Applications of Graphene Oxide Fluorescence Properties in the Biomedical Field: Postprint

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Abstract

Graphene, as an emerging material, has found increasingly broad applications in the biomedical field; graphene oxide (GO), as one of the important derivatives of graphene, benefits from its unique electronic structure and thus can generate fluorescence within a certain wavelength range. It is precisely this property that endows GO with tremendous potential in the biomedical field; this article primarily reviews the applications of GO' s fluorescence properties in molecular detection, disease diagnosis, cell imaging, and other aspects in recent years, and prospects its future development.

Full Text

Preamble

The Application of Graphene Oxide Fluorescence Properties in the Biomedical Field

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Abstract

Graphene has found increasingly broad applications in the biomedical field as an emerging material. As one of its important derivatives, graphene oxide (GO) can produce fluorescence within a certain wavelength range due to its heterogeneous electronic structure. This property endows GO with tremendous potential in biomedicine. This article reviews recent advances in the application of GO' s fluorescence properties in molecular detection, disease diagnosis, and cell imaging, and discusses future development prospects.

Keywords: graphene oxide; fluorescence properties; molecular detection; disease diagnosis; cell imaging

Introduction

In 2004, Geim et al. from the University of Manchester, UK, successfully isolated single-atom-layer graphite—graphene—from layered graphite using micromechanical cleavage, sparking a research boom in graphene. The discoverers of graphene, Andre Geim and Konstantin Novoselov, were awarded the 2010 Nobel Prize in Physics. Graphene features a two-dimensional periodic honeycomb lattice structure composed of carbon hexagonal rings with a thickness of only a single atomic layer, making it the thinnest and hardest known two-dimensional nanomaterial at just 0.335 nm thick[2]. Graphene exhibits excellent optical, mechanical, thermal, and chemical properties, demonstrating promising biomedical applications[3-5].

Chemically derived GO contains both sp2 and sp3 carbon atoms in its molecular structure. The oxygen-containing functional groups (carboxyl, epoxy, and hydroxyl) on its surface facilitate dispersion in various solvents and enable further reactions with other substances, broadening its biomedical applications[6-10]. Additionally, GO' s large specific surface area allows it to adsorb different fluorescent molecules, making it suitable for biological detection and disease diagnosis[11]. Third, its inherent optical properties enable GO to realize bioimaging in living cells[12-14]. GO' s honeycomb planar conjugated structure, reactive oxygen functional groups, good biocompatibility, and large specific surface area confer unique advantages for biomedical applications. Moreover, compared with other nanomaterials, GO can be prepared at low cost, which is beneficial for clinical applications. This article will review research progress on the application of GO' s fluorescence properties in biosensing, disease diagnosis, and biological cell imaging.

1. Overview of GO' s Fluorescence Properties

Fluorescent sensors can be constructed by utilizing GO' s fluorescence and fluorescence quenching properties. However, in sensing processes, GO typically acts as an acceptor for fluorescence quenching rather than fluorescence generation[15-18]. The abundant conjugated sp2 structures on its surface readily interact with fluorescent molecules, diminishing their fluorescence intensity. In recent years, a series of sensors based on GO' s fluorescence quenching characteristics have become a research hotspot[19-21].

Fluorescence resonance energy transfer (FRET) is essentially a fluorescence reabsorption process. When the emission spectrum of one fluorescent molecule (the donor) partially overlaps with the absorption spectrum of another fluorescent molecule (the acceptor) and they are in close proximity (typically less than 10 nm), the donor transfers energy to the neighboring acceptor molecule through dipole-dipole interactions, resulting in decreased donor fluorescence and enhanced acceptor fluorescence compared to when alone. The FRET efficiency between GO and fluorescent molecules is determined by the inverse sixth power of the distance (d) between donor and acceptor, which is significantly stronger

than conventional FRET, giving GO higher quenching efficiency.

GO itself possesses fluorescent properties, but its emission is very weak, limiting its biomedical applications based on photoluminescence. In FRET-based biosensors, GO can serve as either a donor or an acceptor. Through appropriate functionalization, GO's optical properties can be optimized to generate stronger fluorescence signals, enabling it to act as a fluorescent donor. For cell imaging and biological detection, the preparation of fluorescent materials with low toxicity, good biocompatibility, and high brightness creates favorable conditions for biomedical development. Researchers have reported[22] that acyl chloride modification of GO followed by covalent attachment of different alkylamines can reduce non-radiative recombination of electron-hole pairs caused by oxygen-containing groups, transforming weakly fluorescent GO into highly fluorescent GO with high quantum yields. This approach achieved a quantum yield of 13%, representing approximately a 640-fold improvement. By designing metal nanoparticles with spectra matching that of fluorescent GO based on FRET principles, GO fluorescence can be effectively turned off. This provides a novel method for using GO as a fluorescent donor in biomedical applications.

In recent years, graphene and its derivatives have become research hotspots, and GO' s fluorescence properties are playing increasingly important roles in biosensors, disease detection, cell imaging, and other fields.

Overview of GO fluorescence properties applications in the biomedical field

2.1 DNA Detection

GO's surface features a hexagonal ring structure that exhibits strong adsorption toward DNA bases containing hexagonal ring structures. However, GO shows significantly different binding affinities for different DNA molecular structures. For instance, the binding affinity between single-stranded DNA (ssDNA) and GO is much stronger than that for double-stranded DNA (dsDNA). This difference, combined with GO' s excellent fluorescence quenching capability, has led to increasingly widespread applications of graphene oxide in biosensing and molecular detection[23]. GO undergoes strong non-covalent adsorption with ssDNA probes (labeled with fluorescent materials), and through FRET, energy transfer occurs between GO and the fluorescent material, quenching the fluorescence. Target DNA hybridizes with the DNA probe to form dsDNA molecules, separating the fluorescent material from GO. The detection of target DNA is achieved by monitoring the fluorescence recovery from the quenched state.

In 2010, Lu et al.[24] developed a novel fluorescent molecular beacon sensor using GO' s fluorescence quenching effect for DNA sequence detection. Building upon this work, He et al.[25] designed three different fluorescent probes based on varying emission wavelengths to simultaneously detect three target DNA sequences, achieving multiplexed detection of specific DNA sequences with improved sensitivity and significantly reduced detection time. Zhao et al.[26] discovered that GO exhibits stronger affinity for long-chain DNA than for short-chain DNA.

Based on this finding, several GO-DNA fluorescent sensors have been subsequently developed[27,28]. Fan et al.[29] created a GO-DNA sensor that reduces the fluorescence background of molecular beacon sensors while enhancing fluorescent molecular signals, thereby improving detection sensitivity for target DNA. Zhang et al.[30] developed a graphene sensor capable of label-free detection of specific DNA sequences. In experiments by Pang et al.[31], GO was used as a quenching group with fluorescently labeled ssDNA probes to detect characteristic DNA sequences of *Staphylococcus aureus*, leveraging the different adsorption capacities of GO for ssDNA and dsDNA to detect target DNA through fluorescence intensity changes.

DNA (deoxyribonucleic acid) is a primary component of chromosomes and the main genetic material. Qualitative and quantitative detection of DNA is crucial for clinical disease research and biomedical testing.

2.2 Protein and Enzyme Detection

Proteins are fundamental components of organisms that, together with DNA, constitute chromosomes. They are intimately involved in all life activities and play vital roles in metabolism, immune function, and genetic information control. In fluorescent sensors for protein and enzyme detection based on GO, researchers primarily leverage GO' s excellent fluorescence quenching capability combined with relevant chemical reactions to monitor target protein and enzyme concentrations[32].

He et al.[33] established a fluorescent protein detection method based on small-molecule DNA terminal protection using GO as a platform. This design showed sensitivity and selectivity for target proteins in fluorescence-enhanced detection, with a detection limit of 0.77 ng/mL, representing improved sensitivity. The method can be used to detect serum concentrations in actual samples.

Additionally, Lin et al.[34] proposed a novel biosensor for uniform detection of concanavalin A (ConA) using pyrene-conjugated maltose assembled graphene based on FRET. In the presence of ConA, competitive binding between ConA and glucose disrupts the -stacking interaction between pyrene and graphene, leading to fluorescence recovery. The method offers a linear range from 2.0×10^{2} to 1.0 M, a low detection limit of 0.8 nM, and rapid detection within 5 minutes. This approach enables fast, sensitive, and selective ConA detection, demonstrating the tremendous potential of graphene FRET platforms in protein-carbohydrate research with broad applications in drug screening, biomolecular recognition, and disease diagnosis.

Chang et al.[35] developed an aptamer-based sensor using FRET for thrombin detection. Fluorescently labeled aptamers undergo non-covalent assembly with graphene, quenching the dye' s fluorescence. Upon thrombin addition, the dye moves away from the graphene surface, forming a quadruplex-thrombin complex and restoring fluorescence. This method achieves an extremely low detection limit of 31.3 pM, two orders of magnitude lower than carbon nanotube-based

fluorescent sensors, and demonstrates excellent sensitivity and specificity in both buffer and serum.

The integration of GO with biomolecular recognition units for protein and enzyme detection holds broad prospects in clinical diagnosis, and their quantification facilitates further exploration of biological genetics and disease mechanisms. However, this field remains underdeveloped and requires continued research efforts.

2.3 Detection of Other Biomolecules

Various molecules with different biological effects exist in living organisms. Based on the FRET effect between fluorescent molecules and GO, fluorescent sensors utilizing GO adsorption and desorption can be developed to detect biomolecules such as ATP, glucose, and biotin. Leveraging GO' s unique adsorption and quenching properties, Zhu et al.[36] developed a simple, convenient, amplification-free, highly sensitive, and selective fluorescent biosensor for ATP detection based on ATP-dependent enzymatic reactions and the different adsorption affinities between GO and DNA structures.

Glucose is an essential substance in life activities that directly participates in metabolic processes in the human body, and blood glucose is the most important diagnostic criterion for diabetes. Therefore, rapid and sensitive detection of glucose is of great significance. Zhang et al.[37] proposed a GO/DNA composite system using fluorescence enhancement to detect glucose content. In this system, if glucose is present, it is specifically oxidized and catalyzed by glucose oxidase (GOx), generating $\bullet$OH radicals that irreversibly damage and decompose long-chain ssDNA modified with carboxyfluorescein (FAM), ultimately releasing short FAM-ssDNA fragments. Due to the weak interaction between GO and short FAM-linked DNA fragments, DNA fluorescence can be restored by glucose addition. Thanks to GO' s excellent fluorescence quenching efficiency and the specific catalysis of glucose oxidase, this method exhibits extremely high sensitivity and selectivity and has been successfully applied to glucose analysis in serum, with a linear range of 0.5–10 mol/L and a detection limit of 0.1 mol/L.

Biotin (vitamin H) is a water-soluble vitamin that serves as a coenzyme for carboxylases, decarboxylases, and carboxyltransferases[38], playing important roles in metabolism and biochemical processes within living organisms[39]. Mock et al.[40] reported that pregnant women often suffer from biotin deficiency during early pregnancy. Due to biotin' s significant impact on human health, sensitive and rapid detection of its concentration is necessary. Zhang et al.[41] designed a novel fluorescent sensing system for biotin detection based on the strong interaction between GO and DNA, as well as the specific interaction between biotin and streptavidin, achieving a detection limit of 0.44 nmol/L. The proposed fluorescent sensing system was applied to biotin determination in actual samples, and this work could provide a common platform for detecting small biomolecules based on protein-small molecule ligand binding.

The applications described above all involve GO as a FRET acceptor in fluorescent biosensors. However, GO with intrinsic fluorescence properties can also serve as an energy donor. Chen et al.[42] reported a label-free fluorescent biosensor based on photoinduced charge transfer (PCT) using GO for dopamine (DA) detection. Multiple non-covalent interactions between GO and DA, along with the ultrafast decay of GO' s near-infrared fluorescence, lead to effective self-assembly of DA molecules on the GO surface and produce significant fluorescence quenching. This PCT-based biosensor enables direct readout of GO' s near-infrared fluorescence for selective and sensitive DA detection, with a detection limit of 94 nM and a relative standard deviation of 2.0%. The sensor was successfully applied to the quantitative recovery (98–115%) of DA determination in biological fluids.

Although GO' s super fluorescence quenching ability has been widely exploited to develop fluorescent sensors, the potential of its unique intrinsic fluorescence for chemical/biological sensing remains to be further investigated. Compared with traditional sensors, GO can be prepared in large quantities at low cost, and probes do not require labeling with quenching groups. These sensors primarily utilize the stronger adsorption of GO to ssDNA than to dsDNA, detecting various biomolecules through fluorescence intensity changes resulting from energy transfer between donors and acceptors. As an emerging material developed in recent years, GO' s fluorescence properties require further exploration and refinement for biomedical detection applications.

3. GO Fluorescence Properties for Clinical Disease Diagnosis

GO' s fluorescence properties contribute to clinical disease diagnosis. Yue et al.[43] conjugated Cy5 with GO sheets, where fluorescence is quenched under normal conditions due to proximity to GO but is activated upon reaching tumor sites. Both in vitro and in vivo studies have demonstrated the probe' s tremendous potential for tumor diagnosis, leading to the design of a novel probe for tumor tissue detection.

Similarly, based on the high affinity of folate receptors (FR) expressed on the surface of many human cancer cells, Feng et al.[44] developed a fluorescent nanoprobe for targeted imaging of FR-positive cells by covalently linking folic acid (FA) and rhodamine B (RB) to GO through disulfide bonds. This approach not only produces a high signal-to-background ratio but also avoids false-positive results caused by non-specific adsorption.

Gao et al.[45] reacted a GO-multi-walled carbon nanotube aqueous solution with HAuCl to prepare Au NPs/GO-MWCNTs, which were then modified on a glassy carbon electrode to create an -fetoprotein electrochemical immunosensor. With a detection limit of 0.003 ng/mL, the sensor can accurately measure -fetoprotein—a specific tumor marker for liver cancer cells—in serum. The sensor exhibits good selectivity and simple fabrication, holding important application

value in the clinical diagnosis of liver cancer.

Furthermore, Gu et al.[46] utilized a light-addressable potentiometric sensor (LAPS) modified with carboxylated GO to detect circulating tumor cells (CTCs) in prostate cancer, achieving detection of 10 CTCs in 1 mL of blood with high specificity and sensitivity.

In recent years, based on GO' s good water solubility, favorable biocompatibility, fluorescence properties, and ease of surface functionalization, GO has been successfully applied to detect a range of bacteria, cancer cells, and disease-related proteins. However, GO tends to aggregate and its stability needs improvement. Therefore, it is necessary to combine other technologies and integrate multidisciplinary approaches to modify GO' s surface and prepare higher-quality GO.

4. GO Fluorescence for Biological Cell Imaging

The optical properties of graphene and GO can advance biological and medical research, particularly in imaging. In 2008, Sun et al.[47] synthesized and explored the biological applications of nano-GO (NGO), which are single-layer GO sheets with lateral dimensions as small as a few nanometers. They obtained size-separated, polyethylene glycol-modified non-hydroxyl compound sheets that were soluble in buffer and serum without aggregation. The NGO sheets were found to be luminescent in the visible and infrared regions, and their intrinsic photoluminescence (PL) could be applied to near-infrared (NIR) live cell imaging (excitation: 658 nm, emission: 1100–2200 nm) with minimal background interference.

More recently, Li et al.[48] reported that graphene oxide nanoparticles can serve as optical probes for two-photon luminescence imaging and cell therapy. By co-modifying GO with transferrin and polyethylene glycol (PEG) and using it as a probe, they achieved two-photon fluorescence imaging of ex vivo cells.

Furthermore, researchers[49] found that loading photosensitizers onto nano-graphene can be used for multimodal imaging-guided photodynamic therapy of tumors. Photosensitizer molecules (HPPH) were loaded onto PEG-functionalized GO through supramolecular - stacking. The resulting GO-PEG-HPPH complex showed high HPPH loading efficiency. After radiolabeling HPPH with Cu, its in vivo distribution and delivery were further tracked through fluorescence imaging and positron emission tomography (PET).

Compared with other fluorescent materials, GO offers advantages such as relatively stable optical properties, good water solubility, favorable biocompatibility, ease of surface functionalization, and facilitation of cell adhesion. These characteristics establish a solid foundation for GO' s application in live cell imaging.

5. Conclusions and Outlook

GO exhibits low cytotoxicity, good biocompatibility, and fluorescence properties, making it widely applicable in biomedicine and attracting increasing research attention. This review has summarized recent advances in the application of GO' s fluorescence properties in biosensors, biomolecule detection, disease diagnosis, and cell imaging, providing a comprehensive overview of the latest trends in GO fluorescence research in the biomedical field.

As an emerging graphene derivative, research on GO' s fluorescence properties is still in its infancy, with many issues requiring resolution. For example, the interactions between GO and cells/organelles, as well as GO' s in vivo toxicity and metabolic mechanisms, remain unclear. Additionally, the preparation of graphene sheets with appropriate sizes and size control or separation at various length scales is necessary for both in vitro and in vivo applications. While GO is an excellent fluorescence quenching platform, its intrinsic luminescence is weak, necessitating the synthesis and development of more ideal functionalized GO composite materials.

The biological applications of graphene and GO remain largely unexplored. The preparation of GO with better biocompatibility and the ability to self-degrade in biological organisms has become a research hotspot, and the potential of GO' s fluorescence properties awaits further exploration by researchers.

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