Biosensors and Bioelectronics ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Multifunctional graphene micro-islands: Rapid, low-temperature plasma-enabled synthesis and facile integration for bioengineering and genosensing applications

Shafique Pineda [a,b,1], Fabricio Frizera Borghi [a,b,1], Dong Han Seo [a], Samuel Yick [a], Malcolm Lawn [c], Timothy van der Laan [a,b], Zhao Jun Han [a], Kostya Ken Ostrikov [a,b,d,*]

[a] CSIRO Manufacturing, P.O. Box 218, 36 Bradfield Road, Lindfield, NSW 2070, Australia
[b] School of Physics, The University of Sydney, Sydney, NSW 2006, Australia
[c] National Measurement Institute (NMI), Nanometrology, 36 Bradfield Road, Lindfield, NSW 2070, Australia
[d] Institute for Future Environments and Institute for Health and Biomedical Innovation, School of Chemistry, Physics, and Mechanical Engineering, Queensland University of Technology, Brisbane QLD 4000, Australia

ARTICLE INFO

Article history:
Received 23 December 2015
Received in revised form
20 April 2016
Accepted 21 April 2016

Keywords:
Low-temperature plasma
Plasma nanoscience
Graphene
Biosensor
Biocompatibility
Biomaterials

ABSTRACT

Here, we present a rapid, low-temperature (200 °C) plasma-enabled synthesis of graphene micro-islands (GMs). Morphological analyses of GMs by scanning electron microscopy (SEM) and atomic force microscopy (AFM) feature a uniform and open-networked array of aggregated graphene sheets. Structural and surface chemical characterizations by Raman spectroscopy and X-ray photoelectron spectroscopy (XPS) support the presence of thin graphitic edges and reactive oxygen functional groups. We demonstrate that these inherent properties of GMs enable its multifunctional capabilities as a bioactive interface. GMs exhibit a biocompatibility of 80% cell viability with primary fibroblast lung cells after 5 days. Further, GMs were assembled into an impedimetric genosensor, and its performance was characterized by electrochemical impedance spectroscopy (EIS). A dynamic sensing range of 1 pM to 1 nM is reported, and a limit of quantification (LOQ) of 2.03×10^{-13} M is deduced, with selectivity to single-RNA-base mismatched sequences. The versatile nature of GMs may be explored to enable multi-faceted bioactive platforms for next-generation personalized healthcare technologies.

1. Introduction

Graphene, an atomically-thin film of crystalline carbon, has attracted significant interest for biomedical technologies owing to its exceptional physicochemical properties, determined by its unique two-dimensional structure and morphology (Novoselov et al., 2012). However, green and resource-efficient production of graphene and its facile integration into biomedical devices are essential for such technologies to be feasible, which remains a challenge (Zurutuza and Marinelli, 2014).

Recent investigations have demonstrated significant progress in addressing several of these concerns to facilitate the utilization of graphene technologies in biomedical applications. This includes reducing the production cost of graphene films grown by thermal chemical vapor deposition (CVD), by using lower growth temperatures and other carbon precursors (Guermoune et al., 2011; Sun et al., 2010). However, these techniques still involve long processing times and notably high temperatures (~850 °C). Moreover, while graphene dispersions prepared by chemical exfoliation are cheaper alternatives to CVD grown graphene films, their synthesis requires multi-staged complex processing, often in harsh chemical environments (Hernandez et al., 2008). Also, such graphene dispersions tend to agglomerate, and utilization of graphene as a functional surface requires additional chemical binders (Li et al., 2008). While these graphene dispersions have shown good biocompatibility, their uses are limited by a poor electrical conductivity and a resource-consuming integration, which undermines its performance and multifunctionality for applications in bioelectronics.

In addition, there have been efforts to improve the integration of graphene into electronics, by utilizing either dry transfer techniques or improved transfer techniques based on reactive chemical etching (Gao et al., 2012; Suk et al., 2011). However, these approaches are multi-staged and involve complex handling of graphene, and often introduce cytotoxic impurities on the surface of graphene. It is thus important to develop a simple, fast, environmentally-benign, low-temperature graphene production and

* Corresponding author at: CSIRO Manufacturing, P.O. Box 218, 36 Bradfield Road, Lindfield, NSW 2070, Australia.
E-mail address: kostya.ostrikov@csiro.au (K.K. Ostrikov).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bios.2016.04.072
0956-5663/

integration for bioelectronics.

Plasma has been shown to grow multilayer graphene flakes, containing single layer graphene domains of sub-micrometre to just-above-micrometre size (van der Laan et al., 2015). However, such graphene flakes are sparsely grown, and thus, remain unfavorable for use as bioactive coatings and biosensing interfaces, whereby a larger quantity, surface coverage, and better transferability of graphene are required. Herein, graphene micro-islands (GMs) are synthesized by a low-temperature plasma, that is resource-efficient and eco-friendly. We demonstrate the multifunctionality of GMs as a bioactive interfacial material for biocompatible coatings and electrochemical genosensing.

2. Materials and methods

2.1. Plasma-enabled growth of GMs and water-mediated transfer

The deposition of GMs was carried out in a RF inductively coupled plasma CVD system. A copper foil (99.5%, Alfa Aesar) of dimensions 4 cm × 4 cm was used as growth substrate for the GMs. Firstly, a gas mixture of 10 sccm Ar and 90 sccm H_2 was fed into the chamber, and then the plasma was generated at a pressure of 2.0 Pa and RF power of 750 W, respectively. The copper foil was treated with Ar/H_2 plasma for 3 min. Next, 2 sccm of CH_4 was fed into the chamber. Although no external substrate heating was used, during a subsequent 8 min deposition process, the substrate temperature reached ~200 °C due to the plasma-heating effects. Next, the GMs were decoupled from the copper foil by immersion in de-ionized water. GMs floated as a film on the water surface, and were transferred onto a glass microslide for subsequent characterizations.

2.2. Microscopy and microanalysis

Field-emission scanning electron microscopic (FE-SEM) images were obtained by Zeiss Auriga microscope operated at 5 keV electron beam energy with an InLens secondary electron detector. Atomic force microscopy (AFM) images were acquired with an Asylum Research MFP-3D AFM operating in intermittent contact ("tapping") mode with a 5 N/m spring constant cantilever. Image analysis was performed using the Scanning Probe Image Processor (SPIP™) software produced by Image Metrology A/S. Raman spectroscopy was performed using a Renishaw inVia spectrometer with a laser excitation at 514 nm (Ar laser) and a probing spot size of ~1 μm^2. X-ray photoelectron spectroscopy (XPS) spectra were recorded by Specs SAGE 150 spectroscope with the Mg Kα excitation at 1253.6 eV. Both survey and narrow scans of C 1s were conducted.

2.3. Biosensing measurements

The size of each sensing substrate was 2 cm × 1 cm. The biosensing electrode consisted of GMs on aluminium foil. Subsequently, the GMs were treated with 0.05 M *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) and 0.03 M N-hydroxysulfosuccinimide (NHS) in phosphate buffered saline (PBS, pH =7, Sigma Aldrich) for 15 min. This enabled the formation of active ester intermediates via carbodiimide chemistry. Next, the surface of graphene was washed several times with PBS and DI water to remove excess EDC/NHS. Then, NH_2-conjugated miRNAs (probe sequence: 5′-NH_2-AUUUCACGACUGUCACGUCUA-3′, Sigma Aldrich) were diluted in PBS to 0.2 μM, and 50 μL was pipetted onto the EDC/NHS-treated surface. This was left to incubate overnight in a wet environment at room temperature. Next, the sensing surface was washed with 0.05% sodium dodecyl sufonate (SDS) (Sigma Aldrich) in 0.04 M hydroxylamine solution (Sigma Aldrich) to deactivate the remaining carboxyl functional groups and to remove non-specifically bound probe miRNAs. Then, 0.01 M Polyethylene glycol (PEG) (Sigma Aldrich) was loaded on the sensing surface to block the exposed areas of graphene to reduce further non-specific binding. The (biomarker) miRNA sequence (target sequence: 5′-UAAAGUGCUGACAGUGCAGAU-3′, Sigma Aldrich) was dissolve in human serum (Human Plasma AB, Sigma Aldrich) to obtain dynamic concentrations of 1 pM–1 nM, which were pipetted onto the sensing surface. This was left to incubate at 45 °C for 20 min to induce hybridization between the complementary probe and target sequences. Finally, a washing step with PBS/DI water was employed to remove remaining non-specifically bound target miRNAs. To demonstrate sensing specificity, a similar protocol was adopted by replacing the target sequence with a single-RNA-base mismatched sequence (non-complementary sequence: 5′-UAGAGUGCUGACAGUGCAGAU-3′, Sigma Aldrich). This fully assembled device was then utilized in a three-electrode electrochemical cell for biosensing measurements.

2.4. Cytotoxicity testing

The cytotoxicity of plasma-grown GMs was evaluated using CellTiter 96 Aqueous Non-Radioactive Cell proliferation (MTS) Assay (Promega, C#G5421) following the protocol provided by the manufacturer. Primary fibroblasts lung cells (MRC5 cell line) were cultured in 96-well plates at 2 × 10^4 cells/well on control (Tissue Culture Polystyrene – TCPS), glass and glass covered with GMs for 1–5 days. Optical microscopy images were taken after 24 h and 120 h of culture, on the 1 day and 5 days samples, respectively.

3. Results and discussion

3.1. Fabrication and characterization of graphene micro-islands

The procedure for preparing GMs is illustrated in Fig. 1A. A low-temperature plasma process enables the growth of GMs on a copper foil. This approach is typically more resource-efficient compared to chemical vapor deposition (CVD) and represents an environmentally-benign alternative to conventional wet-chemical methods, which involve high temperatures and hazardous chemicals, respectively (Biswal et al., 2013).

During the early growth process (~2 min), the methane is rapidly dissociated into carbon building units by the plasma. Subsequently, these carbon species reorganize into hexagonal carbon-rings forming graphene nanosheets. Such plasma-unique effects are mainly attributed to the strong plasma-matter interactions in the plasma sheath (Ostrikov et al., 2013; Yick et al., 2013). In particular, hydrogen may enhance the surface flux of these building units through the recombination-mediated energy dissipation on the surface and facilitate the nucleation and growth of graphene nanosheets (Seo et al., 2013). Fig. 1A outlines the process for GM synthesis. Initially, graphene nanosheets grow vertically from the substrate due to the electric field in the plasma sheath (Supplementary Fig. S1). Upon water-mediated transfer, the vertically-standing graphene nanosheets collapse to lay horizontally on the transferred substrate, in a two-dimensional open and arrayed network of GMs. Additionally, the controlled growth of GMs and its reproducibility are studied in Supplementary Fig. S2.

The microstructure and morphology of GMs are examined in Fig. 1B–D, with scanning electron microscopy (SEM) and atomic force microscopy (AFM) topography imaging. The GMs are outlined in the high-magnification SEM image (Fig. 1B). GMs are deduced to be composed of graphene domains (each 200–500 nm in dimension), aggregated in an open network of micron-sized

Please cite this article as: Pineda, S., et al., Biosensors and Bioelectronics (2016), http://dx.doi.org/10.1016/j.bios.2016.04.072