Chapter

Theranostics Application of Graphene-Based Materials in Cancer Imaging, Targeting and Treatment

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Abstract

Recent advancements in graphene-based nanomaterials provide the opportunity that compliments the limitations of conventional drug delivery systems (DDSs) through simultaneous targeting of the anticancer drug to the cancer cell by reducing the side effects of other administration routes. Graphene with its extraordinary electronic properties like larger surface area, possibilities of surface modification, can efficiently target the tumor cell. At the same time, nanocarriers have the advantages of immune clearance adulteration of physicochemical properties of anticancer drug. The DDSs can be made by biodegradable nanocarriers such as proteins, peptides, biocompatible polymers, antibodies, polymer-drug conjugates, etc. Graphene-supported DDSs in cancer therapy also supports the co-delivery of therapeutic agents, antioxidants, SiRNA, shRNA, etc. as the co-delivery approach, which provide additive or synergistic therapeutic efficacy and can reduce toxic effects.

Keywords: graphene, nanocarrier, cancer imaging, drug delivery, DDSs

1. Introduction

As a promising interdisciplinary field, nanotechnology acts as a bridge for various disciplines, such as material sciences, engineering, physics and chemistry, and is dedicated to the production of different materials in the nanometer scale (<100 nm), with assorted physical, chemical and mechanical properties. Although nanoscience and nanotechnology are new research interests, the application of nanomaterials for humankind was well-known since ancient times. Among the periodic elements, carbon has the intense ability of catenation and great tendency to form various hybrid orbitals (e.g., sp, sp^2, and sp^3) which results in the formation of various smart compounds having different physical and chemical properties according to their structure [1, 2]. Due to the properties mentioned above, carbon have tendency of forming different allotropes of different dimensions, like quantum dots (0D), carbon nanotubes (1D), fullerenes (0D), graphene (2D), graphite (3D), among which, graphene got lot of attention in the past decade. Graphene has hexagonally packed honeycomb like geometry in which a unit layer of carbon atoms are arranged

in two-dimensional (2D) lattice [3–6]. The hybrid orbital of carbon-carbon atoms are in sp^2 hybridized form, in which the in-plane σ (C–C) bonds are much stronger than the out-of-plane π (C–C) bonds, which is highly accountable for the delocalized array of electrons and come up with the weak polar interaction between graphitic layers of the graphene sheets as well as with graphene and other molecules.

In scheming a potent drug delivery carrier, besides its physicochemical constancy in the biological surroundings, reactivity and toxic issues, diffusivity, immunogenicity, interactions with biological systems, drug loading and release characteristics, blood circulation half-life, drug transportation ability of the biological medium to aim the cells within tissues, etc. are the significant issues (**Figure 1**). Due to the electrostatic interaction and presence of different alkali and alkaline earth metal ions *viz.* Na, Mg, etc. in the physiological medium, the graphene sheets tends to agglomerate which results in reduction of their surface area, decreasing their solubility and increasing their toxicity. Therefore, surface modification of graphene nanosheets is required to overcome such physical and biological effects. Covalent functionalization and noncovalent physisorption are the two well-known strategies universally applied for surface modification to construct desired modified graphene nanosheets [7–9].

Number of research groups have been investigating the surface modification of GO with different kinds of biocompatible polymers as a nanodrug carrier for development of targeted drug delivery systems. The polymers are selectively preferred according to their functional groups, bioavailability and compatibility in the cell medium [10]. Therefore responding to specific stimulus, surface fabrication of GO with various polymers for this particular drug delivery application is limited [11]. For well-organized diagnosis, expressive intracellular drug release is elected over the contemporary arrival of the drug in the system.

With the innovation of the smart material graphene, the curiosity of researchers remarkably moved toward graphene and its oxygenated derivatives from the previously invented other nanomaterials of carbon family, and various scientists are working to organize the surface modification of graphene through the spacious understanding of different functionalization methods [12–14]. From the chemical

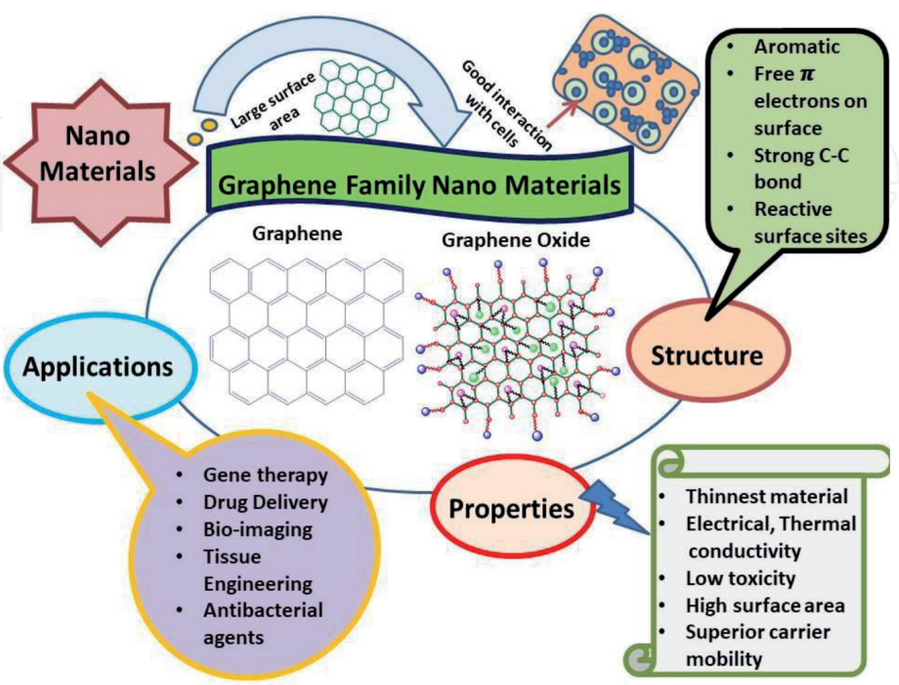

Figure 1.
Properties and applications of graphene oxide.

point of view, production of graphene oxide that possesses many reactive oxygen-containing groups is appropriate for advance modification and relative aspects of GO sheets, or we can say that the reactivity of GO is highly desirable.

Beside this, graphene is sparingly soluble in the water, polar solvents and in the cell environment due to aromatic character [15]. Graphene oxide on the other hand has water contact angle of 30.7° [16] and proficient of composing weak hydrogen bonds and metal incorporated complex ion due to the polar oxygenated groups present on the basal plane and negatively charged carboxylic groups present on the edge site [17–20]. The distinctive arrangement of graphene oxide and its strong carbon-carbon covalent bonding provides outstanding thermal and electrical conductivity with very low thermal expansion quotient. These properties of graphene are also significantly affected by alteration such as edge scattering defect [21] and isotopic doping [22] due to diffusion or localization of phonons at the defect sites. Light absorption and optical imaging are highly dependent on the total number of layers present in GO sheets, as they increase accordingly with the number of layers present in GO [23]. Optoelectronic devices based on GO derivatives are developed as tunable IR detectors, modulators and emitters by electrophysiology and charge multiplexers [24]. This capability to organize the rearrangement and partition of surface electrons can be oppressed in emergent bio-imaging applications [25, 26].

The GO sheets are highly influenced by the divalent ions and some specific polymers which play a crucial role in mechanical properties of GO sheets by inter connecting both the molecules [27, 28]. Due to the superior mechanical properties of GO, it has been reported that by incorporating the different polymers with it, the tensile strength of the respective polymer increases. Graphene supported polymethyl methacrylate (PMMA) and poly-L-lactic acid (PLLA) drastically amplifies the young's modulus and hardness of these polymer nanocomposites for mechanical applications [29].

With reference to different physicochemical properties of nanomaterials of graphene family, it can be projected that they will demonstrate numerous mutual connections with biological moieties such as cells and tissues depending on chemical modification, thickness and dimensions of graphene sheets, etc. [30, 31]. With more supplementary data of graphene application in biomedical field, research on its cellular activity and other intracellular processes is rising. Introduction of polar and reactive oxygenated functionalities give rise to oxidative force in objective cells is supposed to be the effective mechanism for potency of graphene oxide [32, 33]. Due to their dose dependent cytotoxicity and bactericidal activity, graphene-based materials are being explored for applications in antimicrobial products. A number of studies have been performed reporting the antibacterial activities of CNTs, graphene, GO and rGO against *Escherichia coli* and *Staphylococcus aureus* bacteria with rGO having the strongest antibacterial effectiveness [34–38]. With reference to the various studies discussed above, it is apparent that shape and size, of graphene-based materials importantly take part in determining their interactions with cell membrane and intracellular uptake. Moreover *in vitro* studies in various cell lines along with broad perspective of various mechanisms dependent on graphene-based materials are being increasingly explored for various applications in antimicrobial products. In the end, we will briefly discuss the prospects and future challenges regarding graphene-based materials as cancer imaging, targeting and treatment applications.

2. Synthesis of graphene oxide and its surface modification

Different methods are used to set up the preparation of graphene sheets according to its structural and chemical behavior with various biocompatible molecules.

The methods are widely categorized as colloidal suspension (size specific), arc discharge (electric charge specific), and chemical or mechanical exfoliation. For the broad and extensive production of graphene sheets, mechanical exfoliation method were not used as it is expensive but for the fabrication of electronic devices it is widely applied. Graphene oxide (GO) the oxygenated derivative and replacement of graphene is synthesized by the chemical exfoliation method, in which the sp2-hybridized C–C hybrid orbitals breaks and the different oxygenated groups such as hydroxyl, carboxyl and epoxy are introduced [39]. The surface modifications of the graphene sheets are site specific as the bulky group carboxyl attached toward the edges of the sheets while on the other hand hydroxyl and epoxy groups tends to form bond with the basal plane of the graphene sheets [40, 41]. These oxygenated and highly reactive functional groups offers reactive handles for a range of surface-functionalization reactions covalently and non-covalently, which can be used to build up surface modified GO, its biocompatible composites. For the large-scale synthesis of graphene, the most common methods required exfoliation of graphene. The only variances among graphene made by different methodologies are the defect content and yield of their products [42]. Various methods are available for graphene synthesis, but for the large production of graphene oxide (GO) oxidative-exfoliation methods give excellent results. There are some additional treatments required to reduce typically defective graphene-like nanosheet into reduced graphene oxide (RGO) [43]. During the oxidation process of graphene, functional groups containing oxygen attached to the surface increase the distance among graphitic layers and responsible for enhancing the exfoliation by weakening the van der Waals forces [44]. After the oxidation process, several washing steps are required so that oxidizing agents and some other impurities removed from graphite oxide to enhance the exfoliation. For large-scale washing of graphite oxide, different conventional approaches such as filtration process [45], centrifugation process [46], and dialysis process [47] are mostly used. Among all these processes infiltration processes, after some time, particles of graphite oxide choke the filter pores and make it time-consuming process.

2.1 Synthesis of graphene oxide (GO)

For the synthesis of graphene oxide, the most used source of graphite is flake graphite, which occurs as a natural mineral which further use to purify to remove heteroatomic contamination [48]. GO prepared by the use of flake graphite have the property to easily dispersed in water hence used on a large scale [49]. This expanded form of graphite powder has been used for the synthesis of GO sheets by the following method.

2.1.1 Modified Hummer's method

Graphene oxide is synthesized by modified Hummer's method using graphite powder [50]. In this method, in a round bottom flask, a mixture of 1 g of $NaNO_3$ and calculated graphite powder are mixed. In this mixture drop by drop, 46 mL of H_2SO_4 was added in an ice water bath with continuous stirring. After 4 h stirring without any pause at 32°C temperature, 6 g of $KMnO_4$ was poured into a slurry mixture of $NaNO_3$ and graphite. Later 2 h continuous stirring 92 mL of DD water was combined in it at 95°C. Again after 2 h, 200 mL of DD water was poured in it and leave for 1 h for constant stirring. Lastly, at room temperature, 20 ml of H_2O_2 was mixed and mixed it repeatedly for 1 h. The obtained supreme oxidized product washes by 10% HCl solution for purification by abundant quantity by ion free water. Finally, it is filtered by 0.2 μm Nylon membranes until neutralizing the final product.

2.2 Surface functionalization of GO

From the last decades, the interest has increased in the scientific community for the use of graphene oxide (GO) in biological and biomedical field applications [51]. GO is a two dimensional material with a large surface area containing single-layer sheets of carbon atoms with sp^2 hybridization and carbon sites with sp^3 hybridization in which hydrophilic functional groups with oxygen are present [52]. Thus, GO has many possibilities for surface functionalization due to outstanding solubility in water [53]. There are different methods for the functionalization of the surface of graphene oxide; some of them are discussed below (**Figure 2**).

The nanocarrier thus synthesized was characterized by some advanced spectroscopic techniques, like RAMAN (**Figure 3**), Fourier Transform infrared (FT-IR) (**Figure 4**), Transmittance Electron Microscope (TEM) (**Figure 5**). Thermal stability and quantitative analysis were characterized by Thermo gravimetric Analyzer TGA under a nitrogen atmosphere at a heating rate of 10°C/min from 30 to 600°C (**Figure 6**).

2.2.1 Covalent functionalization of GO

During the processes of oxidation and exfoliation of graphite, there is a large extent of carboxylic group forms on the graphene surface. These groups better modified by different methods; one of them is covalent functionalization. In covalent functionalization, graphene is coupled with reagents, such as 1-ethyl-3-(3-dimethyllaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) [54], or can also be converted to acyl chlorides using thionyl chloride (SOCl) [55]. Covalent functionalization is a multipurpose methodology for modification

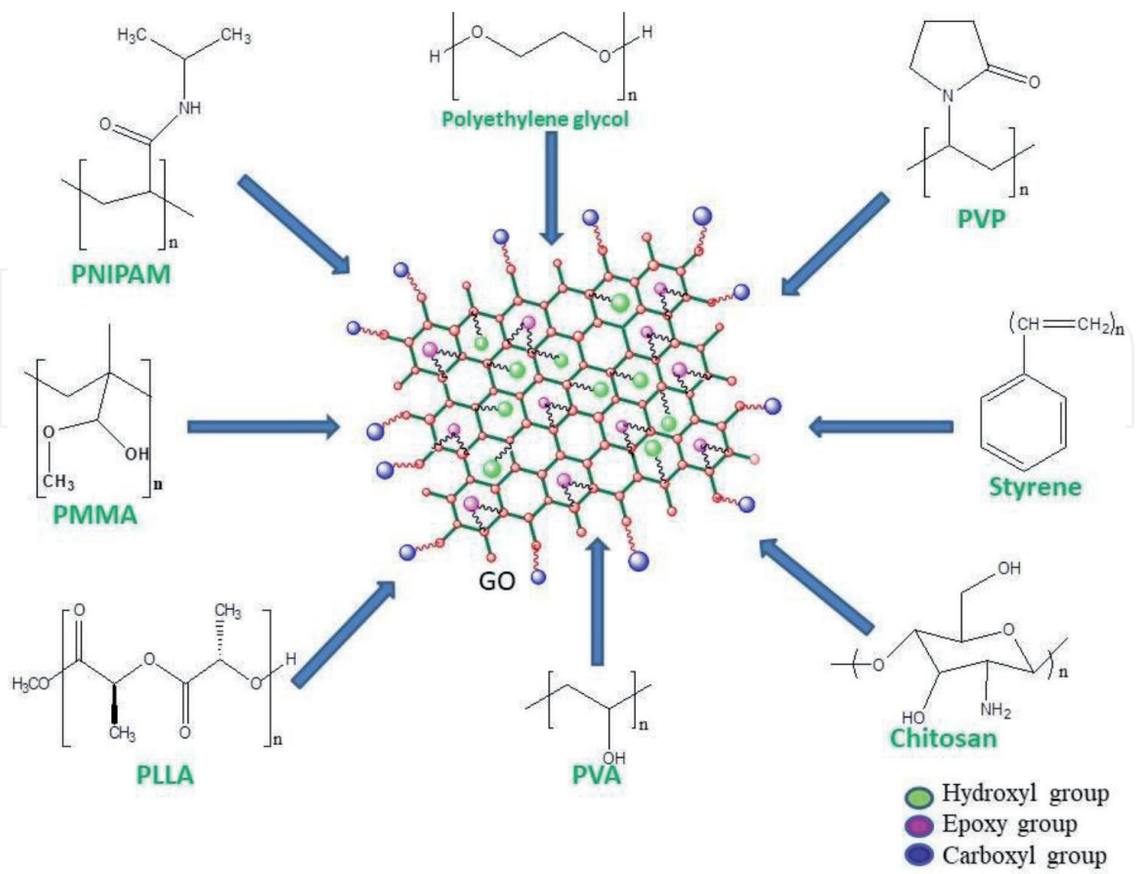

Figure 2.
Surface modification of graphene oxide.

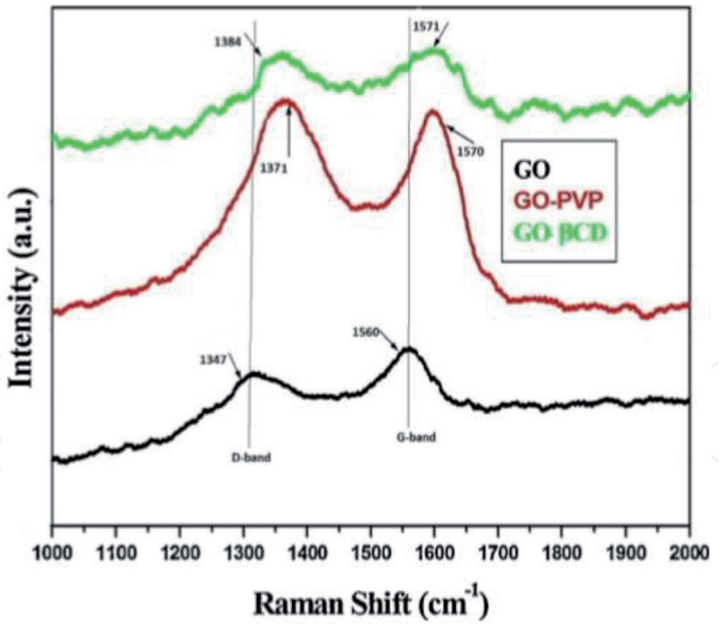

Figure 3.
Raman spectra for GO, GO-PVP, and GO-β-CD.

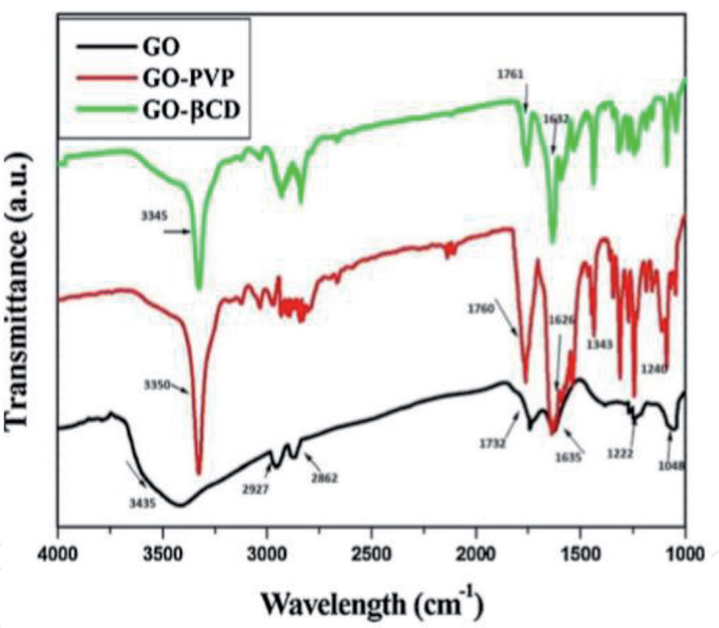

Figure 4.
Fourier transform infrared (FT-IR) spectra of GO, GO-PVP and GO-βCD.

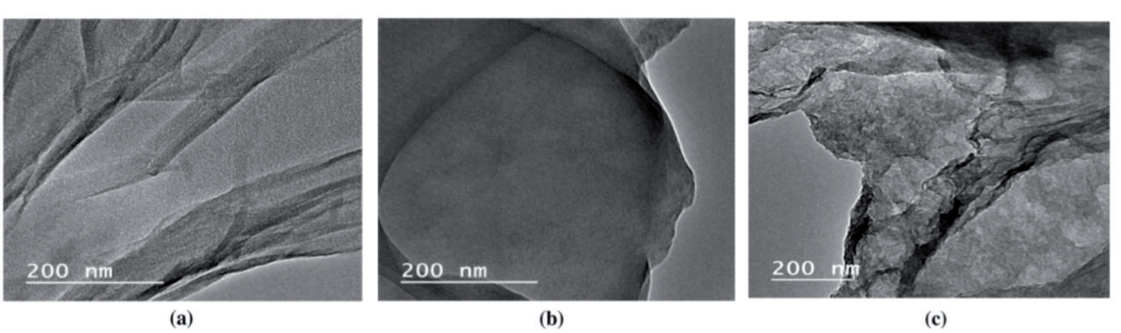

Figure 5.
TEM images of (a) GO (b) GO-PVP, and (c) GO-β-CD.

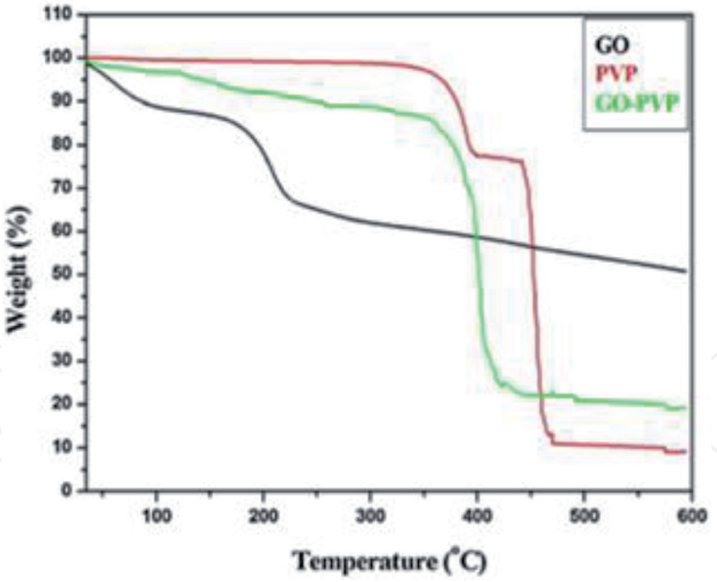

Figure 6.
TGA analysis of GO, PVP, and GO-PVP.

of graphene surface which tailoring the chemical properties as well as electronic properties of graphene [56]. The methodology of GO functionalization by covalent functionalization depends on the environments of the reaction, nature of solvent, temperature condition, different functional groups of the incoming molecules, and other factors like reaction time. When polymer attach to the GO nanosheets it creates stress on GO surface, the covalent mode of functionalization is helpful in controlling the chemical properties of GO and reduces the stress caused by polymer [57]. GO surfaces have the ability for excellent covalent functionalization which makes it a unique nanomaterial which is helpful in developing studies of biological system. According to previous studies functionalization of GO shows excellent results when perform its use in targeted drug delivery applications [58]. There are chances of this because GO surface has the affinity for the adsorption of huge amount of hydrophobic drugs easily and due to specificity of covalent functionalization it releases the drug to particular regions of organisms. This functionalization of GO is also applicable in other biological activities like anti-bacterial activity, bioimaging [59], and photo-dynamic therapy [60]. Even though some procedures of graphene surface modification by covalent functionalization have validated efficient results but some methods generate some supplemental defects on the surface of graphene which are responsible for the changes in graphene structure.

2.2.2 Non-covalent functionalization of GO

Non-covalent functionalization is a more effective method in order to make maximum use of the inherent structure and mechanical properties of graphene oxide or graphene. Non-covalent functionalization is largely preferred in place of covalent functionalization as it does not alter the structure and electronic properties of graphene and it simultaneously introduces new chemical groups on the surface. The most common examples of non-covalent functionalization on graphene surface include polymer wrapping, π-π interactions, electron donor-acceptor complexes, hydrogen bonding, and van der Waals forces. Non-covalent functionalization of graphene results in the enhancement of dispersibility, biocompatibility, and reactivity, binding capacity, or sensing properties. Non-covalent interactions also known as supramolecular interactions are found in all types of materials that experience

attractive as well as repulsive forces between them. These type of interactions are found in many natural and synthetic systems [61, 62]. In comparison to covalent bonds the energies of individual non-covalent interactions are normally lower [63]. In graphene, two types of π-π interactions occur between the electron-rich and electron-poor regions, which influence its interaction with other molecules or nanomaterials. This is commonly seen in the face-to-face and edge-to-face arrangement [64]. Graphene materials, with the π-π interactions have dissociation energies less than 50 kJ mol^{-1}. The weakest forces, that is, London-dispersion forces or van der Waals interactions are responsible for the non-covalent interaction affect all atoms in close proximity. The hydrophobic effects caused by different types of interactions are influence not only dispersibility of GO but recognition interactions [65, 66].

3. Challenges in nanotheranostics designing

In forthcoming nanotheranostics will be accepted as an efficient nanomedicine due to their unique properties like imaging, target selectivity and ability to load the drug in nanocarrier. In the process of nanotheranostics evolution as a potential nanoplatform various challenges encountered for detection of clinical complications. An appropriate technology is required for the treatment and selection of effective therapeutic agents for respective diseases like metallic nanocrystals, image-contrasting agents and choosing an efficient therapeutic agents for corresponding diseases like metallic nanocrystal and concatinate them as nanomaterial. The advanced nanomaterial high selectivity to the target site is required for advance nanotheranostic for excellent delivery of drug targeted nanotheranostic should contain delivery and loading capacity. The biocompatible material should be used in the preparation of nanotheranostics the normal tissue should not damage and easily excreted by human system. Whole designing of nanotheranostics will cheap with no side effects to body.

3.1 Pharmacokinetic and toxicological aspects

The introduction of a new drug to the site is not only expensive, but also time consuming. It includes discovery, clinical testing, development, and approval. Improving safety/efficacy ratio of marketed drugs is more cost-effective. All this can be done by controlling the time, rate, and place of drug release in the body through a drug-delivery system. Hence, a drug-delivery system could be seen as an interface between the patient and the drug [67]. Since past decades, a growing number of drugs were discovered and were optimized for an enhanced efficiency. However, about 40% of the new drugs, especially those based on biomolecules, like peptides, nucleotides, or proteins, often present a low bioavailability and are rejected by the pharmaceutical industry [68]. For controlled release the ideal materials must control certain important issues like easy reach to the target site in the body, ability to transport the necessary volume of active compounds, and a certain level of release with a certain speed, apart from the properties needed to ensure a better and safe interaction with the human body [69].

4. Graphene-based composites in various biomedical application

Graphene is considered as the finest and most durable monolayer capable of free existence. The specific 2D geometry and presence of pi electrons in graphene basal plane further applied for valuable drug loading via hydrophobic interactions and

π-π stacking. Furthermore, large surface area of graphene allows for high density surface fabrication via different surface modification. A number of research on the in vivo behavior and bioactivity of graphene (**Figure 7**) has been investigated previously.

4.1 Drug delivery

Graphene is the finest and most durable monolayer material which is capable of free existence. In graphene, its 2D structure and presence of delocalized π electrons on its surface can be used for effective drug loading via hydrophobic interactions and π-π stacking. In addition to this, large surface area of graphene allows it for high density bio-functionalization via both covalent and non-covalent surface modification methods. Various studies based on the in vivo behaviour and bioactivity of graphene shows that the nanocarriers interact with the cell membranes and enter into the cells by endocytosis. For targeted drug delivery to the cell nucleus, it is essential that the drug carrier escapes endosomal compartment and release loaded drug into the cytosolic compartments [70, 71]. This process proposed a strategy to reverse cancer drug resistance in DOX resistant MCF-7/ADR cells by loading DOX on graphene oxide surface via physical mixing [72]. High pH dependent release for drug loading with of DOX was observed *in vitro*. GO enhanced accumulations of DOX in MCF-7/ADR cells causing higher cytotoxicity in comparison to free DOX. It is well known that pH is acidic in the cancer micro environment, intracellular lysosomes and endosomes. This fact has been exploited to achieve active drug release in the tumor tissue/cells using chemical modification of graphene [73–76]. For chemotherapeutic efficacy use of graphene-based materials has also been explored for co-delivery of multiple drugs. Zang et al. [77] Loading of DOX and CPT in controlled way inside the same drug delivery system resulted in remarkably higher toxicity in MCF-7 cells compared with GO-loaded only with DOX or CPT. Thus, graphene and GO-modified magnetic nanoparticles results in various biomedical applications in the field of drug delivery, MRI and bioimaging.

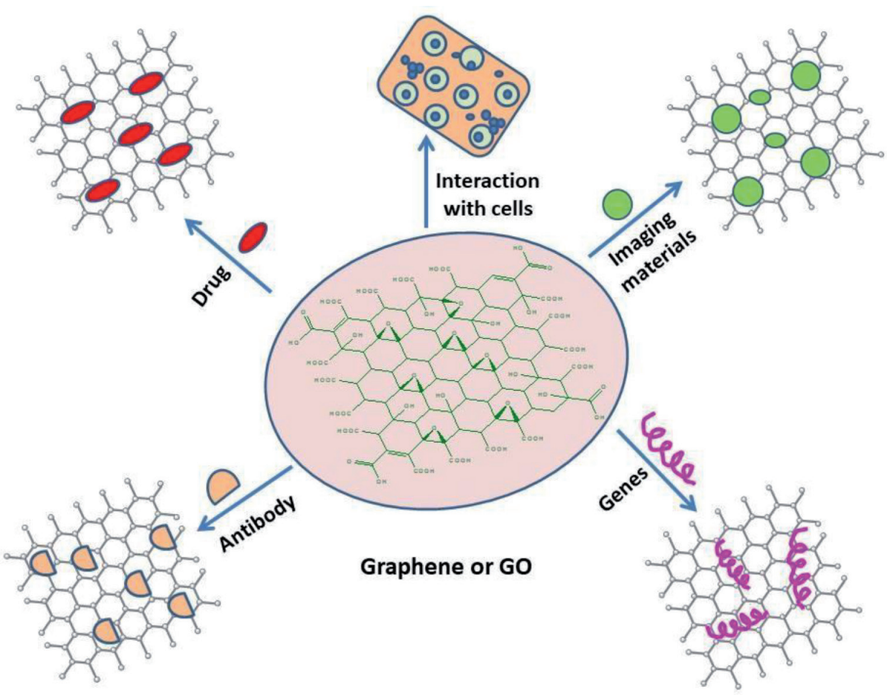

Figure 7.
Graphene-based composites for various biomedical applications.

Attachment of nanoparticles such as iron oxide with graphene-based nanomaterial makes them super paramagnetic in property and can be useful in drug delivery applications [78]. The resulting magnetic hybrids dispersed uniformly in aqueous solution before and after loading of DOX. Magnetic hybrids show agglomeration behavior in acidic medium and redispersion behavior observed in basic medium. This pH triggered magnetic behavior of GO-Fe_3O_4 nanoparticle hybrids can be help in controlled drug delivery. Similar pH-dependent drug release system was reported for 5-FU-loaded nanohybrid system composed of graphene nanosheets (GN), carbon nanotube (CNT) and Fe_3O_4 [79].

4.2 Gene delivery

Gene therapy is used in many expanding area to treat genetic disorders like Parkinson's disease, cystic fibrosis and cancer. An effective gene therapy needs efficient and safe gene vectors that also protect DNA from nuclease degradation as well as facilitate DNA uptake with high transfection efficiency [80, 81]. According to review of literature, graphene has been reported for wide applications in the field of gene delivery, gene-drug co-delivery and protein delivery with. PEI has been extensively investigated as nonviral gene vector having strong electrostatic interactions with negatively charged phosphates of RNA and DNA. Chemical modification is very easy in PEI which offers increased transfection efficiency, cell selectivity and reduced cytotoxicity however low biocompatibility and high toxicity of (Polyethyleneimine) PEI limit its use for biomedical applications [82]. Chitosan-GO complex are also used for simultaneous drug and gene delivery [83]. Chitosan-GO converts pDNA into stable nano-sized complexes. Amine-terminated PEGylated GO was effectively used to deliver high protein payloads due to non-covalent interactions with surface of PEG-GO [84]. Bone morphogenic protein-2 (BMP-2) was loaded onto Ti substrate coated with alternate layers of positively (GO-NH_3+) and negatively (GO-COO-) charged GO nanosheets with high loading efficacy and conserved bioactivity. Osteogenic differentiation of MSCs was enriched on Ti coated GO surfaces carrying BMP-2 than only Ti surface with BMP-2. In vivo studies in mouse also exhibited vigorous new bone formation with Ti-GO-BMP2 implants compared to Ti or Ti-GO or Ti-BMP2 implants and making the new composite a very effective carrier for therapeutic drug delivery [85]. All above studies have highlighted potential of graphene-based materials as drug and gene delivery vehicles *in vitro* studies though there is a necessity to validate their potential in vivo with particular focus on safety, biodistribution and efficacy.

4.3 Tissue engineering

Tissue engineering is an interdisciplinary field that endeavors to develop biological substitutes to resolve, retain or enhance functionality of a tissue or whole organ [86]. Recently, graphene-based materials have been used to treat wound healing, stem cell engineering, regenerative medicine and tissue engineering. Hydrogels have viscoelastic and transport properties to mimicking natural tissues [87], but their weak mechanical properties can limit their use in many tissue engineering applications. Graphene has a platform for tailoring various functionalities on flat surfaces with outstanding mechanical properties like high elasticity, strength, flexibility. Potentially, graphene has a wide range of applications in the field of hydrogels, biodegradable films, electrospun fibers and other tissue engineering scaffolds. When GO incorporated into PVA-based hydrogels it potentially increases tensile stability (132%) and

compressive strength (36%) of composite hydrogel without altering their cytoaffinity [88]. According to Lu et al. graphene-based composite materials are applicable for wound healing by formulating graphene containing chitosan-PVA nanofibrous scaffolds. These three groups, chitosan-PVA-graphene electrospun fibers, chitosan-PVA fibers were also studied without graphene and control (no scaffold), to check wound healing affinity in mice and rabbit [89]. Graphene containing samples healed the wound completely in faster rate in comparison to without graphene-based samples in both mice and rabbit. Graphene-based materials also have applications in the area of musculoskeletal tissue engineering using mouse myoblast C2C12 cell lines [90]. Cellular behavior on graphene derivatives are enhanced by the Surface roughness and surface oxygen content and adsorption of serum proteins. Thus, graphene materials can be useful in reinforcing tissue engineering scaffolds due to its mechanical and electrical properties. Graphene materials have properties like large surface area which adsorb proteins/DNA and can be useful in many therapeutic applications. For instance, Mahmoudi et al. [91] recently reported protective role of GO and protein-coated GO surfaces in amyloid beta fibrillation process, which is implicated in various neurodegenerative disorders. However, along with detailed *in vitro* characterization of scaffolds, more emphasis should be placed on their evaluation in vivo with respect to inflammatory responses, biocompatibility and regenerative potential.

5. Application of graphene in bioimaging

Graphene-based nanomaterials have been widely explored in biomedical fields such as bioimaging, drug delivery, theranostics, and so on. The recent advances in bioimaging of graphene-based nanomaterials, including graphene, graphene oxide, reduced graphene oxide, graphene quantum dots, and their derivatives, the synthetic methods of graphene-based nanomaterials are included in situ synthesis and binding method. The bioimaging modalities, including optical imaging (fluorescence [FL], two-photon FL), positron emission tomography/single-photon emission computed tomography, magnetic resonance imaging, photoacoustic imaging, Raman imaging, and multimodal imaging are highlighted (**Figure 8**).

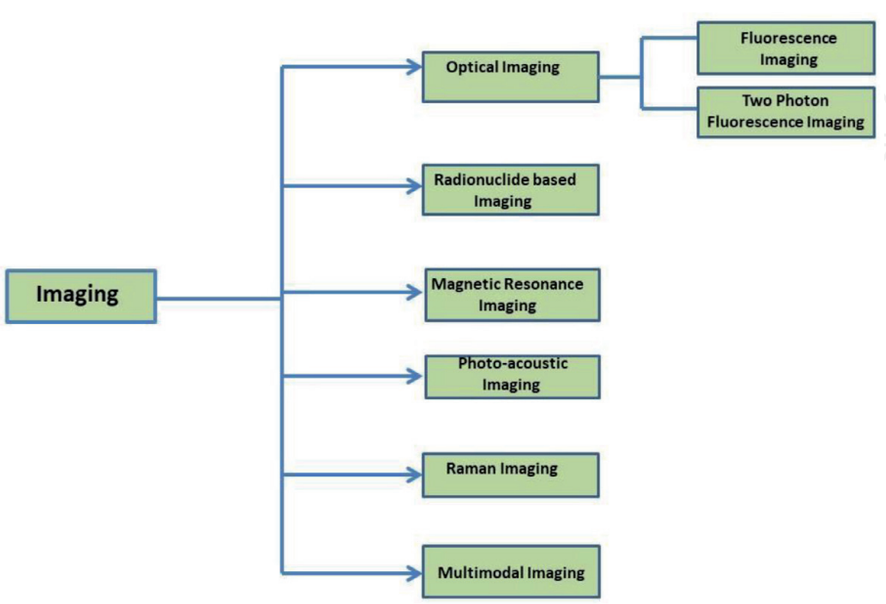

Figure 8.
Different types of bioimaging graphene-based bioimaging.

5.1 Optical imaging

GO- and rGO-based composites are extensively used in the bioimaging field as the arrangement of tissues were comprehend with the help of optical imaging and with the application of unique characteristics of photon-based visible light [92]. As compared to the other progression GO derivatives has immense superiority including high-sensitivity non-ionizing energy, relative and economical advantage, real-time imaging, multiplexing capability, short range free space optical communication [93]. Although, this advancement is affected by low tissue penetration (0–2 cm), high tissue spreading of photons in the visible spectra region and considerable conditions because of tissue auto fluorescence and light absorption by macronutrients, oxygen binding groups and even by water molecules [94]. GO-based composites were dynamically developed for future aspects of different optical imaging techniques, such as (Fluorescence Imaging) FL imaging and (Two-Photon Fluorescence Imaging) TPFI.

5.1.1 Fluorescence imaging

Fluorescence imaging is a nonpersistent technique depends on the intensity of photons emitted from the probe used for fluorescent imaging [95]. A number of research groups focused on organic fluorescent dyes to fabricate GO and rGO *in vitro* and in vivo FL optical imaging. Liu and coworkers subjugated firstly a visible near infrared fluorescent dye, Cyanin 7 (Cy7), complexed with nGO-PEG and form a imaging system (nGO-PEG-Cy7) for in vivo FL imaging of tumor xenografted mice. The nGO-PEG-Cy7 supposed to be enriched in the tumor over time after intravenous vaccination. Prominent uptake of nGO was observed in the infected area compared with other healthy parts of the mouse after 24 h postinjection for different types of tumor modalities. The result shows high tumor accumulation of nGO-PEG-Cy7 based on the improved permeability and retention effect of malignant tumors [96]. Apart from compiling with organic fluorescent dyes, the other non-fluorescence dyes or porphyrin rings are also directly coated on the graphene surface for this particular imaging. Chen and coworkers reported GO conjugated multifunctional system composed of VEGF-loaded (vascular endothelial growth factor) IR-Dye-800 (e.g., GO-IR-Dye-800-VEGF) for fluorescence imaging of ischemic limb cells in the aquatic posterior limb ischemia mold [97]. Further IR-800 dye was firstly compile with the six headed amino groups of poly ethylene glycol (PEG), then later VEGF was loaded onto the two available basal planes of GO via physisorption. The IR-800 shows the same account of FL emission spectra variation even after combing it with various other components at the particular specific maximum wavelength of 800 nm. This specific imaging property of the fluorescence active compounds is used extensively in the imaging application of the GO-based compounds. When we compared the ischemic limbs with the nonischemic limbs the fluorescence intensity ischemic limbs are stronger than that of nonischemic limbs at after intravenous vaccination, explains that GO-IR800-VEGF could exclusively be used to target ischemic limb sowing the enlarged permeability of blood cells. At 24 h time point p.i., the mice were sacrificed and organs of GO-IR800 and GO-IR800-VEGF-injected mice were harvested for *ex vivo* imaging. Interestingly, both of GO-IR800 and GO-IR800-VEGF were mainly distributed throughout the entire ischemic tissue below the ligation site. When we functionalized a biocompatible polymer on the GO surface it acts as a linkage between the GO and these fluorescence active dye [98]. Recently graphene quantum dots are also came into the scenario as they show some photoluminescent properties when incorporated with GO. The lattices inside the GQDs play an important role in

functionalization process as well as show some extraordinary characteristics such as zig zag geometry, electron hole mobile electron carriers, high photostability and lower toxic index [99]. Nahain and its research group demonstrate the particular sized GQDs with the fluid known as hyaluronic acid for proficient CD44-targeted delivery to tumor-effective BALB/c mice, representing the intense fluorescence image of the tumor cell line [100]. However, the QY of GQDs still needs to be improved for further bioimaging applications. Meanwhile, further surface modification is also necessary to improve the optical belongings of GQDs and improve the tumor accrual rate.

5.1.2 Two-photon fluorescence imaging

Due to some background noise disturb the fluorescence imaging due to single photon fluorescence imaging Two-Photon Fluorescence Imaging came into the picture. Two-Photon Fluorescence Imaging has fascinated much concentration because of its potential applications in fundamental study and medical diagnostics [101]. With the help of TPFI more detailed examination of various in vivo activities of deep located tumorous cells. Compared with one photon excitation using simple continuous-wave lasers, two-photon nonlinear excitation usually uses a nonlinear femtosecond laser to obtain a high reflux of excitation photons. Recently, graphene-based nanomaterials aroused considerable interest in the field of TPFI. Wang and Gu et al. first reported transferrin functionalized GO-PEG with strong two-photon luminescence as a nonbleaching optical probe for three-dimensional TPFI and laser-based cancer microsurgery, using an ultrafast pulsed laser as the excitation source [102]. Gong group employed nitrogen doping GQDs (N-GQDs) with an average size of ~3 nm as efficient two photon fluorescent probes for cellular and deep-tissue imaging [103]. Taking dimethylformamide as solvent and nitrogen sources, the nitrogen was successfully doping to GQDs by a facile one-pot solvothermal method. Obviously, the TPFI using N-GQDs as fluorescent probe is particularly suitable for in vivo investigation of biostructures in the 800–1500 μm region.

5.2 Radionuclide-based imaging

Optical imaging cannot provide quantitative results and sometimes may be interfered by FL quenching of fluorescent dyes, light absorption and scattering of tissues, and autofluorescence background. Radiolabeling method would be able to accurately track the labeled substances in vivo in a quantitative manner with excellent sensitivity (~10–11–10–12 mol/L) and nearly no penetration depth limit. The radionuclide-based imaging mainly contains PET and SPECT. PET and SPECT images are acquired over a nominally low background signal and require little signal amplification [104]. Graphene-based nanomaterials as promising nanoplatforms are playing an important role in PET/SPECT imaging. In 2011, Liu et al. reported a method to label nGO-PEG with 125I by anchoring iodine atoms on the defects and edges of GO [105]. After that a number of studies have been developed based on this method. Cai group explored in vivo active tumor targeting using 64Cu-labeled nGO-PEG [106]. In comparison to PET, SPECT is ~10 times less sensitive (~10–10–10–11 mol/L); however, SPECT enables concurrent imaging of multiple radionuclides of different energies [107]. Cornelissen et al. explored the use of anti-HER2 antibody (trastuzumab)-conjugated nGO, radiolabeled with 111In-benzyl-diethylenetriaminepentaacetic acid (BnDTPA) via π-π stacking, for in vivo targeting and SPECT imaging. The radiolabeled nGO-trastuzumab conjugates demonstrated better pharmacokinetics compared with radiolabeled trastuzumab without NGO, with more rapid clearance from the circulation [108].

5.3 Magnetic resonance imaging

Noninvasive technique MRI have been extensive used for the detection of morphological feature of tissue related bioscience in comparison to other optical imaging. But somehow the lower sensitivity for the detection of different concentration and inappropriate signaling time has been assigned as the huge drawbacks of MRI [109]. While the biomolecules and ions with paramagnetic nature of metal ions having manganese (Mn) and gadolinium (Gd) as major contributions are reported as the toxic in most of the cases [110]. Whereas such metallic ions can be incorporated with GO utilizing chelation procedure in between metals and different graphene layers [111]. The Gd (gadolinium) incorporated graphene oxide and amidoamine polymer dendrimer-based composite for the delivery of anticancerous drugs on the targeting sites have been reported by Wei et al. [112]. The composite of nitrogen doped graphene oxide have been studied for the detection of tumor containing sites on the defective cells. Starting from GO andiron hydrate the reduced graphene oxide-based composites were synthesized by Liu et al. following autoclave-based thermal treatment methodology. The hydrophobic interaction-based functionalization of polyetheneglycol and maleic anhydride-alt-1-octadecene molecule with iron-based nanoparticles were reported by the same to restore the magnetic properties along with the enhancement of thermal stability of developed solutions [113]. Graphene oxide/iron oxide nanoparticle-based system was fabricated to study and diagnosis of pancreatic cancer by Fu et al. [114]. The graphene and iron nanoparticle-based composite was reported as the magnificent composite to help the surgeons into the preparations of cancer cells. The dual mapping is main device that can easily radiate the difference in between normal and RLN tissues, thus further the lymph nodes can be treated with laser. The penetration effects of lower energy waves are much larger and deeper, while the radio waves worked as the low scatter for biomedical systems, i.e., tissues, cells or organs, etc. the PAI cells photographic technique, which take advantage of the absorption of longer wavelength containing waves into thermal energy for thermal expansion [115].

5.4 Photoacoustic imaging

The reduced graphene nanomaterials are irreplaceable candidates to absorb near infra-red light in comparison to graphene oxide that reflects sp^2 hybridization of carbon [116]. The reduced nature of reduced GO reflects hydrophobic nature of the graphene oxide thus finally result its poor water solubility. In order to find out a unique solution, the microwave heating-based-reduced graphene sheets having lower oxygen containing functional groups were synthesized by Patel et al. [117]. The Hummers method has been utilized to reduce the major oxygen content present in the graphitic powder, the methodology of Hummers method includes the acidic oxidations. The GO sheets can also be reduced to rGO by single step thermal reduction methodology and reported rGO possess excellent stability and lesser cell toxicity [118].

5.5 Raman imaging

The advanced characterizations technique, i.e., the RAMAN technique is excellently advanced tool for the analytical and experimental extension for the detection of related various biochemical problems. The RAMAN spectrum including D and G bands exactly mentions and elaborates the enhancements of combinations of various nanoparticles [119]. The folic acid hybrid incorporated Ag/GO composite have been developed for specifically targeting of defective cancerous cells [120].

The in situ synthesis of gold- and GO-based composites have been incorporated in HeLa 229 cells which have been found to display excellent peaks and shifts in Raman spectra. The gold nanoparticle incorporated with nitrogen doped graphene oxide was reported by Ma and coworkers. The in situ synthesis of gold nanocomposites were also assumed to have physical forces of attraction between NOPs and gold particles. The further modifications of GO and reduced graphene sheets with 2-mercaptopyridine were reported by the non-covalent linkage [121]. Gold-based GO composites were reported for the development of good substrate than the Au NPs. Similarly, polyethylene glycol functionalized gold/copper nanoparticles along with graphene were incorporated through CVD method by the group of Tan et al. [122]. The unique Raman signals of graphite-based nanoparticles were reported along with further cell labeling and SERS detection. Recently, bio-imaging applications with more modalities have gained excellent popularity in recent decade [123].

5.6 Multimodal imaging

The multimodality of such imaging applications has been referred as the better technique over the individual imaging technique for the higher accuracy and for the better diagnosis [124, 125]. While the multiphase analysis of single agent lack the potential problem on the probe, i.e., tissues blood for the further removal of impurities along with several doses [126]. Liu et al. have developed rGO-IONP for triple modulation, i.e., FL, PA along with MR [127]. The rGO-based composites of iron and GO have been synthesized via hydrothermal methodology where the polyethylene glycol was incorporated with poly(maleicanhydride-alt-1-octadecene) (C18PMH-PEG), further NIR was performed for the detection of magnetic absorbance. Similarly, Chen et al. reported the graphene oxide-based composite of PEG having non-covalent interactions, i.e., π-π stacking for the detection of tumorous cells. The recent reports of Wu et al. have synthesized the BaGdF5 nanoparticles which were reported to formulate on the graphene oxide sheets in moderate presence of polyethylene glycol. In the transmission electron microscopy (TEM), the exact morphology of GO/BaGdF5-based PEG composite was shown. This showed excellent separation of layers, along with the accuracy in size of sheets, where the size was reported to exist as smaller than corresponding pure GO sheets. While the SAED (selected area electron diffraction) spectra showed the excellently good crystal nature of BaGdF nanoparticles having cubic shape [128].

6. Conclusion and future prospects

The unique ability of catenation of carbon and tendency to form various hybrid orbitals results in the formation of various smart compounds with different physical and chemical properties. Its 2D hexagonally packed unique structure of in-plane sigma C–C bonds accounts for certain physical and chemical properties in biological media has led to its varied applications in the field of drug delivery, gene delivery, tissue engineering and various imaging techniques, etc. The electrostatic interaction and presence of metallic ions in biological media tends to agglomerate and reduces the surface area of graphene sheets. Therefore, covalent and non-covalent methods of surface modifications are used to increase the efficacy of graphene sheets. Further surface fabrication of GO with various polymers allows its use in the fields of drug delivery, tissue engineering and different imaging techniques. Surface modification by way of exfoliation is used for large scale synthesis of graphene. Modified Hummer's method is a common procedure of synthesizing GO from the

natural mineral source, i.e., flake graphite. The carboxylic group is found on the surface of graphite during the process of oxidation and exfoliation is modified by covalent functionalization making its use possible in the studies of biological systems and also found applications in biological activities like anti-bacterial activity, bioimaging and photodynamic therapy. Non-covalent functionalization has added advantage of not altering the structure or electronic properties of graphene while introducing new chemical groups on its surface. This results in enhancement of its dispersibility, biocompatibility, reactivity, binding capacity and sensing properties. Graphene has the properties of high surface region, distinctive geometry and structure, flexibility, extra ordinary physico-chemical properties, counting the high fracture strength, high Young's modulus, great thermal and electrical conductivity, highly mobile charge carriers and biocompatibility. All mentioned properties makes graphene a valuable and important material for applications in biological systems and other biomedical processes.

In contrast to pristine graphene synthesized GO has high dispersibility in physiological media leading to better contact with biologically important organic molecules. Outstanding thermal and electrical conductivity and very low thermal expansion quotient of GO allows its use in energy conversion storage devices and bio sensors. GO derivative-based optoelectronic devices have been developed as IR detectors and electrophysiological modulators and emitters. The optical properties like intense light transmittance, fluorescence, photoluminescence and high mobility of charge make graphene an important material for application in MRI and biomedical imaging. Superior mechanical properties of GO like high tensile strength and extensive stiffness has enabled its use in the field of biomedical implant and tissue engineering. Cell-graphene and biomolecule-graphene studies have opened a vast area of GOs exploration in the fields of cellular biology, genomics and development of antibiotics, etc. Despite its varied uses certain challenges still remain in the field of nanotheranostic designing in terms of bioavailability, selectivity, biocompatibility and safety. In the field of pharmacology, better targeted and relatively lower dose drug delivery with graphene complex has proved cheaper and efficient than the discovery of newer drug. Graphene-based materials as drug and gene delivery vehicles have used successfully in *in vitro* studies, however much research in in vivo studies is still in early stages. Many researchers have focused on developing graphene-based materials for wound healing, stem cell imaging, regenerative medicine and tissue engineering. Graphene finds its application in bio imaging by way of optical imaging, fluorescence Imaging (FL) and two photon fluorescence imaging (TPFI), etc. High sensitivity non-ionizing energy, real time imaging, multiplexing capability, short range free space optical communication and economic advantages makes GO derivative superior for use in optical imaging. However, low tissue penetration, high tissue spreading of photons in the visible spectra, tissue auto fluorescence and light absorption by oxygen binding groups and water limits the use of GO derivatives in optical imaging. Labeling of fluorescent dyes on GO surface and their detection by photons emission from probe has been enabled the development of fluorescent imaging technique for the study of biological systems. Recently certain desirable properties of GQDs like luminescence, zig zag geometry, electron hole mobile electron carriers, high photostability and lower toxic index have enabled development of better FL imaging system. The limitations of FL imaging leads to development of TPFI using non-linear femto second laser to obtain a high reflux of excitation photons, thereby enabling the development of better and deeper fluorescent imaging probes. Radio labeling of graphene-based nanomaterials has increased the sensitivity of qualitative imaging studies like PET/SPECT further doped GO has found its application in MRI for detailed study of different tissues in humans. Past few years have witnessed the development of

graphene from a little known nanoparticle to wide spread interest in the field of development of graphene-based nanomaterial for applications in biochemical and biophysical systems and processes. However, much research work is still desired to enable commercial scale applications of GO-based nanomaterials.

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References

[1] Hoboken NJ, Depan D, Shah J, Misra RDK. Controlled release of drug from folate-decorated and graphene mediated drug delivery system: Synthesis, loading efficiency, and drug release response. Materials Science and Engineering C. 2011;**31**(7):1305-1312

[2] Allen MJ, Tung VC, Kaner RB. Honeycomb carbon: A review of graphene. Chemical Reviews. 2010;**110**(1):132-145

[3] Geim AK, Novoselov KS. The rise of graphene. Nature Materials. 2007;**6**:183-191

[4] Novoselov KS, Falko VI, Colombo L, Gellert PR, Schwab MG, Kim K. A roadmap for graphene. Nature. 2012;**490**:192-200

[5] Karki N, Tiwari H, Pal M, Chaurasia A, Bal R, Joshi P, et al. Functionalized graphene oxides for drug loading, release and delivery of poorly water soluble anticancer drug: A comparative study. Colloids and Surfaces, B: Biointerfaces. 2018;**169**:265-272

[6] Romberg B, Hennink WE, Storm G. Sheddable coatings for long-circulating nanoparticles. Pharmaceutical Research. 2008;**2**(1):55-71

[7] Yang K, Hu L, Ma X, Ye S, Cheng L, Shi X, et al. Multimodal imaging guided photothermal therapy using functionalized graphene nanosheets anchored with magnetic nanoparticles. Advanced Materials. 2012;**24**(14):1868-1872

[8] Pal K, Maiti UN, Majumder TP, Debnath SC, Ghosh S, Roy SK, et al. Switching of ferroelectric liquid crystal doped with cetyltrimethylammonium bromide-assisted Cds nanostructures. Nanotechnology. 2013;**24**:125702

[9] Lin J, Chen X, Huang P, Hu SH, Chen YW, Hung WT, et al. Quantum-dot-tagged reduced graphene oxide nanocomposites for bright fluorescence bioimaging and photothermal therapy monitored in situ graphene-based nanomaterials for bioimaging. Advanced Drug Delivery Reviews. 2016;**105**:242-254

[10] Sagadevan S, Pal K. Chowdhury ZZ, Scalable synthesis of CdS–graphene nanocomposite spectroscopic characterizations. Materials in Electronics. 2017;**28**(22):17193-17201

[11] Wong CW, Chan YS, Jeevanandam J, Pal K, Bechelany M, Elkodous MA, et al. Response surface methodology optimization of mono-dispersed MgO nanoparticles fabricated by ultrasonic-assisted sol–gel method for outstanding antimicrobial and antibiofilm activities. Journal of Cluster Science. 2020;**31**:367-389

[12] Yang M, Yao J, Duan Y. Graphene and its derivatives for cell biotechnology. The Analyst. 2013;**138**(1):72-86

[13] Liu K, Zhang JJ, Cheng FF, Zheng TT, Wang C, Zhu JJ. Green and facile synthesis of highly biocompatible graphene nanosheets and its application for cellular imaging and drug delivery. Journal of Materials Chemistry. 2011;**21**(32):12034e40

[14] Feng L, Zhang S, Liu Z. Graphene based gene transfection. Nanoscale. 2011;**3**(3):1252-1257

[15] Taherian F, Marcon V, Van der Vegt NFA, Leroy F. What is the contact angle of water on graphene? Langmuir. 2013;**29**(5):1457-1465

[16] Xue Y, Liu Y, Lu F, Qu J, Chen H, Dai L. Functionalization of graphene oxide with polyhedral oligomeric silsesquioxane (POSS) for multifunctional applications. Journal of Physical Chemistry Letters. 2012;**3**(12):1607-1612

[17] Hsieh CT, Chen WY. Water/oil repellency and work of adhesion of liquid droplets on graphene oxide and graphene surfaces. Surface and Coating Technology. 2011;**205**(19):4554-4561

[18] Hasan SA, Rigueur JL, Harl RR, Krejci AJ, Gonzalo-Juan I, Rogers BR, et al. Transferable graphene oxide films with tunable microstructures. ACS Nano. 2010;**4**(12):7367-7372

[19] Yang ST, Chang Y, Wang H, Liu G, Chen S, Wang Y, et al. Folding/aggregation of graphene oxide and its application in Cu^{2+} removal. Journal of Colloid and Interface Science. 2010;**351**(1):122-127

[20] Cote LJ, Kim F, Huang J. Langmuir-Blodgett assembly of graphite oxide single layers. Journal of the American Chemical Society. 2008;**131**(3):1043-1049

[21] Nika DL, Pokatilov EP, Askerov AS, Balandin AA. Phonon thermal conduction in graphene: Role of Umklapp and edge roughness scattering. Physical Review B. 2009;**79**(15):155413

[22] Jiang JW, Lan J, Wang JS, Li B. Isotopic effects on the thermal conductivity of graphene nanoribbons: Localization mechanism. Journal of Applied Physics. 2010;**107**(5):054314

[23] Kravets VG, Grigorenko AN, Nair RR, Blake P, Anissimova S, Novoselov KS, et al. Spectroscopic ellipsometry of graphene and an exciton-shifted Van Hove peak in absorption. Physical Review B. 2010;**81**(15):55413

[24] Wang F, Hamdi M. Strictly non-blocking conditions for the central-stage buffered clos-network. IEEE Communications Letters. 2008;**12**(3):206-208

[25] Ma X, Tao H, Yang K, Feng L, Cheng L, Shi X, et al. A functionalized graphene oxide–iron oxide nanocomposite for magnetically targeted drug delivery, photothermal therapy, and magnetic resonance imaging. Nano Research. 2012;**5**:199-212

[26] ShenAJ LDL, Cai XJ, Dong CY, Dong HQ, Wen HY, Dai GH, et al. Multifunctional nanocomposite based on graphene oxide for *in vitro* hepatocarcinoma diagnosis and treatment. Journal of Biomedical Materials Research Part A. 2012;**100**(9):2499-2506

[27] Park S, Lee KS, Bozoklu G, Cai W, Nguyen ST, Ruoff RS. Graphene oxide papers modified by divalent ions—Enhancing mechanical properties via chemical cross-linking. ACS Nano. 2008;**2**(3):572-578

[28] Park S, Dikin DA, Nguyen ST, Ruoff RS. Graphene oxide sheets chemically cross-linked by polyallylamine. Journal of Physical Chemistry C. 2009;**113**(36):15801-15804

[29] Barun D, Prasad KE, Ramamurty U, Rao CNR. Nano-indentation studies on polymer matrix composites reinforced by few-layer graphene. Nanotechnology. 2009;**20**(12):125705

[30] Sanchez VC, Jachak A, Hurt RH, Kane AB. Biological interactions of graphene-family nanomaterials: An interdisciplinary review. Chemical Research in Toxicology. 2011;**25**(1):15-34

[31] Bianco A. Graphene: Safe or toxic? The two faces of the medal. Angewandte Chemie, International Edition. 2013;**52**(19):4986-4997

[32] Stone V, Johnston H, Schins RPF. Development of in vitro systems for nanotoxicology: Methodological considerations. Critical Reviews in Toxicology. 2009;**39**(7):613-626

[33] Oberdörster G, Oberdörster E, Oberdörster J. Nanotoxicology: An

emerging discipline evolving from studies of ultrafine particles. Environmental Health Perspectives. 2005;**113**(7):823-839

[34] Kang S, Herzberg M, Rodrigues DF, Elimelech M. Antibacterial effects of carbon nanotubes: Size does matter! Langmuir. 2008;**24**(13):6409-6413

[35] Kang S, Pinault M, Pfefferle LD, Elimelech M. Single-walled carbon nanotubes exhibit strong antimicrobial activity. Langmuir. 2007;**23**(17):8670-8673

[36] Akhavan O, Ghaderi E. Toxicity of graphene and graphene oxide nanowalls against bacteria. ACS Nano. 2010;**4**(10):5731-5736

[37] Hu W, Peng C, Luo W, Lv M, Li X, Li D, et al. Graphene-based antibacterial paper. ACS Nano. 2010;**4**(7):4317-4323

[38] Tazawa H, Tatemichi M, Sawa T, Gilibert I, Ma N, Hiraku Y, et al. Oxidative and nitrative stress caused by subcutaneous implantation of a foreign body accelerates sarcoma development in Trp53+/− mice. Carcinogenesis. 2006;**28**(1):191-198

[39] Bourlinos AB, Gournis D, Petridis D, Szabo T, Szeri A, Dekany I. Graphite oxide: Chemical reduction to graphite and surface modification with primary aliphatic amines and amino acids. Langmuir. 2003;**19**(15):6050-6055

[40] He HY, Klinowski J, Forster M, Lerf A. A new structural model for graphite oxide. Chemical Physics Letters. 1998;**287**(1):53-56

[41] Lerf A, He H, Forster M, Klinowski J. Structure of graphite oxide revisited. The Journal of Physical Chemistry. B. 1998;**102**(23):4477-4482

[42] Banhart F, Kotakoski J, Krasheninnikov AV. Structural defects in graphene. ACS Nano. 2010;**5**(1):26-41

[43] Paton KR. Scalable production of large quantities of defect-free few-layer graphene by shear exfoliation in liquids. Nature Materials. 2014;**13**(6):624-630

[44] Marcano DC. Improved synthesis of graphene oxide. ACS Nano. 2010;**4**(8):4806-4814

[45] Becerril HA. Evaluation of solution-processed reduced graphene oxide films as transparent conductors. ACS Nano. 2008;**2**(3):463-470

[46] Li D, Muller MB, Gilje S, Kaner RB, Wallace GG. Processable aqueous dispersions of graphene nanosheets. Nature Nanotechnology. 2008;**3**(2):101-105

[47] Shih CJ, Lin S, Sharma R, Strano MS, Blankschtein D. Understanding the Ph-dependent behavior of graphene oxide aqueous solutions: A comparative experimental and molecular dynamics simulation study. Langmuir. 2011;**28**(1):235-241

[48] Wissler M. Graphite and carbon powders for electrochemical applications. Journal of Power Sources. 2006;**156**(2):142-150

[49] Ishikawa T, Nagaoki T. Shin tansokogyo (New Carbon Industry). 2nd ed. Tokyo: Kindaihensyusya; 1986. pp. 125-136

[50] Hummers WS, Offeman RE. Preparation of graphitic oxide. Journal of the American Chemical Society. 1958;**80**(6):1339

[51] Xie C, Lu X, Han L, Xu J, Wang Z, Jiang L, et al. Biomimetic mineralized hierarchical graphene oxide/chitosan scaffolds with adsorbability for immobilization of nanoparticles for biomedical applications. ACS Applied Materials & Interfaces. 2016;**8**(3):1707-1717

[52] Loh KP, Bao Q, Eda G, Chowalla M. Graphene oxide as a chemically tunable

platform for optical applications. Nature Chemistry. 2010;**2**(12):1015-1024

[53] Mahmoudi N, Simchi A. On the biological performance of graphene oxide-modified chitosan/polyvinyl pyrrolidone nanocomposite membranes: *In vitro* and *in vivo* effects of graphene oxide. Materials Science and Engineering: C. 2017;**70**(1):121-131

[54] Song MM, Xu HL, Liang JX, Xiang HH, Liu R, Shen YX. Facile fabrication of water-dispersible nanocomposites based on hexa-*peri*-hexabenzocoronene and Fe_3O_4 for dual mode imaging (fluorescent/MR) and drug delivery. Materials Science and Engineering C. 2017;**77**(71):904-911

[55] Panahi F, Fareghi-Alamdari R, Dangolani SK, Nezhad AK, Golestanzadeh M. Graphene grafted N-methyl-4-pyridinamine (G-NMPA): An efficient heterogeneous organocatalyst for acetylation of alcohols. Chemistry Select. 2017;**2**(1):474-479

[56] Paulus GLC, Wang QH, Strano MS. Covalent electron transfer chemistry of graphene with diazonium salts. Accounts of Chemical Research. 2013;**46**:160-170

[57] Lin Y, Ehlert GJ. Superhydrophobic functionalized graphene aerogels. ACS Applied Materials & Interfaces. 2011;**3**(7):2200-2203

[58] Yin T, Liu J, Zhao Z, Zhao Y, Dong L, Yang M, Zou J. Redox sensitive hyaluronic acid decorated graphene oxide for photothermally controlled tumor cytoplasm selective rapid drug delivery. Advanced Functional Materials. 2017;**27**:1604620-1604631

[59] Li A, Ma H, Liu J. Graphene oxide coated Fe_3O_4@$MSiO_2$ Nps for magnetic controlled bioimaging. RSC Advances. 2016;**6**(68):63704-63710

[60] Wei Y, Zhou F, Zhang D, Chen Q, Xing D. Graphene oxide based smart drug delivery system for tumor mitochondria-targeting photodynamic therapy. Nanoscale. 2016;**8**(6):3530-3538

[61] Atabia F, Gargarib SLM, Hashemic M, Yaghmaeia P. Doxorubicin loaded DNA aptamer linked myristilated chitosan nanogel for targeted delivery to prostate cancer. Iran Journal of Pharmacy Research. 2017;**16**(1):35-49

[62] Lehn JM. Supramolecular Chemistry: Concepts and Perspectives. Vol. 99. New York: Wiley; 1995. pp. 4763-4768

[63] Atkins P, de Paula J. Physical Chemistry. New York: W. H. Freeman; 2002

[64] Smithrud DB, Sanford EM, Chao I, Ferguson SB, Carcanague DR, Evanseck JD, et al. Solvent effects in molecular recognition. Pure and Applied Chemistry. 1990;**62**(12):2227-2236

[65] Southall NT, Dill KA, Haymet ADJ. A view of the hydrophobic effect. The Journal of Physical Chemistry. B. 2002;**106**(3):521-533

[66] Zhang ZX, Huang HL, Yang XM, Zang L. Tailoring electronic properties of graphene by pi-pi stacking with aromatic molecules. Journal of Physical Chemistry Letters. 2011;**2**(15):2897-2905

[67] Butte K, Momin M, Kurhade S, Kar S. Intravesical drug delivery system for bladder: An overview. International Journal of Pharmaceutical, Chemical and Biological Sciences. 2013;**3**(3):680-681

[68] Li N, Zhao P, Astruc D. Anisotropic gold nanoparticles: Synthesis, properties, applications, and toxicity. Angewandte Chemie International Edition. 2014;**53**(7):1756-1789

[69] Argyo C, Weiss V, Bräuchle C, Bein T. Multifunctional Mesoporous

silica nanoparticles as a universal platform for drug delivery. Chemistry of Materials. 2013;**26**(1):430-434

[70] Liu Z, Robinson JT, Sun X, Dai H. PEGylatednanographene oxide for delivery of water-insoluble cancer drugs. Journal of the American Chemical Society. 2008;**130**(33):10876-10877

[71] Yang K, Wan J, Zhang S, Zhang Y, Lee ST, Liu Z. In vivo pharmacokinetics, long-term biodistribution, and toxicology of PEGylated Graphene In mice. ACS Nano. 2010;**5**(1):516-522

[72] Jing W, Yin-song W, Xiao-ying Y, Yuan-yuan L, Jin-rong Y, Rui Y, et al. Graphene oxide used as a carrier for adriamycin can reverse drug resistance in breast cancer cells. Nanotechnology. 2012;**23**(35):355101

[73] Depan D, Shah J, Misra RDK. Controlled release of drug from folate-decorated and graphene mediated drug delivery system: Synthesis, loading efficiency, and drug release response. Materials Science and Engineering C. 2011;**31**(7):1305-1312

[74] Zhang L, Xia J, Zhao Q, Liu L, Zhang Z. Functional graphene oxide as a nanocarrier for controlled loading and targeted delivery of mixed anticancer drugs. Small. 2010;**6**(4):537-544

[75] Bai H, Li C, Wang X, Shi G. A Ph-sensitive graphene oxide composite hydrogel. Chemical Communications. 2010;**46**(14):2376-2378

[76] Yang X, Zhang X, Liu Z, Ma Y, Huang Y, Chen Y. High-efficiency loading and controlled release of doxorubicin hydrochloride on graphene oxide. Journal of Physical Chemistry C. 2008;**112**(45):17554-17558

[77] Zhang L, Xia J, Zhao Q, Liu L, Zhang Z. Functional graphene oxide as a nanocarrier for controlled loading and targeted delivery of mixed anticancer drugs. Small. 2010;**6**(4):537-544

[78] Yang X, Zhang X, Ma Y, Huang Y, Wang Y, Chen Y. Superparamagnetic graphene oxide–Fe_3O_4 nanoparticles hybrid for controlled targeted drug carriers. Journal of Materials Chemistry. 2009;**19**(18):2710-2714

[79] Fan X, Jiao G, Gao L, Jin P, Li X. The preparation and drug delivery of graphene-carbon nanotube–Fe_3O_4 nanoparticle hybrid. Journal of Materials Chemistry B. 2013;**1**(20):2658-2664

[80] Shen H, Zhang L, Liu M, Zhang Z. Biomedical applications of graphene. Theranostics. 2012;**2**(3):283-294

[81] Yang ZR, Wang HF, Zhao J, Peng YY, Wang J, Guinn BA, et al. Recent developments in the use of adenoviruses and immunotoxins in cancer gene therapy. Cancer Gene Therapy. 2007;**14**(7):599-615

[82] Jager M, Schubert S, Ochrimenko S, Fischer D, Schubert US. Branched and linear poly(ethylene imine)-based conjugates: Synthetic modification, characterization, and application. Chemical Society Reviews. 2012;**41**(13):4755-4767

[83] Bao H, Pan Y, Ping Y, Sahoo NG, Wu T, Li L, et al. Chitosan-functionalized graphene oxide as a nanocarrier for drug and gene delivery. Small. 2011;**7**(11):1569-1578

[84] Shen H, Liu M, He H, Zhang L, Huang J, Chong Y, et al. PEGylated graphene oxide-mediated protein delivery for cell function regulation. ACS Applied Materials & Interfaces. 2012;**4**(11):6317-6323

[85] La WG, Park S, Yoon HH, Jeong GJ, Lee TJ, Bhang SH, et al. Delivery of a therapeutic protein for

bone regeneration from a substrate coated with graphene oxide. Small. 2013;**9**(23):4051-4060

[86] Langer R, Vacanti JP. Biodegradable polymer scaffolds for tissue engineering. Tissue Engineering. Science. 1993;**260**(14):920-926

[87] Sant S, Hancock MJ, Donnelly JP, Iyer D, Khademhosseini A. Biomimetic gradient hydrogels for tissue engineering. Canadian Journal of Chemical Engineering. 2010;**88**(6):899-911

[88] Zhang L, Wang Z, Xu C, Li Y, Gao J, Wang W, et al. High strength graphene oxide/polyvinyl alcohol composite hydrogels. Journal of Materials Chemistry. 2011;**21**(28):10399-10406

[89] Lu B, Li T, Zhao H, Li X, Gao C, Zhang S, et al. Graphene-based composite materials beneficial to wound healing. Nanoscale. 2012;**4**(9): 2978-2982

[90] Ku SH, Park CB. Myoblast differentiation on graphene oxide. Biomaterials. 2013;**34**(8):2017-2023

[91] Mahmoudi M, Akhavan O, Ghavami M, Rezaee F, Ghiasi SMA. Graphene oxide strongly inhibits amyloid beta fibrillation. Nanoscale. 2012;**4**(23):7322-7325

[92] Janib SM, Moses AS, MacKay JA. Imaging and drug delivery using theranostic nanoparticles. Advanced Drug Delivery Reviews. 2010;**62**(11):1052-1063

[93] Wang J, Mi P, Lin G, Wang YX, Liu G, Chen X. Imaging-guided delivery of Rnai for anticancer treatment. Advanced Drug Delivery Reviews. 2016;**104**(1):44-60

[94] Park K, Lee S, Kang E, Kim K, Choi K, Kwon IC. New generation of multifunctional nanoparticles for cancer imaging and therapy. Advanced Functional Materials. 2009;**19**(10):1553-1566

[95] Baker SN, Baker GA. Luminescent carbon nanodots: Emergent nanolights. Angewandte Chemie, International Edition. 2010;**49**(38):6726-6744

[96] Yang K, Zhang S, Zhang G, Sun X, Lee ST, Liu Z. Graphene in mice: Ultrahigh in vivo tumor uptake and efficient photothermal therapy. Nano Letters. 2010;**10**(9):3318-3323

[97] Sun Z, Huang P, Tong G, Lin J, Jin A, Rong P, et al. VEGF-loaded graphene oxide as theranostics for multi-modality imaging-monitored targeting therapeutic angiogenesis of ischemic muscle. Nanoscale. 2013;**5**(15):6857-6866

[98] Yang HW, Huang CY, Lin CW, Liu HL, Huang CW, Liao SS, et al. Gadolinium-functionalized nanographene oxide for combined drug and microrna delivery and magnetic resonance imaging. Biomaterials. 2014;**35**(24):6534-6542

[99] Liu Q, Guo B, Rao Z, Zhang B, Gong JR. Strong two-photon-induced fluorescence from photostable, biocompatible nitrogen-doped graphene quantum dots for cellular and deep-tissue imaging. Nano Letters. 2013;**13**(6):2436-2441

[100] Abdullah AN, Lee JE, In I, Lee H, Lee KD, Jeong JH, et al. Target delivery and cell imaging using hyaluronic acid-functionalized graphene quantum dots. Molecular Pharmaceutics. 2013;**10**(10):3736-3744

[101] Yoo JM, Kang JH, Hong BH. Graphene-based nanomaterials for versatile imaging studies. Chemical Society Reviews. 2015;**44**(14):4835-4852

[102] Li JL, Bao HC, Hou XL, Sun L, Wang XG, Gu M. Graphene oxide

nanoparticles as nonbleaching optical probe for two-photon luminescence imaging and cell therapy. Angewandte Chemie, International Edition. 2012;**51**(8):1830-1834

[103] Liu Q, Guo B, Rao Z, Zhang B, Gong JR. Strong two-photon-induced fluorescence from photostable, biocompatible nitrogen-doped graphene quantum dots for cellular and deep-tissue imaging. Nano Letters. 2013;**13**(6):2436-2441

[104] Park K, Lee S, Kang E, Kim K, Choi K, Kwon IC. New generation of multifunctional nanoparticles for cancer imaging and therapy. Advanced Functional Materials. 2009;**19**(10):1553-1566

[105] Yang K, Zhang S, Zhang G, Sun X, Lee ST, Liu Z. Graphene in mice: Ultrahigh *in vivo* tumor uptake and efficient photothermal therapy. Nano Letters. 2010;**10**(9):3318-3323

[106] Hong H, Yang K, Zhang Y, Engle JW, Feng L, Yang Y, et al. In vivo targeting and imaging of tumor vasculature with radiolabeled, antibody-conjugated nanographene. ACS Nano. 2012;**6**(3):2361-2370

[107] Sun Z, Huang P, Tong G, Lin J, Jin A, Rong P, et al. VEGF-loaded graphene oxide as theranostics for multi-modality imaging-monitored targeting therapeutic angiogenesis of ischemic muscle. Nanoscale. 2013;**5**(1):6857-6866

[108] Cornelissen B, Able S, Kersemans V, Waghorn PA, Myhra S, Jurkshat K, et al. Nanographene oxide-based radioimmunoconstructs for in vivo targeting and SPECT imaging of HER2-positive tumors. Biomaterials. 2013;**34**:1146-1154

[109] Jiang L, Fan Z. Design of advanced porous graphene materials: From graphene nanomesh to 3D architectures. Nanoscale. 2014;**6**(4):1922-1945

[110] Caravan P, Ellison JJ, TJ MM, Lauffer RB. Gadolinium(III) chelates as MRI contrast agents: Structure, dynamics, and applications. Chemical Reviews. 1999;**99**(9):2293-2352

[111] Yoo JM, Kang JH, Hong BH. Graphene-based nanomaterials for versatile imaging studies. Chemical Society Reviews. 2015;**44**(14):4835-4852

[112] Yang HW, Huang CY, Lin CW, Liu HL, Huang CW, Liao SS, et al. Gadolinium-functionalized nanographene oxide for combined drug and microrna delivery and magnetic resonance imaging. Biomaterials. 2014;**35**(24):6534-6542

[113] Yang K, Gong H, Shi X, Wan J, Zhang Y, Liu Z. In vivo biodistribution and toxicology of functionalized nano-graphene oxide in mice after oral and intraperitoneal administration. Biomaterials. 2013;**34**(11):2787-2795

[114] Wang YW, Fu YY, Peng Q, Guo SS, Liu G, Li J, et al. Dye-enhanced graphene oxide for photothermal therapy and photoacoustic imaging. Journal of Materials Chemistry B. 2013;**1**(42):5762-5767

[115] Huang J, Zong C, Shen H, Liu M, Chen B, Ren B, et al. Mechanism of cellular uptake of graphene oxide studied by surface-enhanced Raman spectroscopy. Small. 2012;**8**(16):2577-2584

[116] Lalwani G, Cai X, Nie L, Wang LV, Sitharaman B. Graphene-based contrast agents for photoacoustic and thermoacoustic tomography. Photoacoustics. 2013;**1**(3-4):62-67

[117] Patel MA, Yang H, Chiu PL, Mastrogiovanni DD, Flach CR, Savaram K, et al. Direct production of graphene nanosheets for near infrared

photoacoustic imaging. ACS Nano. 2013;**7**(9):8147-8157

[118] Sheng Z, Song L, Zheng J, Hu D, He M, Zheng M, et al. Protein-assisted fabrication of nano-reduced graphene oxide for combined in vivo photoacoustic imaging and photothermal therapy. Biomaterials. 2013;**34**(21):5236-5243

[119] Kim YK, Han SW, Min DH. Graphene oxide sheath on Ag nanoparticle/graphene hybrid films as an antioxidative coating and enhancer of surface-enhanced Raman scattering. ACS Applied Materials & Interfaces. 2012;**4**(12):6545-6551

[120] Liu Z, Guo Z, Zhong H, Qin X, Wan M, Yang B. Graphene oxide based surface enhanced Raman scattering probes for cancer cell imaging. Physical Chemistry Chemical Physics. 2013;**15**(8):2961-2966

[121] Ma X, Qu Q, Zhao Y, Luo Z, Zhao Y, Ng KW, et al. Graphene oxide wrapped gold nanoparticles for intracellular Raman imaging and drug delivery. Journal of Materials Chemistry B. 2013;**1**(47):6495-6500

[122] Song ZL, Chen Z, Bian X, Zhou LY, Ding D, Liang H, et al. Alkyne-functionalized superstable graphitic silver nanoparticles for Raman imaging. Journal of the American Chemical Society. 2014;**136**(39):13558-13561

[123] Park K, Lee S, Kang E, Kim K, Choi K, Kwon IC. New generation of multifunctional nanoparticles for cancer imaging and therapy. Advanced Functional Materials. 2009;**19**(10):1553-1566

[124] Huang P, Rong P, Jin A, Yan X, Zhang MG, Lin J, et al. Dye-loaded ferritin nanocages for multimodal imaging and photothermal therapy. Advanced Materials. 2014;**26**(37):6401-6408

[125] Ma J, Huang P, He M, Pan L, Zhou Z, Feng L, et al. Folic acidconjugated Laf3:Yb,Tm@SiO_2 nanoprobes for targeting dual-modality imaging of upconversion luminescence and X-ray computed tomography. The Journal of Physical Chemistry. B. 2012;**116**(48):14062-14070

[126] Cheng Z, Al Zaki A, Hui JZ, Muzykantov VR, Tsourkas A. Multifunctional nanoparticles: Cost versus benefit of adding targeting and imaging capabilities. Science. 2012;**338**(6109):903-910

[127] Rong P, Yang K, Srivastan A, Kiesewetter DO, Yue X, Wang F, et al. Photosensitizer loaded nanographene for multimodality imaging guided tumor photodynamic therapy. Theranostics. 2014;**4**(3):229-239

[128] Wu H, Shi H, Wang Y, Jia X, Tang C, Zhang J, et al. Hyaluronic acid conjugated graphene oxide for targeted drug delivery. Carbon. 2014;**69**(12):379-389