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Review

Graphene oxide: An efficient material and recent approach for biotechnological and biomedical applications

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ABSTRACT

The two-dimensional (2D) derivative of graphite termed graphene has widespread applications in various frontiers areas of nanoscience and nanotechnologies. Graphene in its oxidized form named as graphene oxide (GO) has a mixed structure equipped with various oxygen containing functional groups (epoxy, hydroxyl, carboxylic and carbonyl etc.) provides attachment sites to various biological molecules including protein, deoxyribonucleic acid (DNA), ribonucleic acid (RNA) etc. The attached biological molecules with the help of functional groups make it a promising candidate in research field of biotechnological and biomedical applications. The ease of processability and functionalization in aqueous solution due to available functional groups, amphiphilicity, better surface enhanced Raman scattering (SERS), fluorescence and its quenching ability better than graphene make GO a promising candidate for various biological applications. The amphipathetic nature and high surface area of the GO not only prepare it as a biocompatible, soft and flexible intra/inter cellular carrier but also provides long-term biocompatibility with very low cytotoxicity. Inspite of this, still we lack a very recent review for advanced biological applications of graphene oxide. This review deals the bio application of GO and the recent advancement as a biosensors, antibacterial agent, early detection of cancer, cancer cell imaging/mapping, targeted drug delivery and gene therapy etc.

1. Introduction

A perfect two-dimensional material named Graphene, consisting of a single atomic plane of carbon atoms and fundamental unit of almost all confined carbon based nanostructures has its widespread applications in various area [1–4]. Graphene have extraordinary properties such as (i) large theoretical specific surface area of 2630 $m^2 g^{-1}$ [5] (ii) a room-temperature electron mobility of about 200,000 $cm^2 V^{-1} s^{-1}$ [6] (iii) a Young modulus of 1.0 TPa with 20% third-order elastic stiffness, a tensile strength of 130 GPa [7] (iv) 97.4% transmittance with as low as 125 Ωm^{-1} electrical sheet resistance [8] (v) a room-temperature thermal conduction of 5300 $Wm^{-1} K^{-1}$ [9,10] etc. These interesting and exciting properties were experimentally verified by various researchers [11–14]. Various methods and approaches have been developed so far, for the growth of high-quality, pure and high yield of grapheme. These includes mechanical exfoliation of graphite layers [15], graphene growth on SiC [16,17], large area graphene growth by chemical Vapor deposition on metal substrates [12,18,19] etc. Graphene is also a basic material which after folding in different ways or by aggregation of number of layers results in the carbon allotropes, like 0-D fullerene, 1-D carbon nanotubes, itself 2-D graphene and 3-D graphite [20–29].

The oxidized form of graphene named as “Graphene oxide” (GO) are produced by the oxidation of bulk graphite powders via chemical oxidation processes [30–33]. Graphene oxide have a mixed structure bearing variety of oxygen-containing various functional groups like epoxy (> O), hydroxyl (−OH), carbonyl (C═O) and carboxylic (−COOH) groups as shown in Fig. 1 [34,35]. These functional groups attached on both or either side of the GO sheet stabilizes the sheet on water [36]. These functional groups attached to GO hold great promise for potential applications in many technological aspects as photo catalyst [37–39], photonics [35,40–44], electronics [45–50], composites [51–61], electron field emission [62–64] and energy storage devices [65–75] etc. The fine biocompatibility, easy and efficient transports

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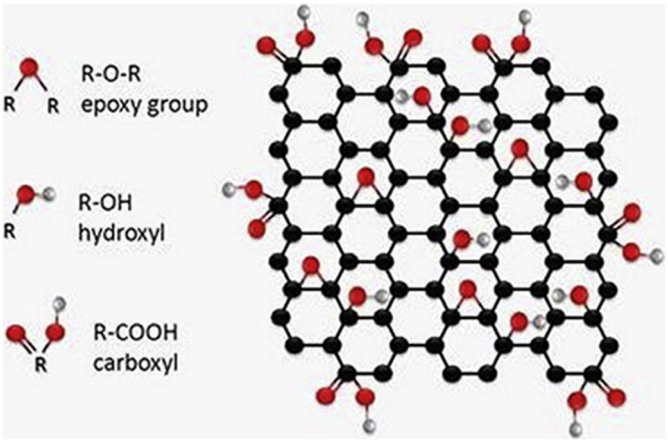

Fig. 1. GO structure containing various functional groups on surfaces and at edges [35]. Reprinted (adapted) with permission from Ref. [35]. Copyright (2013) InTech publisher.

into cells, protecting peptides or DNA from enzymatic cleavage, high fluorescence quenching efficiency [76,77], selective adsorption of nucleotides [78] makes GO, a suitable candidate for potential applications in various frontiers areas of research as mentioned earlier. Unlike the highly crystalline graphene, GO is a unique 2D structure having both crystalline and amorphous defect regions, with the presence of sp^3 carbon and oxygen functional groups [79,80]. However, functionalization disrupts the electronic structure of graphene making GO an insulator rather than a semi-metal and is conceptually different from graphene.

GO and its derivatives have also been successfully tested in numerous applications in bio devices, nanomedicine, biomedical, drug delivery, biotechnology, bioengineering, geno-sensing, imaging of cells, antifungal activity, biosensors, electrochemical sensors and energy storage [81–98]. Bulk graphite oxides material which is simply an accumulation of GO flakes can be easily exfoliated into single layer GO sheets by the ultrasonication in water solution or even with simple swirling. The various functional groups on GO sheets like −OH and −COOH, really work well as a tool for the functionalization of graphene to tune its properties [4,99–101].The various oxygenated functional groups on GO surfaces play very significant and crucial roles towards the properties of GO and hence in the bio-application. The controlled oxidation of GO provides a way to tune its electronic properties, optical transparency, and mechanical characteristics etc. [102,103]. The oxygenated groups increases with increasing oxidation and its electrical properties becomes poorer as compared to highly reduced GO [101]. Thus, the influence of specific different oxygenated groups extremely affects the electrical properties of GO [31]. By taking advantages of this tool various techniques have been adopted to attach variety of molecules to graphene sheets, rendering graphene/GO more versatile precursors for a wide range of applications [104–107]. Multifunctional hybrid nanomaterials with enhanced therapeutic efficiency at physiologically safe dosages for externally triggered, image-guided therapy are highly attractive for nanomedicine. Fig. 2 describes the various bio-applications of GO. The review will mainly focus to these recent bio-applications basically research stepped up till date and future scopes need to step into.

2. Graphene oxide based biosensors

Biosensors with the ability to detect biologically active molecules are of critical importance from both biomedical, environmental, and security point of view [99,108–110]. GO, due to coexistence of both hydrophobic and hydrophilic nature due to the presence of pristine graphite structure and oxygen containing functional groups, is among one of the most attributed materials having new possibilities to develop next, new and advanced level of biosensors [46,111–116]. It exhibits not only good water dispersibility, biocompatibility, but high affinity for specific biomolecules [117,118]. These specific and versatile properties of GO provide a lot of opportunities for the development of novel biological sensing platforms, including fluorescence resonance energy transfer (FRET) based biosensors, surface-enhanced Raman spectroscopy (SERS), electrochemical detection and laser desorption/ionization mass spectrometry (LDI-MS), [119–121].

2.1. Fluorescent sensors for DNA/RNA detection

The surface interaction and their understandings between DNA (containing a negative charge due to phosphate backbone) and GO (containing a various functional groups of −OH, C═O and −COOH groups at their surface and edges) can be of great interest for design and optimization of biosensors and biological devices for disease detections and cure [122]. Recently diverse studies on GO reported the adsorbtion of DNA effectively and hence simultaneously quenched the adsorbed fluorophores with DNA. These interesting behavior of surface adsorption of DNA and quenching of the adsorbed fluorophores opens a new scope to utilize GO as a suitable candidate for DNA based fluorescent biosensors. This DNA and GO interaction strongly depends on temperature, hydrophobic interactions, ion concentration, electrostatic repulsion, pH of the buffer etc.

2.1.1. DNA detection

High sensitivity and selectivity for specific DNA sequence is of immense signification in clinical diagnostics, forensic science, environmental monitoring, and a variety of other biomedical applications [123–129]. In DNA detection in solution, GO act as an excellent acceptor of FRET to quench the fluorescence in dye labeled DNA sequences [130]. A fluorophore-labeled single-stranded DNA adsorbed on GO serves as a sensor because subsequent desorption of it in the presence of complementary target DNA enhances the fluorescence. Wu et al. [131] used different length single stranded DNAs 12-, 18-, 24-, and 36-mer for the adsorption on GO. Several factors like organic solvent, pH and cations affected the adsorption and binding of DNA on GO. Rapid absorption and tighter binding on graphene was observed for shorter DNAs as well as lower pH and higher ionic strength favored it. The ionic strength and hence the adsorption was controlled by the ethanol concentration. When the cDNA was added almost 100% desorption was achieved. Temperature dependent desorption was not effective which indicates the high binding affinity between graphene and DNA. The adsorbed DNA was easily exchanged by free DNA in solution which signifies that GO bonded DNAs are reversible and stable. Fig. 3a shows the schematic representation of FAM-labeled DNA adsorption and desorption on GO. An AFM image (Fig. 3b) showing GO sheets deposited on a silicon wafer with height profile (Fig. 3c) of the line in (b) shows the sheets to be ~1.5 nm in thickness. The fluorescence spectra of 100 nM 18-mer DNA in 25 mM HEPES, 100 mM NaCl, and 5 mM $MgCl_2$ in the presence and absence of 50 μg/mL of GO has been shown in Fig. 3d.

Instead of using monomer only, single- and double-stranded DNAs were used to investigate the interaction with GO [132]. Besides, monovalent and divalent salts were also used to see the effect of binding of nucleic acid to GO. Since divalent ions are efficient to screen the negative charge that is why these ions were much effective than monovalent ions in the adsorption and desorption of ssDNA (single-stranded DNA). The base recognition between the incoming ssDNA and the probe F-ssDNA (FITC-labeled ssDNA; FITC: Fluorescein isothiocyanate) pre-adsorbed on GO was examined by monitoring the increase in fluorescence during the dissociation of probe F-ssDNA from the GO surface. The base complementarity of dsDNA (double-stranded DNA) was analyzed by adding ssDNA (c-ssDNA: complementary-ssDNA or non- c-ssDNA: noncomplementary ssDNA) to the F-dsDNA − GO complex. The results showed that base pairing in dsDNA was exposed to