mice	74
nRGO-PEG-RGD	PTT	U87MG cells	75
nRGO-PEG	PTT	4T1 tumor bearing mouse	76
RGO	PTT	LNCaP cells	77
nGO-ThS	PTT	Amyloid fibrils	78
RGO	PTT	HT29 & SW48	79
Cu ₂ O NC-RGO	PTT	HK-2, MDA-MB-231, A549 cells	80
AuNS@RGO	PTT	HUVECs	81
AuNR@RGO			
AuNR-PEG-GO	PTT	A431 cells and tumors in mouse	82
GON	PTT	AGS cells	83
NGO-HA	PTT	Skin cancer tumors in mouse	84
ZnFe ₂ O ₄ -rGO	PTT	Prostate cancer <i>in vitro</i> , glioblastoma tumors <i>in vivo</i>	85
MUC-1 aptamer-AuNP-GO	PTT	MCF-7 cells	86
GO-Cypate	PTT	4T1 cells <i>in vitro</i> & 4T1 tumor bearing mice	87
ZnPc-NGO-mPEG	PDT	MCF-7 cells	88
Ce6-GO-FA	PDT	MGC803 cells	89
GO-HB	PDT	SGC-7901, SMMC-7721, HeLa, and A549 cells	90
GO-HA	PDT	HeLa cells	91
GO/TiO ₂	PDT	HeLa cells	92
ZnO-GO-FA	PDT	HeLa cells	93
Ce6-GO-HA	PDT	HeLa cells	94
MB-GO	PDT		95

The glucose reduced GO showed excellent biocompatibility and improved NIR absorbance as compared to hydrazine reduced GO, single and multi-walled CNTs and demonstrated efficient photothermal killing of LNCaP prostate cancer cells *in vitro* at very low concentrations (0.05 mg/ml). Li *et al.* exploited the NIR responsive photothermal properties of nano-GO sheets to induce dissociation of amyloid fibrils (A β fibrils) by heat for the treatment of Alzheimer’s disease.⁷⁸ The benzothiazole dye Thioflavin S (ThS) which has an affinity to selectively bind to A β fibrils instead of A β monomers was covalently linked to the nGO sheets resulting in the formation of GO-ThS-A β , which upon irradiation with a low-power NIR laser showed dissociation of the A β fibrils. The dissociation of the fibrils was monitored by the fluorescence change of ThS. In another study, green tea-rGO nanosheets (GT-rGO) were developed and used for the photothermal destruction of HT29 and SW48 colon cancer cells *in vitro*.⁷⁹ Hou *et al.* reported selective photothermal killing of cancer cells *in vitro* under visible light irradiation using water dispersible Cu₂O nanocrystal-rGO hybrids as photothermal agents.⁸⁰ In order to enhance the photothermal properties of GO and rGO, several groups have reported the development of plasmonic

nanoparticle incorporated graphene-based nanocomposites. Lim *et al.* synthesized rGO coated plasmonic gold nanoshells and nanorods and demonstrated enhanced photothermal killing of cells *in vitro* as compared to non-coated or graphene oxide-coated gold nanoparticles.⁸¹ The enhanced photothermal efficiency of the nanocomposites can be attributed to the presence of plasmonic nanoparticles that significantly enhanced the NIR absorption efficiency of the nanocomposites. Gold nanorod-attached PEGylated graphene-oxide (AuNR-PEG GO) nanocomposites were synthesized by Dembereldorj *et al.* and used for photothermal therapy both in *in vitro* and *in vivo*.⁸² The presence of AuNRs significantly enhanced the NIR absorption efficiency of the nanocomposites which resulted in 40% reduction in cell viability of A431 epidermoid carcinoma cells and also reduced tumor volumes in mice. Femtosecond laser beam mediated photothermal destruction of cancer cells was demonstrated by Li *et al.* using graphene oxide nanoparticles (GONs).⁸³ The GONs generated microbubbles upon exposure to femtosecond laser beam that facilitated the killing of cancer cells. Jung *et al.* reported photothermal ablation of skin cancer cells using hyaluronic acid conjugated nano GO sheets.⁸⁴ The NGO-HA conjugates were delivered transdermally to tumor tissues in the skin of mice, where they showed increased tumor uptake and resulted in complete destruction of the tumors in response to a NIR laser without any further recurrence of tumors (Fig. 4(i)). Magneto-PTT of cancers was demonstrated by Akhavan *et al.* using zinc ferrite spinel (ZnFe_2O_4)-rGO nanocomposites (ZnFe_2O_4 -rGO) for carrying out *in vitro* and *in vivo* photothermal destruction of prostate cancer cells and glioblastoma tumors, respectively, under the guidance of a magnetic field.⁸⁵ An aptamer-gold nanoparticle-hybridized graphene oxide nanocomposite was developed by Yang *et al.* for targeted photothermal destruction of MCF-7 cells.⁸⁶ MCF-7 cells showed increased cellular uptake of the nanocomposites via specific interaction between MUC-1-binding aptamer and MUC-1 that are present on the cell membrane of MCF-7 cells. In the presence of NIR irradiation, the nanocomposites exhibited excellent photothermal ablation activity at very low concentrations. Recently, Guo *et al.* reported the synthesis of a novel pH-responsive graphene oxide-based photothermal agent for improved photothermal killing of cancer cells.⁸⁷ A cyanine dye (Cypate) was grafted onto the surface of GO, which participated in enhancing the photothermal properties of the composite via FRET in a pH-responsive manner. Under NIR irradiation, the Cypate molecules emitted near-infrared fluorescence (NIRF) that got transferred to the GO sheets *via* FRET and subsequently got converted into heat for improving photothermal therapy. A single dose intravenous injection of the GO-Cypate nanocomposites into a mouse bearing tumors followed by NIR irradiation resulted in significant photothermal destruction of the tumors.

4.2. Photodynamic therapy

PDT using GO was reported for the first time by Dong *et al.*⁸⁸ They used methoxy PEG conjugated nano graphene oxide (NGO-mPEG) as a platform for loading of a

(i) Photothermal Therapy

Fig. 4. Graphene-based nanomaterials for photo-therapy. (i) Photothermal therapy. (a) Schematic illustration for the transdermal delivery of nanographene oxide-hyaluronic acid (NGO-HA) conjugates into melanoma skin cancer cells and the following photothermal ablation therapy using a NIR laser. Photographs showing the effect of photothermal ablation therapy with NIR irradiation on the tumor growth in SKH-1 mice inoculated with B16F1 cells on both dorsal flanks after topical administration of (b) PBS, (c) NGO, and (d) NGO-hyaluronic acid (HA). (e) Relative tumor volume (V/V_0) with increasing time for a week. (f) Caspase-3 activity in tumor tissues by ELISA with increasing time for a day for the analysis of heat induced apoptosis ($***P < 0.0001$). Reproduced with permission from Ref. 84. Copyright 2014 American Chemical Society. (ii) Photodynamic therapy. MTT cell viability of HeLa cells treated with HA-GO/Ce6 nanohybrids ($\square$) or free Ce6 ($\bullet$) for 6 h, followed by the light irradiation of 1.8 J cm^{-2} (a), or without the light irradiation (b); LIVE/DEAD cell viability of HeLa cells treated with HA-GO/Ce6 nanohybrids or free Ce6 at a Ce6 concentration of $0.1 \mu\text{g mL}^{-1}$ for 6 h of treatment (c). Reproduced with permission from Ref. 94. Copyright 2013 Royal Society of Chemistry.

(ii) Photodynamic Therapy

Fig. 4. (Continued)

photosensitizer zinc phthalocyanine (ZnPc) onto the surface of NGO through π - π and hydrophobic interactions. The ZnPc-NGO-mPEG nanocomposites demonstrated significant cytotoxicity in MCF-7 cell lines in response to Xe light irradiation. Huang *et al.* reported the development of a targeted photodynamic agent by conjugating folic acid with GO sheets and loading it with the photosensitizer Chlorin e6 (Ce6).⁸⁹ The loading efficiency of Ce6 was found to be 80%, with FA conjugation helping in increasing the accumulation of the nanocomposites in MGC803 cells. The nanocomposites exhibited excellent targeted photodynamic killing of MGC803 cells in response to laser irradiation. Hypocrellin A (HA) and Hypocrellin B (HB) are another class of organic photosensitizers that have the potential to be used in PDT, but have gained limited attention due to their stability issues in aqueous conditions.

In order to improve their stability, Zhou *et al.* loaded HA and HB onto the surface of GO nanosheets (GO-HA and GO-HB) through hydrogen bonding and π - π interactions and observed improved stability of HA and HB as compared to their free forms in aqueous solutions.^{90,91} Further, *in vitro* studies using these GO-HA and GO-HB nanocomposites demonstrated excellent photodynamic damage to cancer cells in response to light. In addition to organic photosensitizers, inorganic photosensitizers such as TiO₂ and ZnO have also been explored for UV and visible light mediated PDT. Hu *et al.* developed a GO/TiO₂ hybrid and observed significantly increased production of intracellular ROS by the GO/TiO₂ hybrids when exposed to visible light.⁹² The authors explained that this enhanced photodynamic activity of GO/TiO₂ hybrids could be attributed to the excellent electrical conductivity of GO, which allowed the transfer of the photoelectrons generated by TiO₂ in response to light to the surface of GO that ultimately reacted with oxygen to generate ROS. In another study by the same group, folic acid-conjugated graphene-ZnO nanohybrid were synthesized for targeted PDT which showed efficient generation of ROS in HeLa cells in response to visible light irradiation, that ultimately resulted in apoptosis.⁹³ Li *et al.* reported the synthesis of hyaluronic acid-conjugated GO nanocomposites loaded with Ce6 for targeted PDT of cancer cells *in vitro* and observed 10-fold increase in the PDT efficiency as compared to free Ce6 molecules (Fig. 4(ii)).⁹⁴ Methylene blue (MB) loaded GO nanosheets have also been exploited as a PDT agent by Wojtoniszak *et al.*, where the authors reported enhanced efficiency in singlet oxygen generation by MB-GO as compared to pristine MB.⁹⁵

5. Graphene-Based Nanomaterials for Combined Therapeutic Strategies

5.1. Combined drug delivery and phototherapies

5.1.1. Combined chemo-PTT

The ability to load drugs efficiently onto the surface of graphene-based nanomaterials like GO and rGO due to the presence of a large surface area and also because of the presence of different oxygen containing functional groups, combined with their excellent absorbance in the NIR region have led to the development of multifunctional therapeutic platforms capable of providing synergistic chemo-PTT for better treatment of cancers. Zhang *et al.* developed a simple platform consisting of PEGylated NGO sheets loaded with the anti-cancer drug doxorubicin and demonstrated efficient killing of cancer cells both *in vitro* and *in vivo*.⁹⁶ The therapeutic efficiency of the combined treatment strategy was found to be much higher as compared to chemotherapy and PTT alone. Folic acid conjugated, poly-vinylpyrrolidone functionalized nanographene oxide sheets (FA-NGO-PVP) loaded with doxorubicin were synthesized by Qin *et al.*, which showed highly efficient targeted delivery of drug and generated heat for effective chemo-photothermal

destruction of tumors.⁹⁷ Another targeted platform was developed by Yang *et al.* comprising of PEGylated NGO conjugated with EGFR antibody for targeting and loaded with the anti-cancer drug epirubicin for carrying out a synergistic triple-therapeutic treatment strategy involving growth signal blocking, chemotherapy and photothermal therapy.⁹⁸ The NIR absorbance efficiency of GO and rGO has been found to be enhanced remarkably by incorporating plasmonic nanoparticles like AuNPs into the nanocomposite design. Based on this concept, Xu *et al.* synthesized a highly efficient chemo-photothermal therapeutic agent consisting of gold nanorods (AuNR) encapsulated in NGO shells and conjugated with hyaluronic acid for targeting.⁹⁹ The NGO-HA-AuNR nanocomposites demonstrated 1.5 fold higher killing of Hu-7 cancer cells than chemotherapy alone and four fold higher photothermal ablation as compared to photothermal therapy alone. Wang *et al.* synthesized mesoporous silica coated graphene nanosheets conjugated with a targeting peptide (IP) and loaded with DOX as a chemo-photothermal agent for targeted therapy of glioma.¹⁰⁰ The nanocomposites exhibited high drug loading efficiency along with sustained release profiles in response to both pH and NIR mediated heat. The therapeutic efficiency of the nanocomposites towards killing of glioma cells was found to be significantly enhanced due to the synergistic effect of chemo-photothermal therapy (Fig. 5(i)). A newer nanocomposite referred to as nanocookies was synthesized by Chen *et al.* that comprised of amorphous carbon coated mesoporous silica self-assembled on a nanosheet of reduced graphene oxide.¹⁰¹ These nanocookies were loaded with camptothecin and were used as novel chemo-photothermal agents for tumor treatment. A novel type of nanocapsule made up of lactoferrin shells stabilized by rGO was prepared by Hu *et al.* as a chemo-photothermal agent.¹⁰² Anti-cancer drugs could be efficiently loaded into these nanocapsules and could be used for both *in vitro* and *in vivo* chemo-photothermal treatment of cancer with high therapeutic efficiency. In addition to all these studies, several other graphene-based nanotherapeutic platforms such as CuS nanoparticle decorated GO, PEGylated graphene nanoribbons, Fe₃O₄ nanoparticle loaded GO nanocomposites have been developed for combined chemo-photothermal therapy of cancer.^{103–108}

5.1.2. Combined chemo-photodynamic therapy

GO has also been used as a multimodal therapeutic platform for co-delivery of anti-cancer drugs and photosensitizers to cancer cells and tumors for synergistic chemo-photodynamic therapy of cancer. Miao *et al.* reported the use of PEGylated GO nanosheets for co-delivery of doxorubicin and Ce6 to cancer cells both *in vitro* and *in vivo*.¹⁰⁹ Both *in vitro* and *in vivo* studies showed enhanced therapeutic effect of the combined therapy in comparison to GO mediated chemotherapy and PDT alone. In a similar study, Zhou *et al.* showed a synergistic chemo-PDT of cancer by using GO sheets co-loaded with the anti-cancer drug 7-ethyl-10-hydroxycamptothecin (SN-38) and photosensitizer HA and observed significantly enhanced therapeutic effects of the combined therapy.¹¹⁰

5.2. Combined gene delivery and phototherapies

Graphene-based nanocomposites functionalized with cationic polymers have been developed and exploited as gene delivery vectors. Some very recent works have also exploited these positively charged graphene nanocomposites as multifunctional therapeutic platforms for combined gene-PTT with the ultimate objective of improving the therapeutic efficiency for better treatment of diseases like cancer. Cheng *et al.* reported the synthesis of PEGylated PEI-grafted graphene/Au composites (PPGA) as novel multifunctional gene delivery vectors and demonstrated efficient loading and delivery of Bcl-2 siRNA into HL-60 cells with subsequent

Fig. 5. Graphene-based nanomaterials for combined therapies. (i) Chemo-photothermal therapy. (a) Design of GSPID As a Multifunctional Drug Delivery System for Combined Chemo-Photothermal Targeted Therapy of Glioma (b) Cell viability profiles of glioma cells under different treatments. Reproduced with permission from Ref. 100. Copyright 2013 American Chemical Society. (ii) Chemo-gene therapy. (a) Schematic of the PPG fabrication and MDR reversal. Relative survival rate of MCF-7 cells (b) and MCF-7/ADR cells (c) after being treated with ADR, anti-miR-21PPG, PPGADR and anti-miR-21PPGADR for 24 h. Untreated MCF-7 and MCF-7/ADR cells were served as control in each experiment. Reproduced with permission from Ref. 114. Copyright 2013. (iii) Chemo-gene-photothermal therapy. (a) Targeted gene-mediated thermochemotherapy for drug-resistant tumor. Apoptosis rate of MCF-7/ADR cells treated by (b) PBS (–), (c) Dox (–), (d) PPG-FA (+), (e) PPG-FA/Dox (+), (f) PPG-FA/siRNA/Dox (–) and (g) PPG-FA/siRNA/Dox (+) were tested by flow cytometry. They are measured by the average fluorescent intensity (FL) treated with Annexin V-FITC/PI. (+); 808 nm irradiation (2 W/cm², 5 min); (–); without irradiation. Reproduced with permission from Ref. 115. Copyright 2017. (iv) Photothermal-photodynamic therapy. Photothermally enhanced delivery of PDT by graphene. (a)–(c) Confocal images of KB cells incubated with GO-PEG-Ce6 (5 μM of Ce6 equivalent) at 37°C with (b) or without (a) 808 nm laser irradiation (360 J/cm²) and cells incubated at 43°C in the dark (c). (d) and (e) Cell uptake of GO-PEG-Ce6 under the three conditions at Ce6 concentrations of 2.5 μM (d) and 5 μM (e) determined by the measured fluorescence intensities of cell lysate samples. (f) and (g) Cell viability data of KB cells incubated with GO-PEG-Ce6, free Ce6, or GO-PEG, respectively, at Ce6 concentrations of 2.5 μM (f) and 5 μM (g). Black, red, blue, and green bars represent samples without any light exposure, with both 808 nm (360 J/cm²) and 660 nm (15 J/cm²) light irradiation, with only 660 nm light exposure, and with only 808 nm light exposure, respectively. Relative cell viabilities in all samples were normalized to the control saline-added samples without laser irradiation (100% viability). Reproduced with permission from Ref. 116. Copyright 2011 American Chemical Society.

Fig. 5. (Continued)

downregulation of the Bcl-2 protein expression.¹¹¹ In addition to this, the PPGA nanocomposites showed enhanced photothermal activity in response to NIR irradiation as compared to PPG nanocomposites lacking the AuNPs, thereby making it also an efficient photothermal agent. A PEG and PEI dual-polymer-functionalized GO nanocomposite (GO-PEG-PEI) was developed by Tao *et al.* for the delivery of immunostimulatory oligonucleotides like CpG both in *in vitro* and *in vivo*.¹¹² It was observed that GO-PEG-PEI nanocomposites were successful in promoting the production of pro-inflammatory cytokines as a response to CpGs. In addition to this, the researchers also observed that in the presence of NIR irradiation, the immunostimulation responses were much more enhanced possibly because of the photothermal properties of GO that generated local heat which resulted in accelerating the intracellular trafficking of the nanocomposites. The *in vivo* studies revealed efficient tumor reduction in the presence of NIR irradiation, which can be attributed to combined effects of both immunostimulation and photothermal ablation. In a very recent study published by Yin *et al.*, PEGylated GO nanosheets have been used as a gene delivery vector for carrying out co-delivery of two siRNAs targeting the HDAC1 gene and the G12C mutant K-Ras gene in pancreatic cancer cells MIA PaCa-2.¹¹³ The PEGylated GO nanosheets successfully delivered the siRNAs to the cancer cells *in vitro* which ultimately resulted in induction of apoptosis and cell cycle arrest in the cells. In addition to this, *in vivo* studies demonstrated more than 80% inhibition

in tumor growth under the influence of NIR irradiation due to a combined photothermal-gene silencing effect.

5.3. Combined drug, gene and photo-therapies

Zhi *et al.* developed a treatment strategy to overcome multi-drug resistance (MDR) in breast cancer tumors through co-delivery of an anti-cancer drug with a MDR reversing agent such as miRNA.¹¹⁴ PEI and poly (sodium 4-styrenesulfonates) (PSS) dual-polymer-functionalized GO nanocomposites (PPG) were synthesized and sequentially loaded with adriamycin (ADR) and anti-miRNA-21 to form (anti-miR-21)-PPGADR nanocomplexes. These nanocomposites successfully delivered the therapeutic agents into ADR resistant MCF-7 cells and showed enhanced cytotoxicity to the cancer cells as compared to free ADR. The enhanced therapeutic efficiency of these nanocomposites was due to the combined effects of both chemotherapy and gene silencing, thereby making the ADR resistant cells responsive to the drug (Fig. 5(ii)). In a very recent study, Zeng and co-workers developed a multifunctional PEI-PEG-GO delivery system conjugated with folic acid and co-loaded with a siRNA and Dox for carrying out targeted gene silencing combined with chemo-PTT of drug resistant tumors.¹¹⁵ In the absence of NIR irradiation, the nanocomposites showed gene silencing mediated enhanced chemotherapy of drug resistant cancer cells; while in the presence of NIR irradiation, the overall therapeutic efficiency was further enhanced due to the NIR responsive photothermal properties of GO (Fig. 5(iii)).

5.4. Combined photothermal (PTT) and photodynamic (PDT) therapies

Both visible and NIR responsive properties of GO and rGO have enabled them to be used as phototherapeutic agents for combined PTT and PDT. Tian *et al.* for the first time showed that the photodynamic properties of GO can be significantly enhanced by incorporating the NIR responsive photothermal properties of GO into a single multifunctional therapeutic platform.¹¹⁶ In this study, the authors showed that GO-PEG-Ce6 complexes offered significant photodynamic destruction of cancer cells as compared to free Ce6 molecules. In addition to this, it was also observed that the NIR responsive photothermal effect of GO can be used to promote the delivery of Ce6 molecules, thereby further enhancing the PDT effect (Fig. 5(iv)). In another important study, Sahu *et al.* reported the development of a multifunctional phototherapeutic platform based on nano GO sheets non-covalently functionalized with a Pluronic block copolymer and loaded with the photosensitizer MB via electrostatic interactions.¹¹⁷ *In vitro* studies showed increased cellular uptake of the nanocomposites by cancer cells as compared to normal cells, and significant cell death in response to NIR irradiation. *In vivo* studies also depicted high tumor accumulation of the nanocomposites and complete tumor ablation through a combined PTT-PDT approach. Photothermal enhancement of the photodynamic efficiencies of

graphene-based nanocomposites was also demonstrated by Chen *et al.*¹¹⁸ The group synthesized reduced graphene oxide–ZnO nanoparticles–hyaluronic acid (rGo–ZnO–HA)-based multifunctional nanoplatform for a synergistic PTT-PDT of cancer. Visible light irradiation resulted in the generation of ROS by the ZnO nanoparticles, which helped in sensitizing the cancer cells for a subsequent NIR laser induced hyperthermia that resulted in more efficient killing of the cancer cells. Jiang *et al.* synthesized a water-soluble graphene–phthalocyanine (GR–TSCuPc) hybrid material by a one-step method involving simple mixing of graphene with tetrasodium salt copper phthalocyanine (TSCuPc) through sonication.¹¹⁹ In the hybrid, graphene acted as the carrier of the photosensitizer and as a PTT agent, and TSCuPc served the role of a water-soluble PDT agent. *In vitro* studies showed that the GR–TSCuPc hybrid showed enhanced phototherapeutic effect as compared to free TSCuPc. Table 4 summarizes the applications of different graphene-based nanomaterials for combined therapeutic strategies.

Table 4. Summary of graphene-based nanomaterials for combined therapeutic strategies.

Nanocomposite	Therapeutic modalities	Therapeutic cargo	Reference
PEG-NGO	Drug delivery & PTT	DOX	96
FA-NGO-PVP	Drug delivery & PTT	DOX	97
PEG-NGO-EGFR	Drug delivery & PTT	Epirubicin	98
NGO-HA-AuNR	Drug delivery & PTT	DOX	99
mSiO ₂ -GNS-IP	Drug delivery & PTT	DOX	100
RGO/Carbon/mSiO ₂	Drug delivery & PTT	CPT	101
Nanocookies			
Lactoferrin stabilized RGO nanocapsule	Drug delivery & PTT	DOX	102
PEG-GO/CuS	Drug delivery & PTT	DOX	103
NrGO/PEG	Drug delivery & PTT	Resveratrol	104
rNGO@mSiO ₂ @pNIPAM-co-pAAm	Drug delivery & PTT	DOX	105
NGO-PEG-DA	Drug delivery & PTT	DOX	106
PL-PEG-GONR	Drug delivery & PTT	DOX	107
GO-Fe ₃ O ₄	Drug delivery & PTT	CPT & MTX	108
PEG-GO	Drug delivery & PDT	DOX & Ce6	109
GO	Drug delivery & PDT	SN-38 & HA	110
PEG-PEI-Graphene-Au (PPGA)	Gene delivery & PTT	Bcl-2 siRNA	111
GO-PEG-PEI	Gene delivery & PTT	CpG ODNs	112
PEG-GO	Gene delivery & PTT	HDAC1 & K-RAS siRNA	113
PEI-PSS-GO	Drug delivery, Gene delivery & PTT	ADR & anti-miR-21	114
PEI-PEG-GO	Drug delivery, Gene delivery & PTT	DOX & siRNA	115
PEG-GO	PTT & PDT	Ce6	116
Pluronic F127-NGO	PTT & PDT	Methylene blue	117
rGO-ZnO-HA	PTT & PDT	ZnO NPs	118
GR– TSCuPc	PTT & PDT	TSCuPc	119

6. Graphene-Based Nanomaterials for Bioimaging

In addition to being used as carriers for delivering therapeutic drugs and nucleic acids to cells and as NIR responsive agents for photothermal and photodynamic therapy, graphene-based nanomaterials have also been extensively exploited for bioimaging of cells and tissues. The intrinsic photoluminescence and NIR responsive properties of graphene and its derivatives alongwith its biocompatibility makes them suitable for bioimaging applications. Graphene-based bioimaging can be grouped into two major categories: — optical and non-optical imaging.¹²⁰ Optical imaging includes fluorescence imaging, two-photon fluorescence imaging and Raman imaging, whereas non-optical imaging includes magnetic resonance imaging (MRI), radioactive imaging like PET and SPECT, photoacoustic imaging (PAI) and computed tomography (CT). The following section summarizes the different imaging applications of graphene-based nanomaterials. In this section, studies related to only imaging using graphene and its derivatives have been reported. Studies related to imaging combined with different therapeutic approaches have been summarized in a later section that includes PAI and CT-based imaging using graphene. Table 5 summarizes the applications of graphene-based nanomaterials in bioimaging.

6.1. Fluorescence imaging

The intrinsic optical properties of graphene oxide make them suitable for fluorescence-based bioimaging of cells and tissues. The fluorescent properties of GO and

Table 5. Summary of graphene-based nanomaterials for bioimaging.

Nanocomposite	Imaging modality	Target	Reference
GO, nGO-PEG	Fluorescence	Raji B cells	121
nGO-PEG-Cy7	Fluorescence	Tumor xenografted mouse model	74
GO-PEG-Fluorescein	Fluorescence	HeLa cells	122
GO-IRDye800-VEGF	Fluorescence	Ischemic muscles in a murine model	123
N-GQD	TPFI	Tissue phantom	126
FA-GO-AgNP	Raman	A549 & HeLa cells	130
GO-AuNP	Raman	Ca Ski cells	131
Alkyne-PEG functionalized graphitic silver nano- particles (ACG NPs)	Raman	MCF-7 cells	132
Gd-GNR	MRI		133
AMD-Fe ₃ O ₄ -GO	MRI	HeLa cells	134
⁶⁴ Cu-NOTA-nGO-PEG- TRC105	PET	4T1 murine breast tumors	136
⁶⁶ Ga-NOTA-nGO-PEG- TRC105	PET	4T1 murine breast tumors	137
^{198,199} Au-NGO	SPECT	Fibrosarcoma tumor in rats	138
¹¹¹ BnDTPA-NGO- Trastu- zumab	SPECT	Her2 positive tumors in mouse	139

nano-GO was first reported by the Dai group, where they observed that both GO and nGO-PEG exhibited broad fluorescence emission from the visible region to the IR-A region.¹²¹ They exploited this IR responsive intrinsic photoluminescence property of GO and synthesized Rituxan (anti-CD20 mAb) conjugated nGO-PEG nanocomposites for targeted fluorescence imaging of CD20 positive Raji B cells using a 658 nm excitation laser. The main drawback of this study was that the quantum yield of this intrinsic fluorescence of nGO-PEG was very low, that was not suitable for carrying out further *in vivo* studies. As a result, to overcome this limitation and further improve the fluorescent properties of GO, several different approaches have been carried out which includes loading or linking of organic fluorophores or dyes, inorganic nanomaterials like QDs, upconversion nanoparticles and GQDs onto the surface of GO and rGO sheets. A great deal of attention has been devoted towards the chemistry behind loading and linking these organic and inorganic fluorophores with GO sheets, as direct loading can result in fluorescence quenching of the fluorophore by GO/rGO through energy/charge transfer processes. As a result, spacers such as PEG are used to link these fluorophore moieties to GO/rGO sheets. Yang *et al.* demonstrated *in vivo* fluorescence imaging of tumor xenografted mouse models using a NIR dye (Cy7) conjugated nGO-PEG composite.⁷⁴ The authors observed that with time the nGO-PEG-Cy7 nanocomposites accumulated in the tumor regions through passive targeting and showed intense fluorescence signals upon NIR irradiation. In another study, Peng *et al.* attached fluorescein to PEGylated GO sheets where PEG acted as a spacer between the fluorophore and GO and used it as fluorescent probe for *in vitro* imaging of HeLa cells (Fig. 6(i)).¹²² Sun *et al.* reported synthesis of IRDye800-conjugated GO loaded with VEGF as a targeted fluorescent probe for imaging of ischemic muscles in a murine model.¹²³ In addition to GO and rGO, another class of graphene-based nanomaterials have gained a great deal of attention in the recent years especially in the field of biomedical imaging. GQDs are nanosized materials in the range of 3–10 nm having excellent tunable intrinsic photoluminescence or fluorescence properties in the visible region with high biocompatibility, easy surface functionalization and photostable.¹²⁴ Because of these properties, GQDs have emerged as novel biocompatible imaging and diagnostic agents for both *in vitro* and *in vivo* applications. Zhu *et al.* reported the synthesis of biocompatible, water soluble green fluorescent GQDs by a one-step solvothermal process and used it for labeling and imaging of cells *in vitro*.¹²⁵ In addition to this, there are several reports on synthesis and bioimaging applications of different kinds of GQDs. For a detailed understanding of the bioimaging and theranostic applications of GQDs, readers are requested to refer to the excellent review by Schroeder *et al.*¹²⁴

6.2. Two photon fluorescence imaging

Two photon fluorescence imaging (TPFI) is an improved alternative to single/one photon fluorescence imaging (OPFI) in the field of bioimaging and diagnostics

Fig. 6. Graphene-based nanomaterials for bioimaging. (i) Fluorescent imaging. Confocal fluorescence microscopy images of HeLa cells treated with (a) $2.5 \mu\text{g mL}^{-1}$ PEG-G and Fluo-G suspension for 6 h at (b) 37°C and (c) 4°C . Bright-field images are marked 1, dark-field images are marked 2, and overlap of images of dark and bright field are marked 3. Reproduced with permission from Ref. 122. Copyright 2010 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (ii) Raman imaging. Cell imaging with alkyne-PEG-functionalized ACGs. (a) Raman spectra of alkyne-PEG with (black) and without (red) ACGs. (b)–(f) Raman image of MCF-7 cells treated with alkyne-PEG-modified ACGs using 1 s integration/pixel. BF, bright field; scale bar, $10 \mu\text{m}$. Reproduced with permission from Ref. 132. Copyright 2014 American Chemical Society. (iii) Magnetic Resonance imaging. (a) HeLa cells ($2 \times 10^5 \text{ cells mL}^{-1}$) incubated with the Fe_3O_4 -GO composites at different concentrations for 24 h. (b) Fe_3O_4 -GO composites ($20 \mu\text{g Fe mL}^{-1}$) incubated with HeLa cells at different cell densities for 24 h. Reproduced with permission from Ref. 134. Copyright 2011 American Chemical Society. (iv) Radiolabeled imaging. Serial coronal PET images of 4T1 tumor-bearing mice at different time points postinjection of ^{64}Cu -NOTA-GO-TRC105, ^{64}Cu -NOTA-GO, or ^{64}Cu -NOTA-GO-TRC105 after a preinjected blocking dose of TRC105. Tumors are indicated by arrowheads. Reproduced with permission from Ref. 136. Copyright 2012 American Chemical Society.

Fig. 6. (Continued)

because of the several advantages that TPFi offers over OPFI such as deeper tissue penetration of NIR, minimal NIR absorbance by surrounding tissues, low autofluorescence and negligible photobleaching. Since TPFi operates in the biologically favorable NIR window, so detailed imaging and investigation at deep tissue level to locate inaccessible solid tumors can be done. Liu *et al.* synthesized biocompatible nitrogen-doped graphene quantum dots (N-GQDs) and exploited them as optical probes for TPFi of cells and deep tissues.¹²⁶ The authors reported that the N-GQDs could be identified even at a depth of 1800 μm in the tissue phantom using TPFi as compared to OPFI which could image the N-GQDs only up to a depth of 400 μm , thereby demonstrating the advantage and feasibility of graphene-based TPFi for deep tissue imaging.

6.3. Raman imaging

GO exhibits intrinsic Raman signals at 1600 cm^{-1} (G band) and 1350 cm^{-1} (D band), that can be significantly enhanced by using metal nanoparticles like gold or silver to form Raman probes for imaging of cancer cells and tissues. Many studies have been carried out for developing novel graphene-based Raman probes for bioimaging applications. Graphene-based Raman labels have been synthesized both by the *in situ* reduction of Ag^+ and AuCl_4^- on the surface of GO sheets to form Ag/GO and Au/GO hybrids, respectively, and also by the direct deposition of Au and Ag nanoparticles onto the surface of GO sheets through non-covalent interactions like π - π stacking and other molecular forces to form Au/GO and Ag/GO nanocomposites.^{127–129} Liu *et al.* demonstrated targeted Raman imaging of cancer cells using folic acid conjugated Ag/GO hybrids as Raman probes and observed that cells incubated with the hybrids exhibited much stronger Raman signals from the GO sheets as compared to ones incubated with only GO sheets.¹³⁰ Huang *et al.* studied the cellular uptake mechanisms of GO in the Ca Ski cell line by using Raman probes made up of AuNPs attached to the surface of GO sheets through amide linkages between PEG and DMSA.¹³¹ Song *et al.* reported the synthesis of a new type of graphene-based Raman probe for bioimaging.¹³² They synthesized alkyne-PEG functionalized graphitic silver nanoparticles (ACG NPs) using chemical vapor deposition method. Few layers of graphene were grown on the surface of AgCu NPs by CVD method following which the alkyne-PEG moieties were attached to the surface of GO through non-covalent interactions. These ACG NPs were then used for the Raman imaging of MCF-7 cells, which clearly showed the cellular uptake, localization and distribution of these nanoparticles throughout the cell cytoplasm (Fig. 6(ii)).

6.4. Magnetic resonance imaging (MRI)

Magnetic resonance imaging is a widely used imaging and diagnostic tool that allows for non-invasive visualization of organs and tissues in a quantitative manner. Commonly used MRI contrast agents include paramagnetic metals such as gadolinium and manganese which are somewhat toxic to the human body. As a result, to reduce the toxicity and also to enhance the performance of these existing contrast agents, graphene-based nanomaterials have been explored to act as substrates to carry these agents. Gizzatov *et al.* developed a new type of MRI contrast agent by using carboxyphenylated graphene nanoribbons as substrates to which Gd^{3+} ions were chelated (Gd-GNR).¹³³ It was observed that Gd-GNRs exhibited enhanced MRI contrast and relaxivity as compared to Gd^{3+} -based agents. In another study, Chen *et al.* reported the synthesis of dextran coated Fe_3O_4 NP loaded GO composites as novel MRI contrast agents for bioimaging (Fig. 6(iii)).¹³⁴ Coating of Fe_3O_4 NPs with dextran allowed formation of aggregates of Fe_3O_4 NPs on the surface of GO which in turn helped in enhancing the MRI contrast as compared to single Fe_3O_4 NPs. In addition to paramagnetic metals and iron oxide NPs, a non-magnetic carbon

nanoparticle-based MRI contrast agent was reported for the first time by Hu.¹³⁵ He developed fluorinated GO sheets by creating dipolar C–F bonds which acted as the paramagnetic centers and demonstrated good T_2 contrast.

6.5. Radioactive imaging

Optical imaging techniques such as fluorescence imaging suffers from the limitations of photobleaching, autofluorescence, absorption and scattering of light by surrounding tissues, thereby hampering the quality and contrast of the images. On the other hand, radioactive imaging is a technique that makes use of radioactive substances as labels that can be easily tracked and monitored inside a living system with high sensitivity. The two most commonly used radiolabel imaging techniques are Positron Emission Tomography (PET) and Single-photon emission computed tomography (SPECT). Graphene-based nanomaterials have also been explored as substrates for radiolabel imaging because of the surface properties of graphene that makes functionalization and loading of molecules and imaging agents much easier. Hong *et al.* developed a novel PET contrast agent by labeling nGO-PEG with ^{64}Cu via a 1,4,7- triazacyclononane-1,4,7-triacetic acid (NOTA) linkage.¹³⁶ They further conjugated this nanocomposite with a CD105 specific antibody TRC105 and demonstrated targeted PET imaging of tumor vasculatures in 4T1 murine breast tumors (Fig. 6(iv)). In another study by the same group, the researchers developed another PET contrast agent by labeling nGO-PEG with ^{66}Ga and demonstrated tumor targeted PET imaging.¹³⁷ In addition to PET, graphene-based nanomaterials have also been used for developing SPECT imaging agents. Fazaeli *et al.* developed a SPECT imaging agent by labeling GO nanosheets with $^{198,199}\text{Au}$ and demonstrated targeted SPECT imaging of tumors.¹³⁸ *In vivo* SPECT imaging of Her2 positive tumors was demonstrated by Cornelissen *et al.* using ^{111}In -benzyl-diethylenetriaminepentaacetic acid (BnDTPA) labelled anti-Her2 antibody conjugated nGO as the imaging agents.¹³⁹ The authors observed that the radiolabeled nanoconstructs showed better pharmacokinetic profiles as compared to radiolabeled antibody alone.

7. Graphene-Based Theranostic Platforms

Theranostics refer to diagnostics combined with therapy. Graphene-based nanocomposites have emerged as excellent platforms for theranostic applications in the recent years because of the surface properties of graphene and its derivatives, which allows easy incorporation of multiple modalities into a single platform. Different kinds of multifunctional graphene-based nanotheranostic platforms have been synthesized and exploited for varied biomedical applications by combining imaging techniques with different therapeutic approaches like drug/gene delivery, PTT and PDT. In this section, the theranostic applications of graphene based nanocomposites have been summarized under three main heads- (A) Imaging guided drug/gene

delivery, (B) Imaging guided phototherapies and (C) Imaging guided chemo-photo-therapies, based on the type of therapeutic approach being used. The applications have been further grouped into sections depending upon the type of imaging technique being employed, with the objective of presenting a better categorized appreciation of the immense potential of graphene as nanomedicine. Table 6 summarizes the theranostic applications of graphene-based nanomaterials.

Table 6. Summary of graphene-based nano-theranostic platforms.

Therapeutic modality	Imaging modality	Nano-theranostic platform	Therapeutic cargo	Reference
Drug delivery	Fluorescence	GNC-rGO	DOX	140
Drug delivery	Fluorescence	Graphene-HQDs-Trf	DOX	141
Drug delivery	Fluorescence	cRGD-Chitosan-GO-Fluorescein	DOX	142
Gene delivery	Fluorescence	BPEI-GO	pDNA	54
Drug delivery	Raman	Au@NGO	DOX	143
Drug delivery	Raman	rGO-AuNanostars	DOX	144
Drug delivery	MRI	GO-DTPA-Gd	DOX	145
Drug & Gene delivery	MRI	CMG	DOX, pGFP	146
Drug & Gene delivery	MRI	PAMAM-Gd-NGO	Epirubicin, let-7g miRNA	147
PTT	Fluorescence	CdSe/ZnS QD-rGO		148
PDT	Fluorescence	GO-PEG	DVDMS	149
PTT & PDT	UCL	UCNP-nGO	ZnPc	150
PDT	Fluorescence	GO-SS-Ce6	Ce6	151
PTT & PDT	Fluorescence	GO-HA-Ce6	Ce6	152
PTT	Fluorescence	rGONM-PEG-RGD-Cy7		153
PTT	TPFI	GO-PEG-Tf		154
PTT & PDT	Raman	PEG-Au@GON	ZnPc	155
PTT	MRI	GO/IONP		156
PTT	PA	ICG-GO-FA	ICG	157
PTT	PA	BSA-rGO		158
PTT	MRI & CT	GO/BaGdF5/PEG		159
PTT	MRI & X-ray	GO-IONP-Au		160
PTT	Fluorescence, PA & MRI	C18PMH-PEG- rGO/IONP		161
PTT	Ultrasonic/CT	Polylactic acid (PLA) micro-capsules encapsulating GO and AuNPs		162
PDT	Fluorescence & PET	PEG-GO	⁶⁴ Cu -HPPH	163
PTT & PDT	Fluorescence & PA	PEG-GO	DVDMS	164
PTT & PDT	Fluorescence & MRI	Magnetic fluorescent graphene	SiNc ₄	165
Drug delivery & PTT	Raman & TPFI	Graphene-isolated-Au-nano-crystal (GIAN)	DOX	166
Drug delivery & PTT	X-ray	GO@Ag-DOX-NGR	DOX	167
Drug delivery & PTT	MRI	Magnetic graphene-based mesoporous silica (MGMS)	DOX	168
Drug delivery & PTT	MRI	FeCo/GC nanocrystals	DOX	169

7.1. Image guided drug/gene delivery

7.1.1. Fluorescence imaging-guided drug/gene delivery

Wang *et al.* utilized the NIR photoluminescence properties of gold nanoclusters (GNC) for bioimaging applications and loaded them onto rGO sheets to form GNC-rGO nanotheranostic platforms for carrying out combined drug delivery and fluorescence imaging of cells *in vitro*.¹⁴⁰ Chen *et al.* developed an imaging probe consisting of QDs coated with hybrid SiO₂ shells conjugated to graphene and functionalized with transferrin.¹⁴¹ This functionalized nanocomposite was then loaded with doxorubicin and exploited for QD and DOX mediated dual-colored fluorescence imaging-guided tracking and delivery of drug to cancer cells *in vitro* (Fig. 7(i)). Fluorescein conjugated GO loaded with DOX and coated with cyclic-RGD modified chitosan was synthesized by Wang *et al.* for targeted drug delivery combined with fluorescent imaging of hepatocellular carcinoma cells *in vitro*.¹⁴² Kim *et al.* synthesized a graphene-based gene delivery vehicle by conjugating low molecular weight branched polyethylenimine (BPEI) to GO sheets, thereby imparting a positive surface charge to the nanoconstruct that ultimately facilitated complexation with negatively charged plasmid DNA through electrostatic interactions and showed efficient gene delivery to HeLa and PC3 cells *in vitro*.⁵⁴ In addition to this, the authors exploited the intrinsic fluorescence properties of GO for imaging the cellular uptake of the nanoconstructs.

7.1.2. Raman imaging-guided drug delivery

Graphene-based Raman probes for theranostic applications have also been developed for combining other therapeutic strategies such as drug delivery with Raman imaging into a single multifunctional platform. Ma *et al.* reported the synthesis of nano-GO wrapped AuNPs (Au@NGO) as theranostic tools for Raman imaging and drug delivery to HeLa cells.¹⁴³ Similarly, Wang *et al.* synthesized rGO supported gold nanostars (rGO-NS) as novel sensitive SERS substrates and used them to monitor the loading and release of the drug doxorubicin using SERS. These rGO-NS nanocomposites have the potential to be exploited as a theranostic platform for combined drug delivery and Raman imaging.¹⁴⁴

7.1.3. MRI guided-drug/gene delivery

Zhang *et al.* developed a graphene-based positive T₁ MRI contrast agent through conjugation of diethylenetriaminepentaacetic acid (DTPA) to GO followed by complexation with gadolinium ions (GO-DTPA-Gd) and successfully used it as a theranostic platform for carrying out MRI imaging combined with delivery of doxorubicin to HepG2 cancer cells *in vitro*.¹⁴⁵ The nanocomposite showed improved MRI T₁ relaxivity and better cellular MRI contrast as compared to a commercially used contrast agent. Wang *et al.* combined drug delivery, gene delivery and MRI imaging into a single theranostic platform by synthesizing chitosan functionalized

Fig. 7. Graphene-based nanotheranostic platforms. (i) Imaging guided drug delivery. (A) Preparation of Water Dispersible DOX-Graphene-HQDs-Trf (B) Fluorescent microscopic images of HeLa cells directly labeled by graphene-HQDs-Trf-1 for 4 h (a), and DOX-graphene-HQDs-Trf-1 for 6 h (b), 12 h (c), and 24 h (d). ((i), fluorescent images by blue light excitation; (ii), fluorescent images by green light excitation.) Reproduced with permission from Ref. 141. Copyright 2013 American Chemical Society. (ii) Multimodal imaging guided phototherapies. (A) *In vivo* fluorescence images and (B) ultrasound/photoacoustic images and quantitative analysis of PA signal of PC9 bearing mice treated with GO-PEG-DVDMS (GO-PEG 1 mg kg⁻¹, DVDMS 2 mg kg⁻¹) at 2, 4 and 24 h post-injection. (C) *In vivo* synergistic therapies of GO-PEG-DVDMS. (A) Representative photos of PC9 tumor-bearing mice from different groups after treatment of PDT, PTT or their synergism on day 14. (D) Tumor growth curves of different groups of tumor-bearing mice after different treatments. (E) Body weight curves of PC9 tumor mice after different treatments. Reproduced with permission from Ref. 164. Copyright 2015 Royal Society of Chemistry. (iii) Multimodal imaging guided chemo-phototherapy. (A) Schematic illustration of NIR photothermal enhanced chemotherapy mechanism of GIAN/DOX complexes. (B) UV-Vis characterization of the DOX-loaded GIANs. Inset: digital photo of the DOX, GIAN, and GIAN/DOX solutions. (C) Fluorescence spectroscopy characterization of the DOX loading efficiency. (D) Cell viability of MCF-7 cells with and without NIR laser irradiation after incubation with free DOX, GIAN, and GIAN/DOX, respectively. (E) Bright field microscopy images of trypan blue-stained MCF-7 cells after different NIR photothermal treatments. Scale bar: 50 μ m. (F) and (G), relative cell viability after treatment with different GIAN concentrations and different 808 nm laser irradiation times, respectively. Reproduced with permission from Ref. 166. Copyright 2014 Nature publishing group.

Fig. 7. (Continued)

magnetic graphene nanocomposites (CMG) loaded with the anti-cancer drug DOX and a plasmid DNA encoding GFP and demonstrated efficient delivery of both drug and DNA to A549 lung cancer cells and C42b prostate cancer cells *in vitro* and also to tumor bearing mice *in vivo*.¹⁴⁶ In addition to this, the CMGs acted as strong T2 contrast-enhancing agent thereby enabling MR imaging of the cells and organs. In another study, Yang *et al.* reported the synthesis of poly (amidoamine) dendrimer-grafted gadolinium-functionalized nanographene oxide (Gd-NGO) as theranostic nanocarriers for combined delivery of a chemotherapy drug epirubicin along with Let-7g miRNA.¹⁴⁷ The Gd-NGO/Let-7g/EPI nanocomposites exhibited higher transfection efficiency and better inhibition of cancer cell growth as compared to chemotherapy or gene therapy alone. The nanocomposites were also used as MRI contrast agents for imaging the integrity of blood–brain barrier and tumor tissue uptake.

7.2. Imaging-guided phototherapies

7.2.1. Fluorescence imaging-guided phototherapy

Chen *et al.* exploited the strong fluorescence properties of semiconductor QDs and developed a CdSe/ZnS QD labeled, polypeptide functionalized rGO (QD-rGO) nanotheranostic platform for combined fluorescence imaging and photothermal therapy of cancers.¹⁴⁸ Chen and co-workers demonstrated *in vivo* PDT of cancer combined with enhanced fluorescence imaging by using PEGylated GO loaded with novel photosensitizer sinoporphyrin sodium (DVDMS) and observed intense fluorescent signals in mice from tumor regions showing enhanced uptake of the nanocomposites by tumors through passive targeting and achieved 100% tumor elimination in mice intravenously injected with GO-PEG-DVDMS without any loss in body weight and reappearance of tumors.¹⁴⁹ In another study, Wang *et al.*

reported the synthesis of a novel, multifunctional nanotheranostic platform capable of performing synergistically photothermal and photodynamic therapy combined with imaging.¹⁵⁰ They covalently linked core-shell structured upconversion nanoparticles (UCNP) to the surface of nGO sheets via a PEG spacer, followed by loading a photosensitizer such as phthalocyanine (ZnPc) onto the surface of nGO to form UCNP-nGO/ZnPc nanocomposites. The researchers successfully demonstrated NIR responsive PTT and PDT combined with UCL based imaging both *in vitro* and *in vivo* using this nanocomposite, where, in response to a single light stimulus (NIR) the UCNP generated upconversion luminescence signals for imaging of cells and whole body animals, nGO converted the NIR light into heat for PTT and ZnPc generated cytotoxic singlet oxygen species for PDT. A redox responsive graphene-based theranostic platform was developed by Cho *et al.* by conjugating Ce6 to GO nanosheets via redox-responsive cleavable disulfide linker (GO-SS-Ce6).¹⁵¹ GO-SS-Ce6 in its native form remains non-fluorescent and non-toxic even in the presence of light irradiation due to quenching by GO. However, when these nanocomposites are taken up by cancer cells, the high concentration of glutathione inside cancer cells results in the cleavage of the disulfide bond, thereby releasing Ce6 into the cells. The released Ce6 molecules regain their fluorescent properties and participate in the generation of ROS upon irradiation with light. In a follow-up study by the same group, the researchers reported the synthesis of an enzyme activatable theranostic agent by forming a non-covalent complex between GO and Hyaluronic acid-Ce6 (HA-Ce6) through physical adsorption of HA-Ce6 on the surface of GO (GO-HA-Ce6).¹⁵² Hyaluronidase mediated cleavage of GO-HA-Ce6 inside cancer cells resulted in the release of HA-Ce6 with recovery of fluorescence and ROS generation. In addition to this, the GO-HA-Ce6 nanocomposites also exhibited generation of heat due to the NIR responsive properties of GO resulting in stimulus mediated fluorescent imaging guided dual PDT/PTT of cancer cells *in vitro*. A new class of theranostic platform known as reduced GO nanomesh (rGONM) was developed by Akhavan *et al.* through the photocatalytic degradation of GO nanoplatelets by using TiO₂ nanoparticles.¹⁵³ The rGONMs were functionalized with PEG and conjugated with RGD peptide and cyanine 7 for carrying out targeted fluorescence imaging and photothermal ablation of U87MG glioblastoma tumors in mouse models. The rGONM-PEG-RGD-Cy7 nanocomposites showed 100% elimination of tumors in mice within 48 h of treatment.

7.2.2. Two photon fluorescence imaging (TPFI)-guided phototherapy

Li *et al.* demonstrated for the first time a theranostic strategy based on TPFI coupled with photothermal therapy using graphene-based nanomaterials.¹⁵⁴ They synthesized transferrin conjugated GO-PEG nanocomposites as non-bleaching imaging probes for targeted three-dimensional (3D) TPFI of the gastric cancer cell line AGS, using a low power pulsed laser for excitation. In addition to imaging, the authors also observed that the GO nanoparticles could efficiently induce intensive microbubbling

inside the cells leading to cell death at a laser power as low as 4 mW, owing to the photothermal properties of GO.

7.2.3. Raman imaging guided-phototherapy

Kim *et al.* synthesized a novel theranostic platform for a combined photothermal-photodynamic therapy of cancer guided by Raman imaging.¹⁵⁵ They synthesized core-shell nanoparticles comprising of an Au core surrounded by graphene oxide nanocolloid (GON) shell by a one-pot technique, where GONs acted as both reducing agent and stabilizer for the formation of the AuNPs. PEGylation followed by loading of the photosensitizer zinc phthalocyanine (ZnPc) onto these nanoparticles resulted in the formation of biocompatible ZnPc-PEG-Au@GON nano-theranostic platforms, that demonstrated enhanced photo-therapeutic effects on HeLa cells due to the combination of PTT and PDT, and at the same time acted as an efficient Raman probe for *in vitro* bioimaging of cells.

7.2.4. MRI guided phototherapy

MRI guided detection and subsequent photothermal ablation of regional lymph node (RLN) metastasis of pancreatic cancer was demonstrated by Wang *et al.* using iron oxide nanoparticles (IONP) loaded GO nanocomposites (GO/IONP) as a theranostic agent.¹⁵⁶ The IONPs helped in providing the MRI contrast which was enhanced due to the presence of GO as a substrate, thereby making RLNs easily distinguishable from the surrounding tissues. The NIR responsive photothermal properties of GO was also enhanced by IONP and was used for the photothermal ablation of the tumors.

7.2.5. PAI guided phototherapy

Photoacoustic imaging (PAI) is a non-invasive deep tissue/organ imaging technique that works on the principle of generation of acoustic waves from a sample via absorption of short laser pulses. Biological tissues or organs upon absorbing these short laser pulses tend to generate localized heat that leads to thermal expansion. During contraction, acoustic waves are generated which if recorded by an ultrasonic transducer can result in the formation of an image of the sample by mapping the initial absorbed energy distribution. In this context, RGO have attracted attention as a PA contrast agent because of its better NIR absorbance properties as compared to GO. Wang *et al.* combined photoacoustic imaging with photothermal therapy into a single platform by loading the NIR absorbing dye indocyanine green (ICG) onto GO and demonstrated targeted photothermal ablation of HeLa cells *in vitro* alongwith PAI through conjugation of folic acid as the targeting ligand with the ICG-GO nanocomposites.¹⁵⁷ *In vivo* PAI alongwith PTT was demonstrated by Sheng *et al.* using bovine serum albumin functionalized nano-rGO (BSA-rGO) in a mouse bearing MCF-7 xenograft.¹⁵⁸ Post 2 h intravenous injection of the BSA-rGO

nanocomposites, significant photoacoustic signals were observed from the tumor regions showing increased accumulation of nanocomposites in the tumors. The NIR responsive photothermal property of rGO was then used for the photothermal ablation of the tumors.

7.2.6. Multimodal imaging-guided phototherapy

Graphene-based nanocomposites capable of performing imaging through multiple modalities such as MRI, CT, fluorescence combined with photo-therapies into a single platform has also been explored. Such systems provide the benefits of better diagnosis by incorporating the advantages of each imaging modality for performing improved and enhanced detection. Zhang *et al.* demonstrated dual modal MRI/CT imaging of tumors *in vivo* alongwith photothermal ablation using PEGylated GO nanosheets loaded with BaGdF₅ nanoparticles (GO/BaGdF₅/PEG).¹⁵⁹ The nanocomposites exhibited enhanced magnetic resonance contrast and better X-ray attenuation property than Iohexol which is a standard contrast agent used in radiographic imaging of arteries, veins, ventricles, joints, etc. Intravenous injection of the nanocomposites performed efficient photothermal ablation of the tumors with no toxicity to the host. Shi *et al.* developed a multi-functional magnetic and plasmonic graphene-based nanocomposite by loading IONP onto the surface of GO sheets followed by growing a layer of gold on it.¹⁶⁰ This GO-IONP-Au nanocomposite was then functionalized with PEG and used for photothermal ablation of cancer cells and tumors both *in vitro* and *in vivo* alongwith dual-modal MRI and X-ray imaging. The gold layer helped in enhancing the NIR absorbance of the nanocomposite and IONP and Au together contributed towards contrast generation for imaging. Yang *et al.* developed a graphene-based nanotheranostic platform for triple modal fluorescence, photoacoustic, and magnetic resonance imaging of tumors *in vivo* combined with photothermal therapy and successfully demonstrated multimodal imaging guided photothermal ablation of 4T1-tumors in mice by using C18PMH-PEG functionalized rGO/IONP nanocomposites synthesized *via* a hydrothermal process.¹⁶¹ PLA microcapsules encapsulating GO and AuNPs were developed as nanotheranostic agent by Jin *et al.* for photothermal ablation of tumors under the guidance of bimodal ultrasonic/CT imaging.¹⁶² Rong *et al.* demonstrated bimodal fluorescence/PET imaging guided photodynamic therapy of tumors using PEGylated GO sheets loaded with a radiolabeled photosensitizer ⁶⁴Cu -HPPH.¹⁶³ Similarly, in a separate study, Yan *et al.* demonstrated bimodal fluorescence/PA imaging guided combined photothermal-photodynamic therapy of tumors using PEGylated GO sheets loaded with DVDMS (Fig. 7(ii)).¹⁶⁴ In another study, Gollavelli *et al.* developed SiNc₄ photosensitizer loaded magnetic fluorescent graphene and demonstrated bimodal fluorescence/MRI imaging guided combined photothermal-photodynamic therapy of HeLa cells *in vitro*.¹⁶⁵

7.3. Imaging guided chemo-phototherapies

Bian *et al.* reported the synthesis of graphene-isolated-Au-nanocrystal (GIAN) nanostructures by depositing a thin layer of graphene on the surfaces of Au nanocrystals using CVD method.¹⁶⁶ These nanostructures were used as imaging probes for dual modal Raman and TPF imaging of cells and also as a substrate for loading of anti-cancer drug doxorubicin and NIR mediated photothermal therapy of cancer (Fig. 7(iii)). Shi *et al.* demonstrated tumor targeted chemo-PTT of cancer combined with X-ray imaging of the tumors using GO@Ag nanocomposites.¹⁶⁷ The GO@Ag nanocomposites were loaded with doxorubicin and conjugated with NGR peptide for active targeting of tumors (GO@Ag-DOX-NGR). The GO@Ag-DOX-NGR showed excellent targeted chemo-photothermal efficacy along with X-ray imaging ability both in *in vitro* and *in vivo* studies. Magnetic graphene-based mesoporous silica (MGMS) nanocomposites have emerged as novel theranostic agents for carrying out magnetic imaging-guided therapy. Wang *et al.* developed a theranostic platform based on magnetic graphene-based mesoporous silica for MRI guided targeted chemo-photothermal therapy of gliomas.¹⁶⁸ The MGMS nanocomposites were tagged with a IL-13 targeting peptide and loaded with DOX for targeted delivery of the drugs to U251 glioma cells. The magnetic properties of the nanocomposite due to the presence of IONPs on the surface of GO sheets provided improved contrast for MR imaging as seen from increased T_2 relaxation rates and at the same time enhanced the optical absorbance of the nanocomposites in the NIR region thereby making them suitable for photothermal applications also. Sherlock *et al.* reported a new type of theranostic platform by synthesizing ultrasmall FeCo/graphitic carbon shell nanocrystals (FeCo/GC) having size in the range of 3–4 nm in diameter.¹⁶⁹ The FeCo/GC nanocrystals exhibited excellent magnetization, high doxorubicin loading efficiency and both pH and NIR responsive drug release abilities that resulted in enhanced cytotoxicity towards cancer cells. NIR induced hyperthermia also increased the cellular uptake of these nanocrystals thereby further enhancing the cytotoxic effects. In addition to this, the nanocrystals were also used as MRI contrast agents for imaging their cellular uptake.

8. Conclusion and Outlook

In this review, we provide a systematic and detailed overview regarding the current progress on the different biomedical applications of graphene-based nanomaterials in the field of drug delivery, gene delivery, phototherapy, bioimaging and combined therapeutic and theranostic approaches in a highly categorized manner. The versatility of graphene and its derivatives in the field of biomedical research can be attributed to the unique structural, optical, electrical, thermal and biocompatible properties of these nanomaterials. The monolayer single-atomic 2D nanosheets of graphene oxide and rGO provide ultra-high surface area and numerous functional groups on their surface for highly efficient loading of different kinds of therapeutic

and theranostic cargo such as anti-cancer drugs, nucleic acids like DNA, RNA, siRNA, miRNA, imaging agents like fluorophores, quantum dots, dyes, inorganic materials like plasmonic and magnetic nanoparticles, etc., through hydrophobic and π - π interactions. It also allows easy functionalization with biocompatible polymers like PEG and targeting moieties like peptides, vitamins and antibodies through covalent and non-covalent interactions for the development of novel multifunctional biocompatible theranostic platforms for imaging guided therapy. This ability to combine multiple treatment and diagnostic modalities into a single platform have significantly increased the therapeutic efficiency and importance of graphene in the field of nanomedicine and has made it a material of choice for exploring different novel biological applications. However, in spite of this tremendous progress, most of the research that has been carried out so far on graphene and its derivatives have mainly explored its therapeutic potential at the *in vitro* level, with very little focus on understanding the interactions of graphene with cells and tissues at the organismal level, which is very crucial for a successful translation of a graphene-based nanomedicine from bench to bedside. Detailed mechanistic studies in small as well as large animal models should be done to get a clear understanding of how graphene-based nanomaterials behave once they are inside the body of a host, how they interact with the cells of the immune system and reticuloendothelial system, whether they induce any local or systemic inflammatory response, their pharmacokinetics and biodistribution profiles and most importantly, their toxicity profiles. Some studies have shown that the toxicity and interaction of graphene-based materials with cells and tissues are greatly affected by the size distribution of these materials, with larger size having more toxicity and higher chances of getting phagocytosed and cleared off the circulation by phagocytic cells. However, the large size distribution of these materials that arise in each batch during their synthesis makes it very difficult to correlate size with toxicity and hence requires the development of new synthesis methods that will provide uniformity in the size of these nanoparticles. In addition to synthesis, new surface functionalization strategies for reducing toxicity and improving physiological stability should also be developed. So, by looking at the progress that have been made in the field of graphene research over the years, we can surely say that graphene holds a lot of promise and hope as a future nanomedicine. However, to take it to the clinic a significant amount of effort from scientists and researchers from different disciplines of chemistry, material science, engineering and specially biology is needed to understand the systemic toxicity and immunogenicity of the material.

Acronym list

GO — Graphene oxide
 rGO — Reduced graphene oxide
 GQD — Graphene quantum dot
 NIR — Near infrared

PTT — Photothermal therapy
 PDT — Photodynamic therapy
 PEG — Poly-ethylene glycol
 DOX — Doxorubicin
 PTX — Paclitaxel
 CPT — Camptothecin
 DNA — deoxyribonucleic acid
 RNA — ribonucleic acid
 mRNA — messenger RNA
 siRNA — small interfering RNA
 miRNA — micro RNA
 MRI — Magnetic resonance imaging
 PAI — Photoacoustic imaging
 TPF1 — Two photon fluorescence imaging
 PET — Positron emission tomography
 SPECT — Single-photon emission computed tomography

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