Functionalized Graphene for Drug Delivery Applications

T. K. Henna, K. P. Nivitha, V. R. Raphey, Chinnu Sabu and K. Pramod

Abstract The unique characteristics of functionalized graphene make it a multi-faceted molecule having crucial therapeutic as well as medical significance. Different aspects of functionalized graphene are being discussed here. Functionalization of graphene could even scale up its importance. Functionalization can be done by different methods namely covalent functionalization, covalent functionalization with reaction intermediates, functionalization with nanoparticles, multi-functionalization, substitutional doping. These functionalization strategies mainly aim at reducing the in vivo and in vitro toxicity and agglomeration, moreover the main goal of functionalization is to disperse or solubilize it in different solvents. An Improvised drug and gene targeting nanocarrier system with unique properties have become possible with this graphene functionalization. The anticancer and antibacterial effect and several other applications of functionalized graphene are also being discussed.

Keywords Graphene · Graphene oxide · Functionalization · Covalent and non covalent functionalization · Drug delivery

T. K. Henna · K. P. Nivitha · V. R. Raphey · C. Sabu · K. Pramod (✉)
College of Pharmaceutical Sciences, Government Medical College, Kozhikode 673008, Kerala, India
e-mail: pramodkphd@yahoo.com

T. K. Henna
e-mail: hennatk13@gmail.com

K. P. Nivitha
e-mail: nivitha.kp@gmail.com

V. R. Raphey
e-mail: riyaraphey@gmail.com

C. Sabu
e-mail: chinnu824@gmail.com

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1 Introduction

Graphene is a crystalline two-dimensional allotropic form of carbon, consisting of a single layer of sp^2 hybridized carbon atoms and they are arranged in a hexagonal lattice. Even though they are of single atom scale thickness, they are the hardest material ever known. Thus, they can be processed into thin sheets. They draw attention due to their unique features like high surface area, high strength, high thermal conductivity, electrical conductivity, the excellent transmittance of light, lower toxicity, and low cost [1]. Graphene family of nanomaterials includes single-layer graphene, bilayer graphene, multilayer graphene, Graphene oxide, reduced graphene, and chemically modified graphene. This classification is based on a number of layers, oxygen content, surface chemistry, purity, and their composition. Since graphene doesn't contain any oxygen groups, it possesses high electrical and optical conductivity [2]. Graphene forms stable colloid in various solvents [3]. But graphene oxide have oxygen-containing functional groups, thus they have relatively poor electrical and optical conductivity. Graphene is hydrophobic and poorly dispersible in water. Thus, its biomedical application is limited. It is overcome by converting graphene to graphene oxide. Graphene oxide is hydrophilic in nature, but it is toxic [2]. Functionalization with biocompatible polymers such as PEG reduces the in vivo and in vitro toxicity of graphene oxide [4]. To improve the electrical conductivity of functionalized graphene oxide, it is converted to functionalized graphene by reduction. Hydrazine monohydrate, sodium borohydride, p-phenylenediamine, hydroquinone, and sodium hydrosulfite are used as reducing agents. Since they are hazardous, some other reducing agents are also employed. Acetic acid and HCl act as good reducing agents and they are used as an alternative to hydrazine monohydrate [4].

Reduced graphene possess high conductivity than GO, but less than graphene. And reduced graphene oxide also exhibits high solubility than graphene, but less than graphene oxide. The oxygen-containing functional group is higher in graphene oxide than reduced graphene oxide. The differences in their physicochemical properties are represented in Fig. 1. Crystal structure of reduced graphene oxide is better than graphene oxide [2].

2 Structure

Graphene is a unique single-layered carbon foil having two-dimensional structures. The term graphene originates as a coalition of graphite and the suffix-ene [5]. Usually, graphene consists of six atoms which look like one atom thickness honeycomb network with sp^2 hybridization. The C–C bond length is 1.42 A and bond angle 120°. The structure of graphene is somewhat similar to that of benzene especially the bonding arrangement. Hence graphene can be considered as an enormous polycyclic aromatic hydrocarbon (PAH) [6]. When considering the

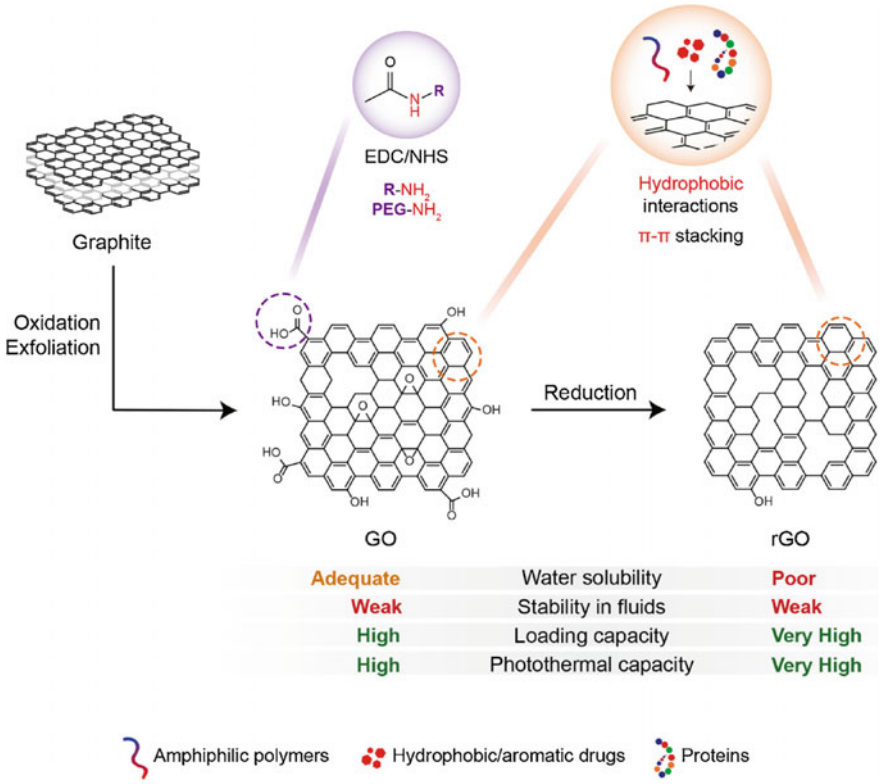

Fig. 1 Schematic representation of the synthesis and physicochemical properties of graphene oxide and reduced graphene oxide. Reprinted from [32], © 2018, with permission from Elsevier

microscopical structure of graphene flakes; one edge is highly dominated by the zig-zag edges and the other side has armchair orientation. It has several functional groups on the surface, such as epoxy, carboxyl, and hydroxyl. The peripheral carboxylate group is responsible for colloidal stability and pH-dependent negative surface charge. Epoxide and hydroxyl groups are uncharged but polar. Thus it allows weak interactions, hydrogen bonding and other surface reactions [7].

3 Synthesis

Pristine graphene is hydrophobic, and its biomedical applications are comparatively limited. Thus an alternative graphene form is discovered [8]. For the synthesis of processable graphene, graphene oxide (GO) has been commonly used as starting material. There are plenty of methods proposed by several researchers about the preparation of reduced graphene oxide (RGO). Graphene can be produced from

graphite and non-graphite sources. The synthesis of graphene can be done by two methods namely top-down and bottom-up. The top-down methods include mechanical cleavage, redox method, and arc discharge. While the bottom-up method includes chemical vapor deposition and organic synthesis [6].

Graphene was first exfoliated mechanically from graphite in 2004 [9]. In redox method, graphite is first treated with an oxidizing agent to change the crystalline shape and structure of graphite and then treated with strong acid to form graphene oxide. This GO is reduced to graphene [6]. In 1859, the first chemical exfoliation of graphite was performed by extensive oxidation of graphite by exposing to a mixture of fuming nitric acid, potassium chlorate for few days [10]. In 1898, this method is modified by exchanging 2/3rd of fuming nitric acid to sulphuric acid and used multiple aliquots of potassium chlorate. This addition of potassium chlorate leads to explosion [11]. Thus, this is again modified in 1958 by reacting graphite powder (100 g) with a mixture of sodium nitrate (50 g), sulphuric acid (2.3 L) and potassium permanganate (300 g) [12]. Hummer's method is the most commonly used method for graphene oxide synthesis in laboratory scale. The drawback of this method is that it produces toxic gases like NO_2 and N_2O_4 and can't be synthesized in large scale [13]. Arc discharge method was used in large-scale preparation, in this method electric current is passed between two electrodes made of graphite at a particular pressure. During this process, the anode will be consumed and the product graphene is obtained on the cathode. Graphene obtained by this method exhibits better electrical conductivity, high crystallization, and thermal stability [6]. Chemical vapour deposition method is the most promising method. It is also known as surface segregation method on a solid surface. The principle involved is that, when hydrocarbon (usually methane CH_4) exposed to heated metal surfaces like nickel and copper under vacuum, a carbon film with single crystal size was formed. The metal leads to the loss of hydrogen and dissolves the carbon to form a metal carbide layer. As the temperature decreases, saturation of metal carbide surface layer occurs which leads to precipitation of graphite carbon from carbide sol [14]. Diluted methane gas (CH_4) is used as a carbon precursor and produced a single-layered graphene. Copper can control the layer formation because of its lowest carbon solubility, thus it is used to prepare large sheet (75 cm) of single-layered graphene. Nickel can't control the number of layers, but the alloy form of nickel gives a better result. Cu–Ni alloy can be used to prepare single-layered graphene [15]. The thickness and width of graphene nanoribbon can be adjusted by changing annealing temperature, time of exposure and nature of substrate metal [16].

4 Functionalization

Since graphene is hydrophobic, it aggregates in an aqueous medium such as proteins, salts, buffer or cell medium, which may be lead to toxicity. This is due to the electrostatic interactions and non-specific interactions between charged graphene

and proteins. So the surface of graphene should be functionalized or modified chemically [2]. Suitable chemical functionalization protects graphene from agglomeration and maintains its inherent properties. Graphene oxide exhibits toxicity. Functionalization with biocompatible polymers such as PEG reduces in vivo and in vitro toxicity. The main purpose of functionalization is to dissolve or solubilize them in a variety of solvents such as water, DMF, chloroform, THF etc. [4]. Graphene can be functionalized via covalent and non-covalent methods by using poly ethylene glycol (PEG), poly ethylene imine, gelatin, chitosan or sulfonic acid groups. When GO is functionalized with polar polymers such as chitosan, polyethylene glycol and polyethyleneimine (PEI), their half-life in systemic circulation is increased and identification by the reticuloendothelial system may be decreased [17]. Functionalization can be performed by two methods, covalent and non-covalent modifications.

4.1 Covalent Modification

To improve the stability of graphene oxide or reduced graphene oxide in a physiological medium, some organic molecules are covalently attached to their surface. Biocompatible PEG was the first molecule attached covalently [2]. Covalent functionalization of graphene solves the issue of poor dispersibility in certain solvents [18]. The mechanism of covalent bond formation involves, (a) Covalent bond formation between free radicals or compounds that readily reacts with dienes and C=C bonds present in graphene or (b) Formation of a covalent bond between organic functional groups and oxygen groups present in GO [19]. GO can be functionalized with D-mannose using mannosylated ethylenediamine. This reduces toxicity to red cells [20]. The covalent modification can be achieved by four methods, nucleophilic substitution, electrophilic substitution, condensation, and addition.

Nucleophilic substitution

The reactive site for the nucleophilic substitution is mainly epoxide groups on the GO. The modifying agent used is amine terminal groups such as amino acids or alkyl amines. The lone pair electron present on the amine ($-NH_2$) group of modifier attacks the epoxy group of GO. Nucleophilic substitution methods are commonly used functionalization techniques because it is very simple, it can be performed in room temperature. This method is employed as large-scale production of GO.

Amines and amino acids are used for functionalization of graphene. Functionalization by small chain primary amines was carried out at room temperature and long-chain aliphatic amines by heating the reaction mixture and refluxed for 24 h [21].

Electrophilic substitution

In this reaction, the hydrogen atom is displaced by an electrophile. Consider, spontaneous grafting of diazonium salt of p-nitro aniline to graphene surface.

Condensation reaction

In the condensation reaction, two molecules combine together to form a single molecule with a loss of small molecules. Through amide and carbamide ester linkages condensation reaction occurs by agents such as isocyanate, diisocyanate, and amines.

Addition reaction

In an addition reaction, two or more molecules combine to form a large molecule. Maleic anhydride can be added to graphene surface in the presence of free radicals. They are highly soluble in THF and are more stable [4].

4.2 Non-covalent Modification

The non-covalent modification is the physical adsorption of certain molecules on graphene surface through π–π stacking interaction, hydrophobic, van der Waals, and electrostatic forces. It is performed through polymer wrapping, adsorption of surfactant or small aromatic molecules and interaction with porphyrins, DNA or peptides. First non-covalent functionalization of graphene nanosheets was performed by using poly (sodium 4-styrene sulfonate). It is prepared by exfoliation and simultaneous reduction of GO using PSS and the obtained product was found to be highly dispersible in water [22]. Sodium deoxycholate, protein, DNA, tween can also be used for non-covalent modification [2]. Nafion graphene nanocomposite was prepared by dispersing nafion in an aqueous solution of graphene and it is reduced by hydrazine. This exhibits excellent electrical conductivity [23]. Non-covalent functionalization has several advantages such as it minimizes the chemical reaction, reduces purification step and it retains the physical properties of GO. Additionally, non-covalent functionalized graphene oxide can work as both a stabilizer for preventing aggregation and as a targeting agent [24]. But the disadvantage is that non-covalent functionalization is not as strong as covalent and it may undergo some variations in the external environment. It may lead to less stable drug delivery. And the drug loading capacity of non-covalent functionalized graphene oxide is less. It is experimentally proved by using covalent and non-covalent modified PEI-GO. The results show that non-covalent modified PEI-GO was less stable [2].

4.3 Covalent Functionalization with Reaction Intermediates

Free radicals, nitrenes, and carbenes are major reaction intermediates. Free radicals are most commonly used for graphene functionalization and are produced from diazonium salt and benzoyl peroxide. During the reaction, electron transfer occurs from graphene to aryl diazonium ion to form aryl radicals that are added to graphene to form covalent adducts. Nitrenes are another reaction intermediates, used for graphene functionalization, formed by thermal or photochemical activation of organic azides. Carbenes undergo CH insertion and C=C cycloaddition reactions with graphene, but they are less frequently used than nitrenes [25].

4.4 Functionalization with Nanoparticles

Graphene is also functionalized with metal nanoparticles, metal oxide nanoparticles, quantum dots, and some other nanoparticles. Noble metals such as Pt, Au, Rh, Pd are used for the functionalization of graphene. Gold nanoparticles on graphene sheets are synthesized by direct reduction of $AuCl_4$ by $NaBH_4$ in a suspension of GO THF. It is found that Pd has a greater affinity towards graphene than Au; the reason suggested is partial covalent binding between Pd and graphene. Metal oxides such as Tin oxide (SnO_2), manganese oxide (Mn_3O_4), cobalt oxide (Co_3O_4) and titanium oxide (TiO_2) nanocrystals are also deposited on graphene surface and are used as anodes in Li-ion batteries. Graphene quantum dots are formed when quantum dots deposit on graphene surface and they have wide range of applications in drug delivery, biological labelling and can be used solar cells and LED preparation [19].

4.5 Substitutional Doping

sp^2 hybridized carbon atoms of graphene lattice can be substituted by nitrogen or boron atoms. By controlling the degree of doping, the electrical properties of graphene can be adjusted. In nitrogen doping, the lone pair of nitrogen atoms conjugates with π system of graphene. N-doped graphene sheets are electron rich, thus n-type semiconducting behavior is expected. Wang et al. performed N-doping of graphene nanoribbons by using high power electrical joule heating in ammonia gas. They also conducted N-doping and simultaneous reduction of GO during annealing in Ammonia. GO was heated from 300 to 500 °C and small amounts of N-doped GO was produced by this method. The efficiency of N-doping depends upon the number of oxygen groups at the edge sites of GO [19]. Functionalization of graphene is very essential to prevent aggregation, but it reduces the electrical conductivity. But, the separation of the excess chemical modifying agent from the functionalized graphene is difficult [20].

4.6 Multi Functionalization

Graphene can also be functionalized by two or more molecules. PEG and PEI were covalently conjugated to graphene oxide through amide bonds. It gives physiologically stable ultra small size conjugate [26]. Multi-functionalized graphene oxide has several advantages and is extensively used in targeted drug delivery. They are described in the application section of this paper.

5 Properties of Functionalized Graphene

Graphene is a compound possessing honeycomb shaped crystal lattice and is highly bonded to each other via sp^2 bonds. Studies have shown that it is the strongest material that has ever tested i.e. 200 times stronger than steel. Graphene is a semiconductor with zero band gaps. Other dominant characters include high thermal conductivity $\sim(4.84 \pm 0.44) * 10^3$ to $(5.30 \pm 0.48) * 10^3$ Wm K^{-1}. The boundary molecular electrons of different organic molecules interact with the π electrons in the graphene molecule which becomes the main reason of preferring electrophilic substitution over nucleophilic substitution. And other reactions include cyclo-additions, click reactions, carbene-insertion reactions [4]. Focusing on to the mechanical properties, graphene has high Young's modulus. The effect of temperature on mechanical strength was studied, and the research shows that temperature decreases the mechanical strength. They also concluded that functionalization of graphene with certain groups have a negative effect on mechanical strength. Young's modulus decreases by adding certain groups. It is in the order of Y (NH_2) > Y (C_6H_5) > Y (OH) > Y (CH_3). Physicochemical properties such as large specific surface area, unique 2D structure, and π electron cloud make graphene suitable for interaction with a different organic molecule and thereby proving that it has wide application in different drug delivery systems [27].

Another excellent property of Graphene is its optical characteristics. It was found that single-layer graphene transmits 97.7% of the total incident light and also the light absorption capacity increase as the number of layers increases. By using this unique property it has been widely used in different electro-optical devices like tunable IR detectors, modulators and emitters by electrical gating and charge injection. Other optical properties include great light transmittance, photoluminescence, and better charge mobility make graphene a significant biomedical tool and can take part in different imaging techniques like magnetic resonance imaging (MRI) and biomedical imaging [7].

6 Characterization of Functionalized Graphene

Focusing on the characterization of single-layered graphene that exhibits unique features when compared with double or multi-layered graphene is considered more relevant in an application level [4]. Various methods that prevail for the characterization of functionalized graphene are discussed in this section.

X-ray diffraction

Graphene oxide is having a broad peak at 10.9°, whereas the pure graphite powder is exhibiting a peak at 26° [28]. Although X-ray diffraction is informative, these studies are limited in the identification of single-layered graphene. The similar X-ray diffraction pattern is exhibited by single-layered graphene oxide as well as the single-layered graphene [4]. Exfoliation of graphite oxide into single-layer graphene oxide gives a straight line in the X-ray diffraction pattern with no diffraction peak.

UV-visible spectroscopy

UV-visible spectroscopic methods are successful in providing information regarding the number of layers and the graphene formation. An absorption peak at 262 nm is exhibited by 2-dimensional graphene and a peak at 230 nm by single-layered graphene oxide in the UV-visible spectrum. And this peak is due to the π–π* transition of the aromatic C–C bonds. At a wavelength of the 550 nm monolayer, graphene nanosheets are having a transmittance of 97.1% [4].

Transmission electron microscopy

Transmission electron microscopy gives an accurate idea regarding the thickness and the number of layers present in the graphene sample. An analysis of the TEM images (Fig. 2) obtained by keeping the folded graphene sheets parallel to the electron beam will provide information about the number of layers present in graphene. The small area electron diffraction (SAED) ensures more accurate data [4].

Raman spectroscopy

Raman spectroscopy is the most effective and fast approachable method for the determination of the quality of exfoliated graphene and serves as a characterization tool for the analysis of graphene-based materials. The method is used to analyze the molecular functionalization, doping effects, oxidation and number of layers on graphene. For this purpose, Raman spectroscopy uses its most significant indicators namely-D, G and 2D modes [29]. The vibration of the sp^2 bonded carbon atoms in the two-dimensional hexagonal lattice a peak at 1576 cm^{-1} is exhibited by chemically reduced graphene. The peak obtained at 1326 cm^{-1} is the result of the defects and disorders chemically reduced graphene. Raman spectroscopy is highly efficient in distinguishing single layered, double layered and other multilayered graphene sheets. A G band and the 2D band are given by graphene at 1580 and 2700 cm^{-1} respectively in the Raman spectra [4].

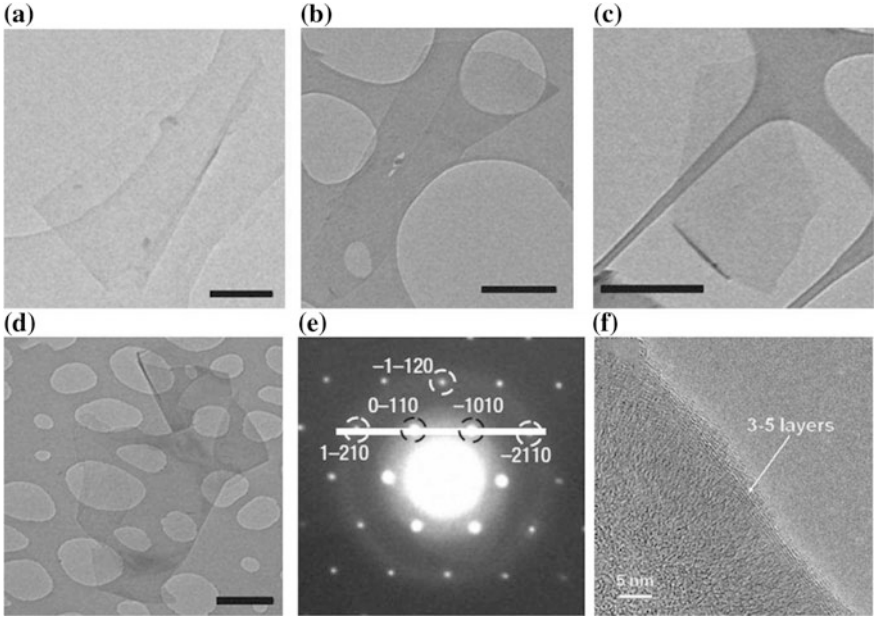

Fig. 2 TEM and HR-TEM of Graphene. Reprinted from [4], © 2012 with permission from Elsevier

Fourier transforms infrared spectroscopy (FTIR)

The Fourier transform infrared spectroscopic method provides all the information regarding the functionalities of graphene oxide. In FTIR spectra of graphene oxide, it exhibits a peak at 3400 and 1620 cm^{-1} because of the stretching vibration of OH groups and the skeletal vibration of graphene oxide sheets respectively [30].

7 Applications

7.1 Functionalized Graphene in Drug Delivery

The two dimensional sp^2 hybridized carbon network of graphene has a tremendous range of applications in drug delivery, biosensors, nanoelectronics, and polymer nanocomposites. Aggregation and processing problems of pure graphene can be overcome by using functionalized graphene which will pay a huge path in the research field as well as in the therapeutic field. Graphene oxide is the derivative of graphene-based materials, which have the good biocompatibility, hypotoxicity, so many functional groups on the surface (epoxide, hydroxyl and carboxyl groups), high strength, surface area, electrical and thermal conductivity, flexibility,

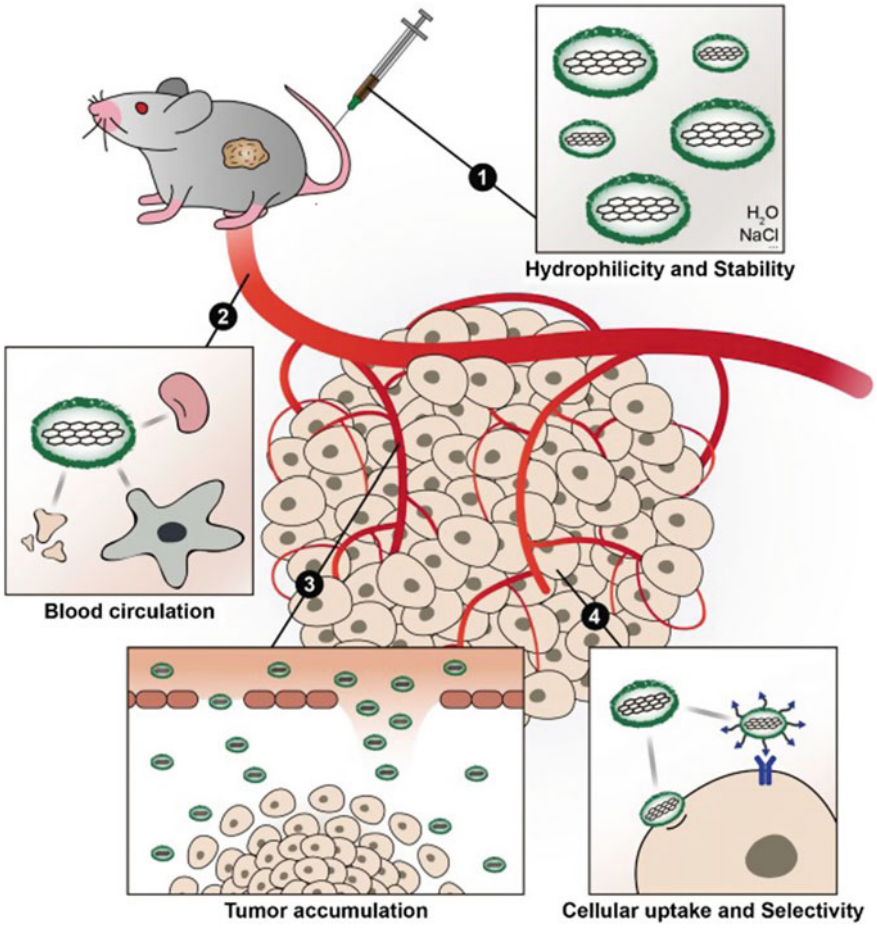

Fig. 3 Schematic representation of the different barriers and barrier crossing by functionalized graphene in order to accumulate in the tumor cells and internalized by cancer cells. Reprinted from [32], © 2018, with permission from Elsevier

transparency and low cost [31]. Functionalization of graphene facilitate easy delivery of different drug molecules and other nanomaterial's and help to cross different biological barriers which enable an efficient and site-specific accumulation at tumor cells as shown in Fig. 3 [32].

Moreover the toxicity studies of pristine graphene and carboxylated graphene (COOH–GO) in monkey kidney cell. It is noticed that pristine graphene was accumulated in the cell membrane and leads to destabilization of F-actin alignment and COOH–GO internalized by the cells and accumulated in the perinuclear region without causing any membrane destabilization even at higher concentration [33]. Pristine graphene generates intracellular reactive oxygen species and it causes damage to DNA and protein and finally leads to cell death via apoptosis or necrosis.

Sharp edges of graphene may cause membrane destabilization and loss of cell integrity when they become direct contact with the cell surface. *A*dsorption of GO on the surface of RBC causes loss of cell membrane and leads to hemolysis [34]. Immunotoxicity study of GO and PVP functionalized GO against human immune cells such as T-lymphocyte, dendritic cell and macrophages were performed. The result shows that PVP functionalized GO have lower immunogenicity than pristine graphene oxide [35]. These studies reveal that functionalization reduces the toxicity of graphene. For the researchers in the pharmaceutical field, graphene opens a new world of drug delivery. Their high dispensability in polar solvents, ability to undergo chemical modifications and excellent loading ability for aromatic drugs, functionalized graphene is recognized as the ideal candidate for drug delivery [36].

Recently, the graphene oxide based targeted drug delivery has become a great interest of topic for many researchers. Drug or gene can be loaded to modified graphene-based nanomaterials by hydrophobic interactions, π–π stacking, and electrostatic interactions. Carriers are made to respond to internal stimuli such as body temperature, pH, specific chemical reaction or external stimuli such as light, field, and ultrasound. The pH of a tumor micro environment, intracellular lysosomes and endosomes are acidic. Thus, pH responsive graphene-based nanocarriers are developed for cancer therapy. For achieving targeted therapy, targeting molecules like folic acid, antibody, carbohydrate, peptide, protein, and aptamers are coupled on the surface of graphene oxide. The Photosensitizer can be loaded on the graphene-based material surface to use in photodynamic therapy. Chemotherapic agent, 1,3-bis (2-chloroethyl)-1-nitrosourea was loaded into PAA-GO via ester linkage for malignant brain tumor therapy. It improves the thermal stability and half-life of the loaded drug. The drug release is controlled by the hydrolysis of ester linkage [2]. Studies on aptamer-conjugated magnetic graphene oxide nanocarriers for specifically targeting the tumor cells loaded with an anticancer agent has provided an entrapment efficiency of 95.75% and good release percentage also [37].

A novel advancement in graphene-based drug delivery is the approach to develop a dual faced graphene oxide by simultaneous grafting of different polymers with different hydro-affinity. This dual drug loaded graphene-based system is a promising approach for combination therapy, that could completely circumvent chemotherapeutic drug resistance in cancer therapy [38]. With the idea of developing a pH sensitive drug carrier, carboxy methylcellulose modified graphene oxide has been developed that could provide a controlled and sustained drug release with small side effects and improved drug bioavailability [39]. In order to overcome the compromised biocompatibility of graphene oxide hyperbranched polyglycerol conjugated graphene oxide has been synthesized. The chemotherapeutic effect was evaluated after loading an antitumor drug to the surface modified graphene oxide, the carrier [40]. Rivastigmine loaded hydrogel beads based on ion-crosslinked gum tragacanth and graphene oxide was prepared. In this preparation, the use of GO increased swelling capacity, entrapment efficiency and controlled release [41]. Since fluorinated graphene is hydrophobic, a nanosized water-soluble fluorinated graphene oxide (FGO) sheets were developed, which shows bright fluorescence used for controlled and targeted drug delivery. FGO can be modified with folic acid

for targeted cancer therapy. The small size enables them to easily endocytosed into cells. They also possess photothermal effects, thus doxorubicin loaded FGO shows synergistic chemo-photo thermal effects in cancer therapy. Their study concluded that FGO can be used as a drug carrier and photothermal agent and it can overcome the systemic drug-related toxicity [42]. Graphene can be surface functionalized by different functionalizing agents like hydroxyl (OH), carboxyl (COOH), methyl (CH_3) and amine (NH_2) groups. A comparison study reveals that G–COOH is considered as strongest. The study was conducted by using doxorubicin as a drug model and the studies have shown that G–COOH adsorbs DOX more effectively because of its high surface binding energy [43]. Zwitter ion modified graphene oxide was developed by using a kind of zwitterion based saline, 3-(dimethyl(3-(trimethoxysilyl)propyl)-amino)propane-1-sulfonate (SBS). They exhibit more stability in both sera free and serum containing solution. Drug-loaded SBS-GO shows thermosensitivity and sustained release nature. Doxorubicin-loaded SBS-GO can be easily internalized by HepG2 cells and exhibits remarkable cytotoxicity [44]. Functionalization of graphene with sulfonate groups and grafting with polyurethane yields polymer with improved properties. It shows high corrosion resistance and is used as filler. It releases cancer drug in a sustained manner [45].

Graphene oxide–galic acid nano-delivery system can release anticancer agent galic acid in a sustained manner. It is effective against liver cancer cells (HepG2) [46]. Dopamine (DA) functionalized graphene oxide nanocarriers are developed and loaded anticancer methotrexate. This is effective against DA receptor-positive human breast cancer adenocarcinoma cell line. Since it is dopamine functionalized, it can be easily targeted to the cell [47]. Considering the high potential of graphene oxide, especially when conjugated with polyethyleneimine for stem cell-induced osteogenesis during fracture healing and tissue engineering also the ability of graphene oxide for promoting the efficient loading and controlled release of aspirin from titanium implants, the surface modification of titanium with graphene oxide could lead a path for the improved success rate of titanium implants in patients [48].

A synergistic anticancer activity was developed by combining the therapeutic actions of curcumin and graphene oxide. The curcumin uptake was found to be improved with the number of oxygen functional groups [49]. Another highly potent nanocomposite of curcumin, paclitaxel and graphene oxide of size 140 nm was synthesized to get synergistic action towards lung and breast carcinoma cells. The higher curcumin load was an advantage of using graphene oxide as the nanocarrier it also can improve the bioavailability of the curcumin-paclitaxel drug complex. The reduced graphene oxide carrier was further improvised with an amphiphilic polymer P-127 [50]. The protein delivery in various genetic and refractory diseases has been compromised due to the proteolytic cleavage resulting in short half-life and instability. A new protein nanocarrier was developed combining graphene oxide with chitosan that could avoid the proteolytic cleavage. Studies were conducted by loading the novel protein nanocarrier with bovine serum albumin and this could provide protection against proteolytic cleavage. Thus this chitosan modified graphene oxide nanocarrier was proven as the effective platform for protein delivery [51]. A new hypothesis has been improvised for targeting the lung carcinoma cells

by synthesizing the molybdenum disulfide conjugated graphene oxide. This molybdenum disulfide-graphene oxide nanocomposite is exhibiting an excellent tumor targeting action along with a high drug loading capability. Additional advantages of good biocompatibility and aqueous dispersibility have been obtained with these nanocarriers [52]. Sodium alginate was covalently linked to graphene oxide with the help of adipic acid dihydride, and doxorubicin was loaded to this. pH responsive release of DOX is achieved at a faster rate. It has a cytotoxic effect against HeLa cells. This nanocarrier enters into the cell by receptor-mediated endocytosis [53].

Graphene functionalization for the development of stimuli-responsive nanocarriers in order to achieve the goal of nuclei based targeted drug delivery is one of the most researched areas. Extensive studies have been conducted by utilizing acidic pH, high intracellular levels of glutathione and near-infrared for the cytosolic delivery of DOX. Doxorubicin-loaded graphene oxide has been developed as a solution for the cancer drug resistance in DOX-resistant MCF-7/ADR cells. And this strategy of DOX delivery by graphene oxide surface loading could satisfactorily improve the cytotoxic effect. The surface functionalization of graphene with natural biopolymers like gelatin and chitosan is useful in reducing the toxic effects of graphene and enhancing the drug loading capacity of graphene. Studies on the development of DOX-loaded GO-folic acid β-cyclodextrin complex, the co-delivery of multiple drugs using graphene oxide and enabling the controlled release of various kinds of drugs from chitosan-GO complex itself explains the significance of graphene as a drug delivery system or the drug carrier [7].

Numerous studies are still going on in the cancer treatment. The great challenge existing is, to target the drug to the affected tissues or cells specifically and to protect normal cells from the damage. The study of the anticancer activity of graphene nanosheets was done by exfoliating graphene sheets by using polyvinyl pyrrolidone which is having an average particle size of 42 nm. The study was performed on various cells like HCT-116, HeLa, SCC-9, NIH-3T, and HEK-293. Researchers have shown that the combination of graphene–polyvinyl pyrrolidone nanoparticle have anticancer activity and act as a pH-sensitive drug carrier for certain anticancer drugs like doxorubicin, as shown in Fig. 4. The graphene-PVP complex loaded with drug by tip ultrasonication thereby forming a stable complex.

Doxorubicin release from the complex in a sustained fashion about 2–3 days in normal as well as in acidic pH It has been found that an anticancer drug-loaded graphene-PVP complex is found to be a most innovative method to treat cancer even though long-term exposure (48–72 h) to graphene-PVP cause mitochondrial toxicity in normal cells. Exfoliated graphene nanosheets based drug carriers will make a revolution in the field of future oncology studies and other drug delivery systems [54]. A schematic representation of the mechanism of graphene-PVP-NP induced cytotoxicity in cancer cells is shown in Fig. 5.

In an earlier study, it was shown that β-cyclodextrin is not considered as a suitable carrier for the poorly water-soluble anticancer drug. But it can be combined with a magnetic compound to produce β-cyclodextrin (β-CD) grafted magnetic graphene oxide nanocarrier and used for the delivery of both hydrophilic and

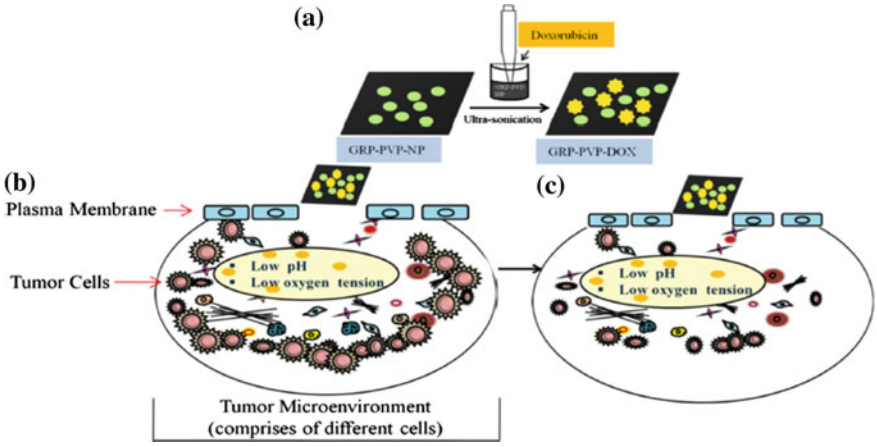

Fig. 4 **a** Loading of doxorubicin in the graphene-PVP complex; **b** release of drug from the complex at low pH and low oxygen concentration which is corresponding to the environment in tumor cells; **c** reduction in the cancer cell growth. Reprinted from [54], © 2017, with permission from Elsevier

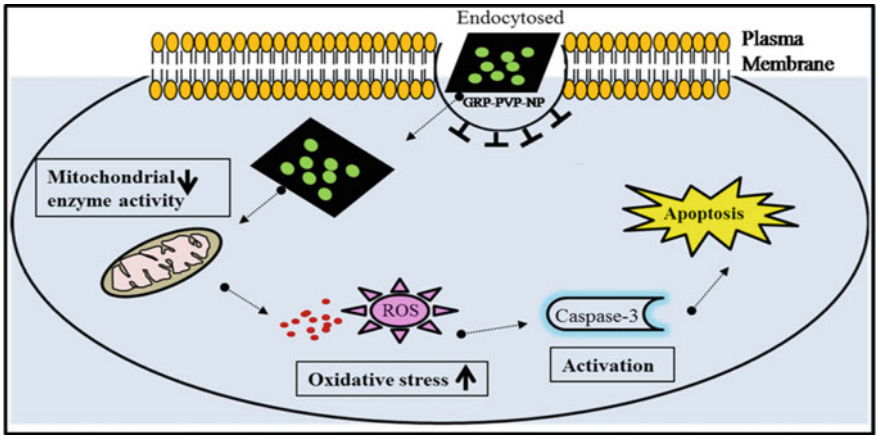

Fig. 5 Schematic representation of the mechanism of graphene-PVP-NP induced cytotoxicity in cancer cells. Reprinted from [54], © 2017, with permission from Elsevier

hydrophobic drugs. MG can be prepared by inverse chemical co-precipitation iron on GO [55]. Chemical co-precipitation of Fe_3O_4 magnetic particles on the surface of GO nanoplatelet leads to the formation of magnetic graphene oxide (MGO). It is then modified with chitosan and MPEG via a covalent linkage to form MgoC-PEG. The anticancer Irinotecan or doxorubicin can be successfully loaded to this magnetic carrier system and are effectively targeted magnetically to U87 cells. PEG enhances the circulation time and decreases the reticuloendothelial system uptake

[56]. Similarly, another magnetic nanocarrier was developed by mounting supra magnetic iron oxide nanoparticle on graphene oxide and then coating PVA. To this carrier system, 5-fluoro uracil was loaded, which releases the drug in controlled release at acidic pH 5.8. PVA increases the circulation time of the drug. This system acts as both imaging and drug delivery system.

Change in pH is a marker of tumor environment, thus a pH sensitive PAA functionalized graphene oxide is used to carry gemcitabine, an analog of deoxycytidine nucleoside for the treatment of ovarian and metastatic breast cancer [57]. Hypericin, a natural photosensitizer, isolated from *Hypericum perforatum* can act as a ligand to target mitochondria. It can penetrate the mitochondrial double membrane. Hypericin-functionalized graphene oxide loaded with doxorubicin gives synergistic anti-tumor effect [58]. Similarly, synergistic chemo and phototherapy by formulating polylysine functionalized graphene were also demonstrated. The photosensitizing agent Zn (II)-phthalocyanine and chemotherapeutic agent doxorubicin was loaded to biocompatible poly-L-lysin functionalized graphene. This nano complex shows high solubility and stability in biological solutions [59]. Pirfenidone is a drug used for the treatment of subarachnoid hemorrhage. It prevents secondary bleeding and cerebral infarction. This activity of Pirfenidone can be improved by loading into a functionalized graphene. Graphene oxide is first treated with transcription activator peptide (Tat) and then functionalized with methoxy Polyethylene glycol and then Pirfenidone was loaded. This carrier system can easily penetrate the blood-brain barrier (BBB) due to the presence of transcription activator peptide. It has a good loading capacity and a fast release at lower pH. Pirfenidone loaded to functionalized graphene oxide shows better activity than single pirfenidone [60].

Aptamer coated dextran functionalized graphene oxide was developed for the targeted drug delivery in cancer therapy. Dextran (DEX) was covalently bonded to graphene oxide nanosheet surface. Then AS1411 aptamer was linked to the hydroxyl group of dextran. To this nanocarrier system, curcumin (CUR), a hydrophobic natural anticancer agent was loaded by π–π stacking interactions. The AS1411 aptamer is a nucleolin recognizer. Thus GO-DEX-Apt-CUR nanosystem having less than 200 nm size can effectively enter into 4T1 and MCF-7 nucleolin overexpressed cancer cells [61].

Doxorubicin acts only after reaching to the nucleus of the cell. pH-responsive charge reversed polyelectrolyte and integrin $\alpha_v\beta_3$ monoantibody functionalized graphene oxide was used to deliver anticancer doxorubicin. DOX released to the cytoplasm of the cancer cell and then moves to the nucleus [62]. Carboxymethyl chitosan functionalized graphene conjugated with hyaluronic acid (HA) and then fluorescein isothiocyanate (FI) can be used to load doxorubicin. The drug encapsulation efficiency was found to be 95%. It can target HeLa cells overexpressing CD44 receptors and suppress their growth. GO-CMC-FI-HA-DOX can release the drug in controlled rate in lower pH tumor cell microenvironment [63]. Graphene functionalized with a polyoxyethylene bi amine and is used to carry silver nanoparticles to form GO-PEG-AgNPs. It is active against *E. coli* and *Staphylococcus aureus*. And it is more effective than silver nanoparticles alone [64]. Carboxylated GO functionalized with β-cyclodextrin by covalent bonds via

esterification. This is used as a drug carrier due to its higher dispersibility in water and is stable for 12 months [65]. Antibodies can be used as targeting ligands. *A* targeting system was developed by the conjugating monoclonal antibody on graphene, which acts against the follicle stimulating hormone receptor. This system is a best tumor targeting agent because of its higher stability, specificity towards FSHR and rapid tumor uptake. Since graphene has a large surface area, they are used for multifunctional drug delivery system. Chemo-photothermal targeted therapy of glioma cells are successfully developed by using IL-13 as targeting ligand, since IL-13 is overexpressed in malignant tumors. And GO is used to absorb NIR lasers because of its good heat transfer for photothermal therapy. It gives a higher effect than single chemotherapy [6]. Certain drugs and polymers have the ability to respond to specific pH of the tumor microenvironment. These drugs or polymers are conjugated to graphene to form a pH targeted system. *G*elatine functionalized graphene nanosheets are loaded with methotrexate, which shows pH-dependent release. The drug is released in acidic pH than neutral [66].

Multifunctional targeted drug delivery system can be developed by using a bio-targeting ligand and a superparamagnetic iron oxide for magnetic targeted delivery. Lactoferrin functionalized graphene oxide iron oxide is effective for glioma targeting. Here, lactoferrin is the bio-targeting material; it is an iron transporting serum glycoprotein, which binds to receptors overexpressed at glioma cell surface and brain tumor. When doxorubicin loaded to this system, it effectively delivers the drug to the tumor cells [67].

Graphene was functionalized with heparin (Hep) via a linker, adipic dihydrazide (ADH). It is then loaded with doxorubicin to form GO-ADH-Hep-DOX nanocomposite; it provides pH sensitive sustained release. It is non-toxic, stable, blood compatible and biocompatible [68]. The hybrid molecule obtained when the thiolated graphene covalently functionalized with luminescent, subnanometer clusters of silver protected by glutathione is having a wide possibility in targeted drug delivery [69]. Biocompatible polymer poly(2-diethylaminoethyl) methacrylate is conjugated with graphene oxide via in situ atom transfer radical polymerization reaction and then loaded camptothecin, which is noncytotoxic and good biocompatible [70].

7.2 Multi Functionalized Graphene in Drug Delivery

A Novel method of cancer treatment using graphene oxide nanoparticle functionalized with polyethylene glycol and folic acid using camptothecin as an anticancer drug. According to the study, functionalizing the Drug-GO-NP complex with PEG and folic acid will markedly increase the uptake of the drug by cancer cells. The development of nanoparticle with folic acid is a good candidate for an effective drug delivery system for cancer therapy. The drug-loaded complex could deliver the drug in a sustained fashion and also have an advantage of reduced dosing frequency when considering the therapeutic viewpoint [71]. Another study shows the loading

capability, drug release and cytotoxic effect of poor water-soluble drug (e.g. camptothecin) loaded GO-PVP/β-cyclodextrin complex. Among the two complexes (GO-PVP and GO-β CD), poorly water-soluble drug loaded GO-PVP was found to be the most guaranteed system for drug delivery and have wide therapeutic applications mainly in the field of oncology [72]. Hydroxypropyl-β-cyclodextrin (HP-β-CD) GO conjugates are also used as carriers. Later this carrier was used for Paclitaxel delivery for ovarian, breast and lung cancer. HP-β-CD chemically bonded onto –COOH group of GO. The paclitaxel-loaded HP-β-CD nanospheres exhibit excellent stability and sustained release of ant cancer agents [73]. Even though graphene oxide based drug delivery for cancer therapy found to beneficial but its action can be modified even more i.e. natural peptide protamine sulfate and sodium alginate can be used; these are adsorbed on to the GO which will give high dispensability and stability to the complex [74]. Functionalized graphene oxide is used as a nanocarrier of new copper (II) complexes for targeted therapy in nasopharyngeal carcinoma. Folic acid and poly ethylenimine (PEI) functionalized GO (FA-PEI-GO) used as a carrier for two new copper complexes to folate receptor-positive nasopharyngeal carcinoma. Folic acid is the ligand interacting with the folate receptor, which can be easily conjugated with GO. Copper complexes have broad-spectrum anti-cancer activity by cell apoptosis mechanism. Thus, this FA-PEI-GO therapy with copper complexes is an efficient carrier for targeted drug delivery [75]. Folic acid—bovine serum albumin (BSA) conjugated GO nanocomposite is used for the delivery of doxorubicin. Here, FA-BSA acts as both stabilizing agent and targeting agent. Doxorubicin is targeted to the folate receptor-rich MCF-7 cell. The clear concept is shown below in Fig. 6. This system reduces the toxicity of GO, increases stability and biocompatibility in physiological fluids [24].

In HER 2 overexpressed breast cancer, nuclear accumulation of drug is achieved by anti-HER 2 antibodies conjugated poly-L-lysine functionalized reduced graphene oxide. Anti HER 2 antibody was bonded to the amino group of poly-L-lysine via glutaraldehyde bifunctional linkage. It is reported that this method of drug delivery shows 7 folds benefit than reduced graphene oxide-PLL nanocarrier delivery.

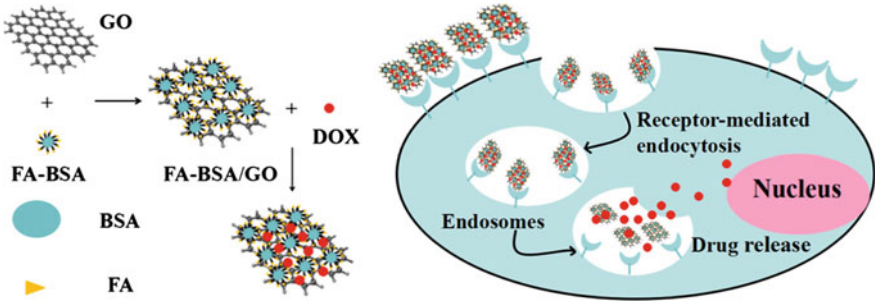

Fig. 6 Schematic representation of Doxorubicin targeted delivery by FA-BSA-GO carrier. Reprinted from [24], © 2016, with permission from Elsevier

It delivers drug rapidly to the nucleus within 4 h by energy-dependent macropinocytic internalization [76]. Phospholipid and PEG-modified graphene nanoribbon loaded with Doxorubicin are active against U87 glioma cells. This activity is 6–7 times higher than free doxorubicin [77]. A detailed study on the functionalization of graphene oxide (GO), by using lactobionic acid (LA) and carboxymethyl chitosan (CMC) was conducted to achieve targeted drug delivery. Here LA acts as a carrier for targeting GO on cancer cells mainly on liver cells (SMMC-7721) and can induce cell death when loaded with drugs like Doxorubicin. In FGO-LA-CMC-DOX complex, LA allows the selective uptake of cancer cells without disturbing normal cell system [78].

Dual-functionalized graphene oxide is used for better siRNA delivery to breast cancer cells. Here reduced graphene oxide (rGO) based nanocarrier was dual functionalized by a phospholipid-based amphiphilic polymer and R8 cell penetrating peptide (CPPs) i.e., for improved hydrocolloid stability and better siRNA transfection ability towards MCF-7 breast cancer cells. By incorporating R8-CPP, it will provide a high positive surface charge which will facilitate easy loading and condensation of siRNA, as well as helps in penetration [79].

7.3 Functionalized Graphene; a Vector for Gene Delivery

The utility of graphene in gene delivery is another field that has to be explored more. Since DNA/RNA are negatively charged, a cationic polymer is used as a vector. The capacity to deliver the gene effectively and the ability to facilitate the gene uptake with high transfection efficiency make graphene a satisfactory vector for the gene delivery and gene-drug co-delivery. The polyethyleneimine, a cationic polymer functionalized GO-based gene delivery thus enables the gene delivery with high transfection efficiency and provide reduced toxic effects associated with polyethylenimine. Graphene oxide surface conjugated with chitosan is another complex carrier widely accepted for the gene delivery and the gene-drug co-delivery [7]. The cell penetrating peptide (CPP) has received immense attraction when conjugated with graphene oxide to generate the CPP-GO complex as the nanocarrier exhibiting excellent intracellular delivery for genes [80].

Oxidized graphenes are efficient nonviral vectors for genetic materials. They are easily uptake by HeLa cells and HUVEC cell and released into the cytoplasm and enter into the nucleus. Graphene oxide functionalized with polyamidoamine (PAMAM) dendrimer and Oleic acid is used as a biocompatible gene vector. It is developed by chemical adsorption of oleic acid and covalent linkage of PAMAM to graphene oxide. GO functionalized with both PAMAM and oleic acid shows higher transfection capacity than GO functionalized with PAMAM only [2]. PEG functionalized graphene oxide is used to deliver molecular beacon (MB), as a model oligonucleotide directed to HeLa cells to detect targeted mRNA. PEG also protects MB from enzymatic cleavage and are less cytotoxic [80].

7.4 Dual Gene/Drug Delivery by Graphene Nanocarriers

The therapeutic efficiency can be enhanced to a higher extent by co-delivery of nucleotide-based therapeutic agent and chemical-based drug simultaneously. Since graphene has a large surface area, both agents are easily loaded to graphene-based nanocarriers by physical or chemical interactions [80]. Since the drug resistance is a major challenge in chemotherapy, an effective strategy of co-delivery of the gene, small interfering RNA(siRNA) along with the drug Doxorubicin using functionalized graphene as the dual nanocarrier has been developed. This could silence the expression of efflux transporters and can be a remedy for the drug resistance during chemotherapy. The folic acid (FA)-conjugated polyethyleneimine-modified PEGylated nanographene exhibited a high DOX and siRNA loading capability along with effective intracellular DOX delivery and gene silencing effect [81]. PAMAM dendrimer functionalized graphene oxide is used for co-delivery of MMP-9 shRNA plasmid and doxorubicin. It increases the efficiency of breast cancer therapy [82]. A complex of PEI and poly-sodium-4-styrene sulfonate (PSS) functionalized GO (PPG) is used for co-delivery of doxorubicin and miR-21 targeted siRNA for the treatment of cancer. Here, first doxorubicin is loaded on PPG by physical mixing and then anti-miRR-21 by electrical adsorption. This system gives synergistic effect by gene silencing of miR-21 and accumulation of doxorubicin in tumor cell [83]. PEI grafted graphene oxide nanocarriers are developed for co-delivery of Bcl2 targeted SiRNA and doxorubicin. It reduces the cytotoxicity and enhances anticancer effect [80].

7.5 Anticancer Activity of Functionalized Graphene

Functionalized graphene is not only a drug carrier but also exhibits anticancer activity itself by exerting inhibitory effects on tumor cells [84]. Resveratrol functionalized graphene oxide induces membrane leakage and oxidative stress in ovarian cancer cells and induces apoptosis [85]. PEG functionalized GO inhibits breast cancer cell metastasis by downregulating the expression of multiple mitochondrial OXPHOS-related proteins and ATP production in cancer cells selectively [86]. Experiments are conducted with graphene to prove its anticancer effects by using glioblastoma multiform tumor cells as a model. But pure graphene is known to have agglomeration and processing problems. In order to increase the anticancer activity and to overcome the disadvantages, the graphene is functionalized with arginine. Studies were made on both normal cells and tumor tissues. After the study, they concluded that both graphenes have anticancer activity and functionalized graphene shown to have more distributed in tumor cells without any agglomeration and arginine suppress the tumor invasiveness. Hence, graphene in its reduced form i.e. rGO-arginine complex is proved to have beneficial against glioblastoma multiform tumor cells [87].

The strategy of functionalization of graphene surface with amino acids for enhancing the surface charge and facilitating the easy uptake by the tumor cells as these cells are negatively charged could synergize anticancer action of graphene. The anticancer activity of ginsenoside Rh2–treated graphene oxide (GO-Rh2), lysine-functionalized highly porous graphene (Gr-Lys), arginine-functionalized graphene (Gr-Arg), Rh2–treated Gr-Lys (Gr-Lys-Rh2) and Rh2–treated Gr-Arg (Gr-Arg-Rh2) were subjected to various cytotoxic assays and these nanostructures were found to be exhibiting satisfactory antitumor activity also these nanostructures is having effect on blood coagulation system [88]. Silver decorated reduced graphene oxide nanocomposites have apoptosis-inducing ability in A549 lung cancer. This is prepared by using *Pulicaria glutinosa* extract (PGE) as reducing agent. The anticancer activity of PGE-HRG-Ag-2 was studied in 5 human cancer cell lines, MCF-7 (breast), A549 (lung), HeLa (cervical), DU-145 (prostate) and HepG2 (liver) and tamoxifen were used as standard reference drug. The results show that PGE-HRG-Ag-2 possesses higher activity than reference tamoxifen against human lung cancer cell line, A549 [89]. *A* unique field of therapeutics i.e. photothermal therapy (PTT) based cancer treatment was developed by using functionalized graphene as a suitable vehicle. The PTT will produce ablations in the cancer cells, eventually tumor growth inhibition and cell death. Graphene oxide (GO), reduced graphene oxide (rGO) and GO-nanocomposites are mainly used. Large specific surface area, abundant functional groups which are available for bioconjugation, drug loading, and targeting make them suitable candidates for PTT. There were a number of comprehensive researches on PTT, and many of them were succeeded and later concluded that it is a great tool for cancer treatment. The main mechanism behind PTT is that they utilize an optical absorbing agent along with electromagnetic radiation for treatment of cancer moreover they induce apoptosis rather than necrosis so there will not be any inflammatory response [90].

7.6 Antibacterial Activity of Functionalized Graphene

The research works has been shown that graphene possess a broad spectrum of antibacterial activity and functionalization of graphene with different organic molecules especially with amine/amide groups which itself having anti-inflammatory activity are found to be tremendously effective against different disease-causing bacteria like Gram-positive (*E. coli*, *Pseudomonas aeruginosa*) and gram-positive (*Staphylococcus aureus*) [91]. *A*ntibacterial activity was found in graphene oxide functionalized with guanidine polymer i.e. with polyethylene glycol (PEG) and polyhexamethylene guanidine hydrochloride (PHGC). Here we get a dual functionalized graphene-GO-PEG-PHGC complex. The complex is incubated with both Gram-positive and Gram-negative bacteria. The results reveal that the complex interact with the cell contents and other nanoparticles present in the bacteria causing greater damage to them leads to cell death. The complex is found to effective against both gram-positive and gram-negative bacteria. The GO-PEG-PHGC can be used as

a disinfectant which is capable of inhibiting the bacterial growth and propagation [92]. Functionalized graphene possesses toxicity against bacterial cell. Thus, they can be used as effective antibacterial agents. Polyethyleneimine functionalized reduced GO coated with silver nanoparticles (PEI-rGO-AgNPs) show antibacterial activity against *Escherichia coli* and *Staphylococcus aureus.* It shows >90% reduction in cell viability. The mechanism involved is that the sharp blade like edges of GO interacts with bacterial cell wall and is damaged. And then, silver ions interact with intracellular components. This leads to bacterial death [93]. A similar investigation was conducted by replacing polyethyleneimine with L-cysteine (L-Cys) and decorating with silver nanoparticles. The GO-Ag-L-Cys complex has high bactericidal activity especially against gram-negative bacteria by disrupting the cell wall integrity while bacteriostatic effect against gram-negative bacteria by inhibiting their cell division. They are active even at low concentration [94]. Thin film and solution of poly (*N*-vinyl carbazole) graphene (PVK-G) have activity against *E. coli* and *Bacillus subtilis* at 1 mg/mL concentration. This shows 80% reduction in bacterial growth [95]. rGO functionalized with polysulfone (PSU) have activity against *Bacillus subtilis and Escherichia coli.* The investigation reveals that this complex has high antibacterial activity because of the production of reacting oxygen species. Another advantage is that the presence of shorter polymer chain which allows greater contact with the bacteria on the surface of graphene [96]. Chlorophyllin and zinc functionalized graphene oxide also exhibit antimicrobial activity. The incubation of bacteria with this functionalized graphene oxide causes loss of cellular integrity and cell death [97].

Amino acid arginine functionalized mono-layer graphene shows excellent antibacterial activity, and it can overcome the silver nanoparticle-induced toxicity [98]. Effect of the size of graphene sheets in its antibacterial effect was studied, and graphene sheets with smaller size show higher antibacterial activity [99]. Noncovalent functionalization of graphene with surfactants shows antibacterial activity. *A* study was conducted by using different surfactants such as tween 80, cetrimide, sodium dodecyl sulfate and reported that cetrimide functionalized graphene possess antibacterial activity. Antibacterial activity of cetrimide functionalized graphene against *S. aureus h*as improved 16 and 65 folds against *P. aeroginosa* than cetrimide alone [100].

7.7 Other Applications

Graphene-based non-materials have a wide variety of biomedical applications which is represented in Fig. 7. By bio-functionalization of graphene-based nonmaterial with various biomolecules, other cells open great field biomedical applications such as bio-imaging, electronic devices, biological sensing, drug/gene delivery, a biocompatible scaffold for cell culture; stem cell differentiation e.g. functionalized graphene is adsorbed with fluorescent-tagged DNA. Both single-stranded (ss) DNA and double-stranded (ds) DNA are adsorbed. If ssDNA having

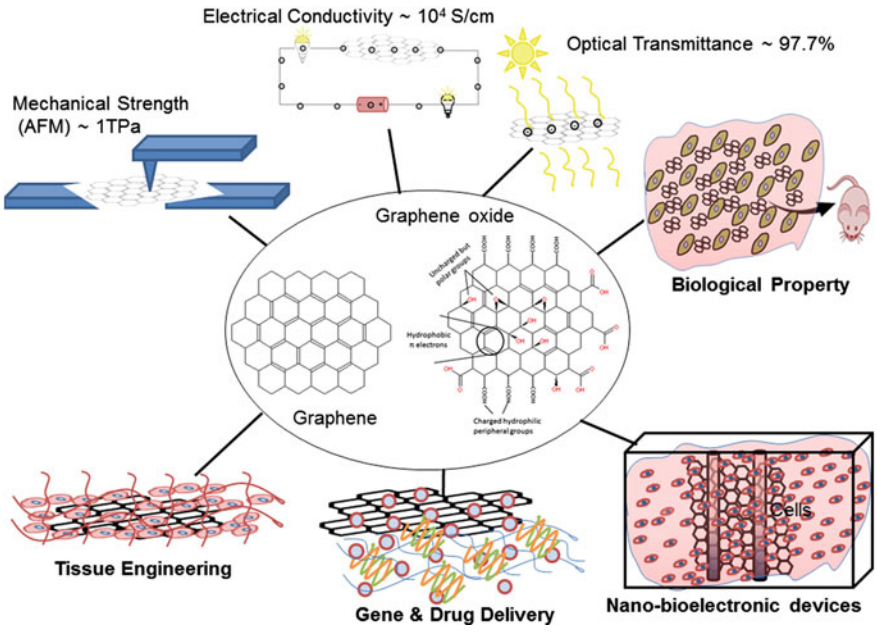

Fig. 7 Schematic representation of applications of graphene. Reprinted from [7], © 2013 with permission from Elsevier

stronger interaction against dsDNA, the fluorescence on the ssDNA darkens more, thus indicating the bio-sensing capability [101]. Graphene can be made luminescent, and it is the best candidate for bioimaging. Fluorescein functionalized pegylated graphene oxide is used in intracellular imaging. Here PEG is used to prevent GO-induced quenching of conjugated fluorescein. Laser irradiation in the presence of nanographene oxide (NGO) creates micro bubbling, which causes immediate cell damage at the specific site, thus it offers localized therapy [19]. Functionalized graphene enhances the properties of the polymer-based nanocomposite. These nanocomposites are used in electronics and sensors in industries and pressure sensors in the biomedical field. Incorporation of poly (vinylidene fluoride-chlorotrifluoroethylene) nanocomposite to hyper branched poly ethylene-graft-poly (trifluoroethyl methacrylate) copolymer functionalized graphene shows increased dielectric property and high energy density. Thus, it can be used in electronic devices, sensors and biomedical applications [102]. The functionalization graphene can be done with different polymers. The polymer functionalized graphene may have advantages in different aspects like it improve mechanical, electronic, optical, thermal and magnetic properties significantly if used appropriate polymer. Polyaniline, polypyrrole called as conducting polymers when used to functionalize graphene, the resultant complex can be used as supercapacitors which have a high capacitance of about 424 F g^{-1} [103].

Graphene has been used for wound healing, regenerative medicine, stem cell, and tissue engineering. A chitosan-PVA nanofibrous scaffolds containing graphene is used for wound healing and is proved in mice and rabbit. Functionalization of graphene with proteins and peptides is used for tissue engineering applications. Graphene materials have the ability to absorb DNA/proteins due to their large surface area, thus it is used in many therapeutic applications [7]. Graphene oxide nanocomposites were developed by functionalizing lactobionic acid and carboxy methyl chitosan and they are used for doxorubicin delivery. It readily releases at lower pH cancer cell environment than at general physiological pH. Cancer cells uptake them and minimal uptake by the non-cancerous cell [78]. A sensing array system in order to differentiate the cancer cells, circulating tumor cells and different cell types based on the functionalized graphene elements electrochemical mechanism has been developed. This innovative approach can be considered as a new pavement in future clinical cancer diagnosis [104]. Encapsulating the MCM-48, mesoporous molecular sieves with either reduced graphene oxide or graphene oxide can be used in remediation of caffeine and phenacetin from aqueous solution [105]. A conductive biodegradable scaffold has been generated by modifying the graphene oxide-gold nanosheets with the natural polymer chitosan. This innovation was sufficient enough to overcome the abnormal conduction and electrical impulse propagation in the heart after myocardial infarction [106]. Circulating tumor cells (CTC) are the main reason for widespread cancer cells. The researchers have demonstrated the isolation of CTC using functionalized graphene oxide nanosheets molded in gold plates. This discovery very effective in therapeutically, i.e. early detection of rare CTC in blood hence spreading of cancer [107]. The chemical functionalization graphene will cause the destruction of sp^2 network and to get converted to sp^3 hybridization. During this conversion the metallic graphene changes to an insulator. Specifically, graphene on appropriate surface modification can be converted to a semiconductor which will lead to new novel nanoelectronic and nanophotonic applications of graphene [4]. Capacitor developed by modifying 3D self-assembled graphene hydrogel by using multi redox anthraquinone derivative alizarine via noncovalent functionalization [108]. Oxygen functionalized graphene shows higher specific capacitance (up to 189 F g^{-1}) than graphene (165 F g^{-1}). This is due to additional pseudo capacitance effect of oxygen-containing functional group, mainly carbonyl and hydroxyl groups. They show large pseudocapacitance and less aggregation. Heteroatom doping alters the physical or chemical characteristics of graphene. Nitrogen doping increases the energy density of graphene supercapacitor in both aqueous and electrolyte solution [109]. Single or multi hetero atom functionalized graphene is used as charge extraction materials to improve solar cell performance [110]. Carboxylated graphene is used as a sensitive electrochemical biosensor [111]. Reduced graphene functionalized with 1,6-diaminohexane and loaded with silver nanoparticles. This is effective against bacterial coliform infection and thus it is used for water disinfection [112].

8 Conclusion

This chapter mainly emphasizes graphene functionalization and the application of functionalized graphene in drug delivery. Chemical modification on graphene helps in enabling additional properties of dispersibility in different solvents, reduced toxicity of graphene and also prevents agglomeration. Covalent and noncovalent modification methods of functionalization along with multi-functionalization, substitutional doping, and nanoparticle functionalization has been described. Functionalization with multi molecules makes graphene an efficient candidate for targeted drug delivery. A wide range of applications of functionalized graphene as carrier for the drug in targeted therapy has been highlighted in this chapter. The tremendous cell targeting ability and the high drug loading capacity opens a wide range of application possibilities as a drug carrier for functionalized graphene. Along with that, the capability of functionalized graphene for gene delivery and for the co-delivery of both drug and gene, the anticancer effect and antibacterial effect has also been pointed up.

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