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Applications of Functionalized Graphene Oxide in Drug Delivery and Biomedical Imaging

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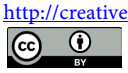

Abstract

Functionalized graphene oxide (GO), known for its unique physicochemical properties and biocompatibility, has demonstrated significant potential in the realms of drug delivery and biomedical imaging. This review initially presents the chemical functionalization approaches for GO, including surface modification and doping techniques, which notably enhance its stability and functionality for biomedical applications. Subsequently, it delves into the applications of functionalized GO in drug delivery systems, especially highlighting its advantages in targeted and controlled drug release. Moreover, the article outlines the application of functionalized GO in biomedical imaging, emphasizing its effectiveness as a contrast agent in Magnetic Resonance Imaging (MRI) and optical imaging. Finally, the challenges currently faced in these applications and potential future directions are discussed. By synthesizing existing literature, this paper aims to provide an in-depth understanding of the development of functionalized GO in these fields and to lay a theoretical foundation for further research and clinical trials.

Subject Areas

Drugs & Devices

Keywords

Graphene Oxide, Functionalization, Drug Delivery, Biomedical Imaging, Controlled Release, Targeted Therapy

1. Introduction

Graphene is a single layer of carbon atoms arranged in a hexagonal lattice,

connected by sp^2 hybrid bonds. In 2004, Novoselov and others prepared small amounts of single-layer graphene flakes using mechanical exfoliation methods [1]. Graphene's unique two-dimensional structure and geometry endow it with remarkable physicochemical properties, such as high hardness [2], thermal conductivity, electrical conductivity, and large specific surface area. These exceptional qualities make graphene widely applicable in the fields of nanodevices [3]-[5], chemical energy storage [6] [7], and biomedicine. However, its hydrophobic nature and tendency to agglomerate limit its widespread use.

Graphene oxide (GO) is a derivative of graphene that exhibits superior properties compared to graphene itself. The oxygen functional groups on the surface of GO (e.g., -OH, -COOH) not only impart GO with certain solubility but also significantly alter the van der Waals interactions between graphene sheets. Functionalized graphene oxide is derived from GO by further modifying its properties, involving the introduction of additional chemical species or functional groups via covalent or non-covalent bonding, such as doping with elements like nitrogen and boron or grafting with polymer chains. This allows functionalized graphene oxide to not only retain the excellent dispersibility of GO but also to gain specific physical or chemical properties to meet the demands of particular applications. For example, partially restoring its conductivity can be achieved by removing some oxygen-containing groups through a reduction process, thereby producing materials with tailored electrical properties. Certain functionalization modifications can further enhance its dispersibility in specific solvents, and even achieve stable dispersion in non-polar solvents. Currently, functionalized GO-based nanomaterials are widely used in the fields of biomedical imaging [8], drug delivery [9] [10], and therapeutics.

Research on functionalized graphene as a drug delivery vector primarily aims to enhance the efficiency and therapeutic effectiveness of drug delivery (See **Figure 1**). Through specific functionalization modifications, the interaction between graphene and drug molecules can be optimized, thereby improving drug loading capacity and release controllability. Moreover, the surface of functionalized graphene can be modified to target specific types of cells, reducing the impact on normal cells and thereby minimizing side effects during treatment. This targeted delivery system can improve the bioavailability and efficacy of drugs while also reducing the required dosage.

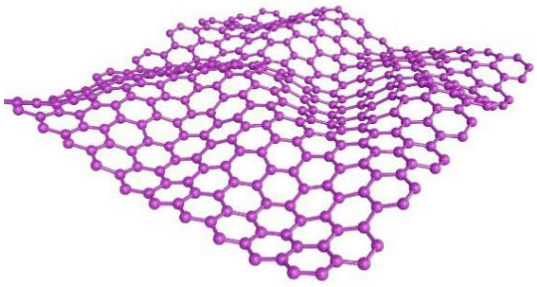

Figure 1. Graphene.

In the field of biomedical imaging, the purpose of research on functionalized graphene is to leverage its unique optical and physical properties to enhance the sensitivity and resolution of imaging techniques. Functionalized graphene can serve as a contrast agent, utilizing its excellent near-infrared absorption and fluorescence characteristics for applications in photoacoustic and fluorescence imaging. Additionally, functionalized graphene can carry specific biomarkers for targeting particular tissues or tumor cells, aiding in the early diagnosis and real-time monitoring of diseased tissues. By increasing the precision of imaging technologies, healthcare professionals can more accurately locate disease sites, assess the progression of conditions, and monitor the effectiveness of treatments.

2. Preparation of GO

Traditional methods for preparing GO include Brodie's method, Hummers' method [11], etc. However, these conventional graphene oxide preparation methods generally suffer from complex purification steps, the production of toxic gases, and insufficient oxidation levels. Brodie's method uses fuming nitric acid and potassium chlorate to prepare GO, achieving high oxidation levels but generating harmful gases like NO_2 and N_2O_4 over a long and hazardous reaction process. Hummers' method is shorter and does not produce the toxic gas ClO_2, making it widely used for preparing GO. Nevertheless, it poses risks of heavy metal contamination and has a high explosion risk.

With deeper research into GO, scholars have explored more effective preparation methods. Marcano [12] improved Hummers' method by omitting $NaNO_3$ and increasing the amount of $KMnO_4$, using a mixture of H_2SO_4/H_3PO_4 in a 9:1 ratio as the oxidant, enhancing oxidation efficiency without producing toxic gases and reducing explosion risks. Li Peng [13] used the strong oxidizing agent K_2FeO_4 to obtain single-layer GO within 1 hour, a method that recycles sulfuric acid to eliminate pollution and avoids introducing heavy metals. These cleaner, more efficient, and cost-effective methods have paved the way for GO's applications.

3. Functionalization of GO

Functionalizing GO can improve its stability and dispersibility. There are mainly two methods for the functionalization of GO: covalent modification and non-covalent modification.

3.1. Covalent Modification

The surface of GO contains a large amount of oxygen-containing substances, so functionalization can be achieved through a wide range of reactions with chemical substances via carboxyl, hydroxyl, and other functional groups, enhancing graphene's biocompatibility, solubility, and stability.

3.2. Non-Covalent Modification

Non-covalent modification mainly uses hydrophobic interactions, π-π stacking,

and hydrogen bonding to modify graphene. This method does not alter the original structure of graphene.

4. Applications of Functionalized GO in Drug Delivery

Over the past few decades, functionalized GO, with its low cytotoxicity, good stability, biocompatibility, and high solubility, has become a popular nanocarrier, playing a crucial role in targeted drug delivery systems [14]-[16].

4.1. Delivery of Cancer Drugs

Surface-functionalized nano-GO, due to its low cytotoxicity and ability to greatly retain cancer drugs, has been frequently used as a carrier for cancer drugs in the past decade. Erqun *et al.* [17] prepared a novel hyaluronic acid (HA)-modified GO nanohybrid targeting drug delivery system primarily for controlling the release of the anticancer drug doxorubicin (DOX). DOX was initially loaded onto the GO nanocarrier through π-π stacking and hydrogen bonding interactions, followed by covalent modification with HA to form the HA-GO-DOX nanohybrid. HA not only provided the system with targeting capability, stability, and dispersibility but also increased the loading efficiency of DOX (up to 42.9%). Compared with free DOX, the GO-DOX hybrid showed a significantly higher tumor inhibition rate in mice bearing H22 liver cancer. Shariq Yousuf *et al.* [18] loaded synthesized GATPT onto GO with a loading efficiency of up to 80%. Confocal microscopy demonstrated that GATPT delivered by GATPT-GO could be effectively internalized into the nucleus of Du145 cancer cells, exhibiting significant cytotoxicity by inhibiting endocytosis and transcription, while being non-toxic to PNT2 cells at low concentrations. Moreover, GATPT-GO released more efficiently in acidic media, and since the pH at tumor sites is lower than at normal sites, this feature could further enhance the targeted therapeutic effect. Hadi *et al.* [19] modified GO with arginine (Arg) and lysine (Lys) and loaded it with a new natural anticancer agent, Rh2. GO functionalized with Arg and Lys exhibited significantly lower side effects than non-functionalized GO, while Gr-Arg-Rh2 and Gr-Lys-Rh2 showed higher activity against cancer cell lines and lower toxicity to normal cells.

4.2. Delivery of Chemically Synthesized Drugs

Beyond the delivery of cancer drugs, functionalized GO is also used for the delivery of chemically synthesized drugs. Pan *et al.* [20] synthesized nano-GO using an improved Hummer's method and ultrasonication, then functionalized GO's surface with taurine (Tau) to enhance its dispersibility and biocompatibility in water. 5-Fluorouracil (5-FU) was loaded onto taurine-modified GO (Tau-GO) through π-π stacking and hydrogen bonding interactions, forming 5-FU-Tau-GO. The loading efficiency (LE) was evaluated by measuring the concentration of unbound drugs, indicating an LE of 33.7%, meaning 508.52 μg of 5-FU could be adsorbed on 1mg of Tau-GO. Therefore, Tau-GO represents a promising drug carrier

capable of achieving a high drug load. Cell viability assays in HepG2 cells using the MTT method showed that Tau-GO did not exhibit significant cytotoxicity at various concentrations but demonstrated a dose-dependent inhibitory effect on cells when loaded with 5-FU. Thus, this nanocarrier has the capability to deliver anticancer drugs, and the toxicity of 5-FU-Tau-GO was higher than free 5-FU. This might be due to taurine's ability to induce apoptosis in tumor cells, thereby indirectly enhancing 5-FU's inhibitory effect on cells. Furthermore, in vitro release experiments showed that 5-FU loaded on Tau-GO could gradually release within cells, extending the effective action time of the drug and thus producing a better inhibitory effect.

5. Applications of Functionalized Graphene Oxide in Biomedical Imaging

5.1. Magnetic Resonance Imaging (MRI)

MRI is a widely used imaging technique for disease diagnosis due to its ability to display soft tissue contrast [21] [22]. Ashwini *et al.* [23] used GO-$CoFe_2O_4$ composites as contrast agents, with GO serving as a base to effectively fix nanoparticles and enhance the biocompatibility of the contrast agent. Moreover, the proton relaxation value of GO-$CoFe_2O_4$ composites was 361 $mM^{-1}{\cdot}s^{-1}$, significantly higher than that of ferrite nanoparticle-based contrast agents, further illustrating the potential of GO-$CoFe_2O_4$ composites as MRI contrast agents. However, due to GO's high chemical reactivity, which carries the risk of deactivation, Amira, Alazmi, *et al.* [24] used reduced graphene oxide (rGO) as a substitute. By lowering the chemical reactivity while retaining the GO sheet structure, the cobalt ferrite nanoparticle-loaded composite material was made more stable.

5.2. Fluorescence Imaging

Inspired by single-layer carbon nanotubes [25], Dai *et al.* [26] were among the first to use GO photoluminescence for biological imaging. Dai and colleagues functionalized nanosized GO flakes obtained via ultracentrifugation with polyethylene glycol, then covalently bonded them with anti-CD20. The resulting complex could selectively bind to B-cell lymphoma cells. Nanosized GO is luminescent in the visible and near-infrared regions, enabling optical identification of cancer cells. In 2013, AA Nahain *et al.* [27] prepared rGO under mild alkaline conditions and functionalized it with spiropyran conjugated hyaluronic acid (HA-SP) to produce functionalized graphene (rGO/HA-SP) fluorescent nanoparticles. Containing HA as a ligand, rGO/HA-SP could bind to CD44 cell receptors. SP acted as a photochromic dye in the complex. Using Balb/C mice as models, in vivo fluorescence imaging of spiropyran was achieved by administering an MC solution of rGO/HA-SP. By optimizing the surface drug release mechanism of rGO/HA-SP, this material could not only serve as a fluorescent probe for diagnosis but also as a drug carrier in drug release systems.

5.3. Radionuclide Imaging

Radionuclide imaging (RAI) is widely used for in vivo labeling and quantitative analysis of substances due to its low penetration power and high sensitivity. Previous studies found that functionalized GO or RGO could be labeled with radionuclides, serving as radioactive tracers for targeted tumor imaging [28] [29]. Lei Chen *et al.* [30] developed a radiolabeled graphene derivative as a therapeutic agent for combined photothermal therapy (PTT) and internal radiation therapy of cancer, using ^{131}I labeling. Polyethylene glycol (PEG)-coated rGO (^{131}I-RGO-PEG) completed in vivo imaging-guided combined PTT. Compared with free radionuclides, ^{131}I-RGO-PEG provided the ability for in vivo tracking and imaging, achieving synergistic therapeutic effects in cancer combination therapy using a single nanoscale therapeutic agent. Moreover, radiotherapy, blood chemistry, and histological examinations showed that ^{131}I-RGO-PEG at therapeutic doses had no apparent toxicity to mice.

6. Conclusions

This review systematically explores the applications of functionalized graphene oxide (GO) in drug delivery and biomedical imaging. Due to its unique physicochemical properties and tunable surface functionalization, GO has emerged as a multifunctional and efficient carrier, demonstrating tremendous potential in the fields of drug delivery and biomedical imaging. Functionalized GO can be utilized as a carrier for various drugs through non-covalent or covalent modifications, offering advantages such as enhanced drug stability, improved drug solubility, and prolonged plasma half-life, thus broadening the possibilities for drug delivery. Furthermore, owing to GO's excellent optical properties, biocompatibility, and imaging capabilities, functionalized GO has been extensively applied in medical imaging, especially showing unique advantages in fluorescence imaging, magnetic resonance imaging (MRI), and radionuclide imaging.

However, current research faces several challenges and issues, such as insufficient studies on its biotoxicity and long-term biosafety, and clinical translation still encounters a series of difficulties and limitations. Therefore, future research needs to focus on the biosafety evaluation and clinical application prospects of functionalized GO to ensure its safe and effective use in the medical field.

In summary, functionalized graphene oxide, as an emerging biomedical material, holds broad prospects in drug delivery and biomedical imaging, playing a crucial role in the diagnosis and treatment of diseases. With the rapid development of material science, it is certain that materials with lower toxicity and better biocompatibility will be used to modify GO, resulting in stable, safe, and nontoxic functionalized GO. This will enable its use as a safer and more effective material in a wide range of clinical research.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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