Sensors and Actuators B 210 (2015) 281–289

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Facile synthesis of 2-dimensional transparent graphene flakes for nucleic acid detection

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ARTICLE INFO

Article history:
Received 13 September 2014
Received in revised form
10 December 2014
Accepted 11 December 2014
Available online 2 January 2015

Keywords:
Graphene
Flakes
Biosensor
Nucleic acid
Tuberculosis

ABSTRACT

We report on the synthesis of 2-dimensional transparent graphene flakes (GF; 2.2 μm × 50 μm), onto indium-tin-oxide (ITO) coated glass plates, by electrochemical exfoliation of graphite rods using in situ intercalation of potassium ions. Raman spectroscopy, Fourier transform infra-red (FT-IR) spectroscopy, scanning electron microscopy and transmission electron microscopy, are used to probe the formation of graphene structures, revealing the generation of GF. Synthesized GF are employed as DNA immobilization platform for genosensor design for *Mycobacterium tuberculosis* detection. This GF based biosensing electrode exhibits high sensitivity, fast response and wide detection range etc. These findings are important for cost-effective strategy for the production of GF for application to advanced biosensors, and to understand graphene-based biosensing mechanism specifically for nucleic acid detection.

1. Introduction

Tuberculosis (TB) is a worldwide health problem with a massive global burden [1]. *Mycobacterium tuberculosis* is the world's greatest infections that mainly affect the women of reproductive age and is the leading cause of death among people with HIV/AIDS [2]. WHO records estimated about 8.6 million new cases of TB in 2012, among which 1.3 million people are approximated to have died of TB. Research in new diagnostics, drugs and vaccines for TB is happening at an unprecedented rate to achieve the desired target of *tuberculosis*-free world. Tuberculin skin test, sputum smear microscopy, commercial broth based culture systems, LED fluorescence microscopy, nitrate reductase assay, Xpert MTB/RIF test are among the most explored diagnostic tools for TB detection [3]. However, current diagnostic tools for tuberculosis are highly expensive, often lack sensitivity and can be time consuming. Hence, an efficient diagnosis device is urgently required for timely and effective diagnosis of this disease.

Graphene, a monolayer thick 2-dimensional sp^2 carbon material, has recently gained enormous scientific interest owing to its promising electrical, optical, and mechanical properties [4,5]. Graphene is the most fundamental form of all sp^2-hybridized carbon materials viz. 3-dimensional graphite, 1-dimensional carbon nanotube and 0-dimensional fullerene. Bloch wave-function analysis and tight bonding model have shown that in graphene, the valence and conduction bands intersect at single point of zero states, called as Dirac point (K-point). This junction at the intrinsic Fermi level leads to its zero band gap semiconducting nature and semi-metallic properties [4]. Electrons in graphene behave as massless Dirac fermions, which lead to extremely high carrier mobility (10^6 cm^2 V^{-1} s^{-1}), responsible for its application toward high-speed electronics [4,6]. Room temperature ballistic transport and high Fermi velocity of electrons (10^6 m/s, 1/300th the speed of light) in graphene are some of the additional outstanding properties. This suggests that graphene is an excellent material for electronics [7].

Owing to such exceptional electrical properties, graphene based devices can perhaps be an alternative for fast bimolecular electronic devices including biosensors [8]. However, for the biosensing devices, the applicant material must also contain chemical functionalities viz., hydroxyl, epoxides, carbonyl carboxyl groups, on

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http://dx.doi.org/10.1016/j.snb.2014.12.054
0925-4005/

its surface for biomolecule immobilization. That is why graphene oxide (GO), that contains many oxygen rich functionalities, is a rapidly rising star on the horizon of all aspects of materials science including next generation bimolecular electronics devices [9–12].

Current approaches to synthesize graphene including chemical vapor deposition and thermal decomposition of silicon carbide wafer offer graphene without such functional groups and have many issues such as process complexity, high temperature processing, low yield, cost and quality [13,14]. Besides this, graphene sheets, due to their high specific surface area, tend to form irreversible agglomerates or even restrict to form graphite by *van der Waals* interactions. Thus, the synthesis and processing of graphene sheets with minimum aggregation continue to be a challenge. Graphite exfoliation in liquids via covalent and non-covalent interactions provides an alternative method for preparing reproducible and scalable single and a few layer thick graphene films [15]. Recently, Parvez et al. have demonstrated the fabrication of graphene by electrochemical exfoliation of graphite for its application to organic electronics [16]. They realized that the graphene produced by this method contains certain amount of oxygen functionalities on its surface. In view of these interesting developments, the electrochemical exfoliation of graphite can perhaps be the most simple and best method to produce low cost graphene and at the same time offer an opportunity to modify surface with desired biomolecules for sensing application.

In the present work, we demonstrate a facile and cost effective approach for the synthesis of GF on indium-tin-oxide (ITO) coated glass plate using electrochemical exfoliation process to explore its application for nucleic acid detection. Our efforts are especially dedicated to develop highly sensitive and cost effective diagnosis strategy for *M. tuberculosis* detection.

2. Materials and methods

2.1. Reagents

Sodium dodecyl sulphate (SDS), triethanolamine (TEA), potassium iodide (KI), N-hydroxysuccinimide (NHS), N-ethyl-N-(3-dimethylaminopropyl carbodiimide) (EDC), oligonucleotide probe sequence specific to *M. tuberculosis*, complementary target, one-base mismatch, and noncomplementary DNA have been procured from Sigma–Aldrich, Milwaukee, WI. All solutions and glassware have been autoclaved prior to being used, and desired reagents (molecular biology grade) are prepared in deionized water (Milli Q 10 TS). Oligonucleotide sequences used in this work are:

Biotinylated Probe: biotin-5′-GGTCTTCGTGGCCGGCGTTCA-3′
Complementary: 5′-TGAACGCCGGCCACGAAGACC-3′
One-Base Mismatch: 5′-TGAACGCCGACCACGAAGACC-3′
Noncomplementary: 5′-ATGTCTCAAGCCAGCTGCTG-3′

2.2. Synthesis of graphene flakes

GF have been synthesized/deposited on ITO coated glass substrates by electrochemical exfoliation of graphite rod using a three electrode system with graphite rod as counter electrode, and Ag/AgCl as the reference electrode. Chronoamperometric exfoliation of graphite has been carried out at a potential of +1.5 V for 60 s in aqueous solution containing KI (intercalating agent), SDS (stabilizing agent) and TEA (complexing agent) in varying molar concentration ratios. Such electrochemical synthesis strategy can offer the presence of functional groups on the surface of graphene based nanostructures as predicted by Parvez et al. earlier and corroborated by us in this work by Fourier transform infrared (FT-IR) spectroscopy [16].

2.3. Immobilization of DNA specific to M. tuberculosis onto GF/ITO electrode

Probe DNA (ssDNA) specific to *M. tuberculosis* (15 μL) has been covalently immobilized onto GF/ITO electrode via amide bond formation between —COOH group of GF and —NH_2 group of DNA using EDC (0.4 M) as the coupling agent and NHS (0.1 M) as activator [17]. The bioelectrode (DNA/GF/ITO) thus fabricated is washed thoroughly with autoclaved phosphate buffer (50 mM, pH 7.0) and is stored at 4 °C when not in use.

2.4. Instrumentation

Raman spectrum has been recorded at room temperature by exciting the sample with wavelength 514.5 nm using argon laser, Model Innova 70, Coherent, USA. A notch filter has been used to eliminate the Rayleigh line from the scattered radiation. The scattered light has been detected by a single stage Jobin Yvon-Spex HR 640 Czemy-Turner monochromator along with a cooled photomultiplier tube (PMT) operated at voltage of 1600 V. FT-IR spectroscopic investigations have been recorded using Perkin-Elmer FT-IR model "Spectrum BX" equipped with the specular reflectance accessory. Morphology of the deposited samples has been examined using high resolution transmission electron microscopy (HR-TEM), model JEM-2100F (JEOL, Japan), and scanning electron microscopy (SEM), model LEO 40, respectively. Electrochemical response studies of DNA-GF/ITO bioelectrode have been carried out using Autolab Potentiostat/Galvanostat (Eco Chemie, Netherlands), in a conventional three-electrode electrochemical cell consisting of Ag/AgCl as reference electrode and platinum foil as the counter electrode, in phosphate buffer saline (PBS) solution (0.05 M, pH 7.0, 0.9% NaCl) containing MB as a redox indicator.

3. Results and discussion

3.1. Electrochemical exfoliation of graphene flakes

Graphene flakes rich in oxygen containing functionalities on its surface have been obtained by chronoamperometric exfoliation of graphite rod at +1.5 V at the optimized molar ratio of KI, SDS and TFE as 10:1:2 with pH of the electrolytic aqueous solution maintained at 9. The overall exfoliation strategy toward 2-dimensional graphene flakes is outlined in Fig. 1. Exfoliation of GO sheets has been initialized and facilitated with intercalation of the potassium ions (K^+) within the layers of graphite rods due to the smaller size of the K^+ ions. K^+ ions emerging from potassium iodide get intercalated between the outer and penultimate planes and weaken the *van der Waals* attractive forces between the graphitic planes. Once the penultimate and outer graphite planes get loosened up from each other, the outer plane can be easily leached out in the solution as GO flakes under the influence of applied external electric field. Being part of the electrolytic solution, SDS molecules help in stabilizing the GO flakes and prevent its aggregation (Fig. 1). TEA has been employed to assist K^+ ion to penetrate in graphitic plane by complex formation. Surfactant, moisture and other chemical impurities have been removed from the deposited GO flakes by sonicating the electrodes for about 15 min followed by the heat treatment at 100 °C for 20 min. GO flakes are reduced to GF by hydrazine (NH_2NH_2) treatment for 4 h at 80 °C.

3.2. SEM and TEM analysis

SEM and TEM investigations have been carried out to analyze structural and textural behavior of electrochemically fabricated films of graphene deposited on ITO. In order to find the optimal conditions for the formation of transparent graphene flakes, the