Biosensors and Bioelectronics 65 (2015) 265–273

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Highly sensitive reduced graphene oxide microelectrode array sensor

Andrew M.H. Ng [a,b], Kenry [b,c,d], Chwee Teck Lim [b,d,e], Hong Yee Low [a,f,*], Kian Ping Loh [b,**]

[a] Institute of Materials Research and Engineering, A*STAR (Agency for Science, Technology and Research), 3 Research Link, Singapore 117602, Singapore
[b] Graphene Research Centre, Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore
[c] NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore 117576, Singapore
[d] Department of Biomedical Engineering, National University of Singapore, Singapore 117576, Singapore
[e] Mechanobiology Institute, National University of Singapore, Singapore 117411, Singapore
[f] Engineering Product Development, Singapore University of Technology and Design, 20, Dover Drive, Singapore 138682, Singapore

ARTICLE INFO

Article history:
Received 11 August 2014
Received in revised form
6 October 2014
Accepted 20 October 2014
Available online 23 October 2014

Keywords:
Graphene
Microelectrode array
Biosensor
Nanoimprinting

ABSTRACT

Reduced graphene oxide (rGO) has been fabricated into a microelectrode array (MEA) using a modified nanoimprint lithography (NIL) technique. Through a modified NIL process, the rGO MEA was fabricated by a self-alignment of conducting Indium Tin Oxide (ITO) and rGO layer without etching of the rGO layer. The rGO MEA consists of an array of 10 μm circular disks and microelectrode signature has been found at a pitch spacing of 60 μm. The rGO MEA shows a sensitivity of 1.91 nA μm^{-1} to dopamine (DA) without the use of mediators or functionalization of the reduced graphene oxide (rGO) active layer. The performance of rGO MEA remains stable when tested under highly resistive media using a continuous flow set up, as well as when subjecting it to mechanical stress. The successful demonstration of NIL for fabricating rGO microelectrodes on flexible substrate presents a route for the large scale fabrication of highly sensitive, flexible and thin biosensing platform.

1. Introduction

A microelectrode has many advantages which include enhanced signal to noise ratios, increased mass diffusion, ease of miniaturization and operation in highly resistive media (Huang et al., 2009; Montenegro et al., 1991). However one of the disadvantages of reducing electrode sizes to sub-millimeter length scales is the reduced signals associated with the reduction in active area, where the current levels are reduced to nano and pico amp ranges. A commonly reported approach to enhance the signals is to functionalize the active layer with nanoparticles (Stern et al., 2006; Walcarius et al., 2013). Another method is to fabricate the sensor device into an array to allow an increase in total effective active area, thereby increasing the sensitivity of the sensor (Montenegro et al., 1991). In such an approach, the spacing between each microelectrode should be optimized so that the boundary layer of each microelectrode where concentration of the analyte differs from the concentration in bulk solution, also known as a diffusion hemisphere, do not overlap (Bard and Faulkner, 2000), as the latter occurrence will compromise the fast radial diffusion characteristics of an MEA (Henstridge and Compton, 2012; Tomčík, 2013).

In sensors, besides the electrode, the other critical component is the material that allows electrochemical reactions to occur – the active layer. Conventionally, gold, platinum and silver metals have been the predominant materials used since the active layer needs to be electrically conductive (Finklea et al., 1993; Jung and Wilson, 1996; Lian et al., 2009; Lupu et al., 2009; Reed and Hawkridge, 1987). However there are other emerging organic electro-active materials such as poly(ethylenedioxythiophene), glassy carbon electrodes, carbon nanotubes, graphene, and reduced graphene oxide (rGO) (Chopra et al., 2003; Hsiao et al., 2013; Huang et al., 2013; Pron et al., 2010; Yuan and Shi, 2013). These materials are generally lower in cost to use and potentially allow for more useful fabrication techniques in contrast to conventional metal sputtering/lithography methods, generating research interest in utilizing them in electrochemical applications (Forrest and Thompson, 2007).

There are several advantages of using rGO as an active layer in sensing applications. rGO is prepared by the chemical or thermal reduction of GO. Following the reduction process, oxygen atoms are partially removed from the surface and the sp^2 conjugation is recovered to some extent, thus allowing the rGO layer to be electrically conductive (Chua and Pumera, 2013). When used as an electrode, rGO can oxidize or reduce the target analyte depending on the analytes formal potential. rGO has a large electrochemical

* Corresponding author at: Engineering Product Development, Singapore University of Technology and Design, 20, Dover Drive, Singapore 138682, Singapore.
** Corresponding author.
E-mail addresses: hongyee_low@sutd.edu.sg (H.Y. Low), chmlohkp@nus.edu.sg (K.P. Loh).

http://dx.doi.org/10.1016/j.bios.2014.10.048
0956-5663/

potential window, easily solution processable, has oxygen moieties strewn across its carbon lattice that allow electrochemical reactions to take place. These attributes have been extensively covered in literature (Cai et al., 2014; Chen et al., 2013; Li et al., 2011; Ng et al., 2014; Penmatsa et al., 2012; Wei et al., 2012). Most previous studies focused on planar, macroscopic electrodes made of drop-casted or spin-coated rGO multilayer films onto pre-fabricated electrode platforms such as a glassy carbon electrode (GCE) (Saby et al., 1997; Wang et al., 2002). The solution processable nature of rGO allows it to be readily functionalized, creating an active material that can be highly tuned to specific analytes ranging from proteins, DNA and enzymes. Further in-depth reading into the detection properties of rGO has been published in the review article by Wu et al. (2013). However, addressing the problems of MEA fabrication of thin film active layers on rGO has been scarce due to the difficulties in integrating these into MEA configurations. One method to increase the currents is to fabricate the microelectrodes into an array format. In an array configuration with multiple microelectrode openings, these active layers work in parallel to increase the overall current in the sensor. However conventional microelectrode array fabrication methods have been developed mainly for thick metal active layers (Frazier et al., 1993), and the fabrication challenges for rGO MEAs arise from the difficulty in controlling the depth of etching for thin 2-D materials like rGO.

Li et al. previously reported a rGO MEA based on coating a substrate with a GO layer and using hydrazine filled polydimethylsiloxane (PDMS) wells to generate a spatial array of electrochemically active rGO disks. However one drawback with this method is that the initially insulating GO layer becomes electrochemically active due to inevitable electrochemical reduction during actual usage, which will lead to a loss of well-defined electrochemically active areas on the surface (Li et al., 2011). He et al. reported patterning micron-size wide bands of GO using capillary actions through a PDMS stamp followed by its chemical reduction (He et al., 2010). However, this method is limited in terms of the shape and size of the rGO layers.

NIL is a method to create micro to nano scale surface patterns using thermal or UV curable resists. It is a mechanical replication process where the negative of the relief structure on a mold (typically Si or quartz) is transferred to a resist either at elevated temperature or under the exposure of UV radiation. The pattern resolution in NIL is governed primarily by the pattern resolution of the mold, and the imprint fidelity is mainly dependent on material properties and process optimization. NIL allows patterning of surfaces on a large scale with the patterned area only limited by the size of the mold. Most importantly, NIL is a versatile lithographic technique where various resists materials can be used and the imprint process window is tuneable according to the material properties. NIL presents a suitable fabrication method to create micro-array patterns (Chou et al., 1996; Guo, 2007). Through a process modification, we have recently reported the fabrication of size and shape tunable graphene and graphene oxide (Ng et al., 2014). Building on the earlier process development, here we report the fabrication of a biosensor based on microelectrode array of rGO and shows that these can function as highly sensitive biosensors.

2. Materials and methods

All chemicals were purchased at Sigma-Aldrich unless otherwise stated.

2.1. GO preparation

GO was produced by adding 1.5 g of Graphite powder, 1.5 g of $NaNO_3$ and 69 ml H_2SO_4 into a conical flask and stirred in an ice bath. 9 g of $KMnO_4$ was slowly added to the mixture which was cooled in the ice bath. Solution was then transferred to an oil bath at 35 °C. 120 ml of deionized (DI) water was added to the flask. Further stirring for 30 min as temperature of oil bath increased to 90 °C. 300 ml of water was slowly added. 9 ml of H_2O_2 was added to the mixture. The solution was then filtered and washed through with DI water, leaving GO powder.

2.2. rGO MEA device fabrication

10% Polyvinylalcohol (PVA) was spin coated on rGO at 3000 rpm for 30 s. An Obducat nanoimprinter was used to imprint PVA at 105 °C, 50 bar for 10 min. The imprinted PVA layer was then etched using an Oxford RIE using 10 sccm of O_2 for 8 min. 1 μm thick Poly methylmethacrylate (PMMA) from Microresist Technology was then spin coated on top of the patterned and etched PVA layer at 3000 rpm for 30 s. The PMMA layer was then etched in an Oxford RIE using 10 sccm of O_2 for 5 min. The exposed PVA layer was then dissolved in DI water for 2 h.

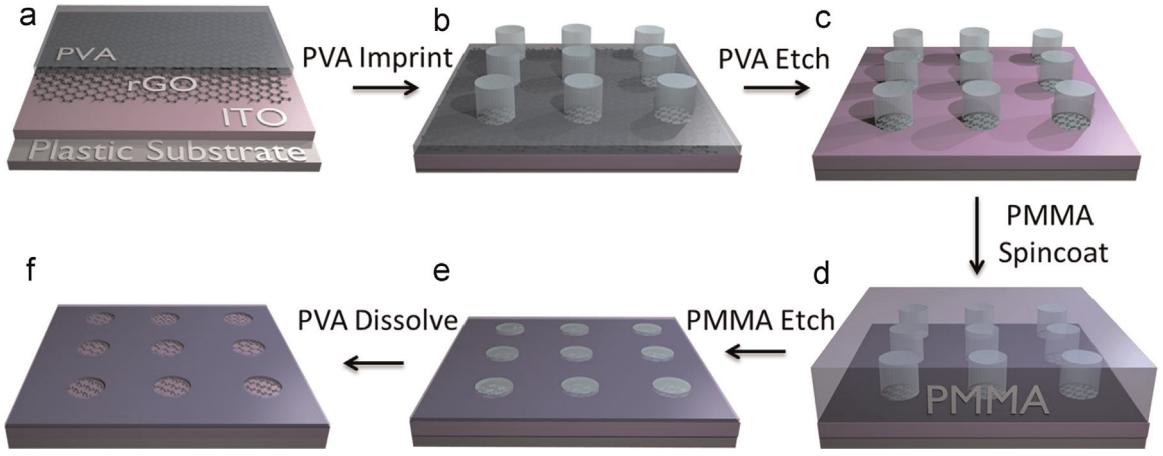

Fig. 1. (a) PVA is spin coated onto electrochemically reduced graphene oxide (rGO). (b) PVA layer is patterned into a pillar array through NIL. (c) Patterned PVA layer is etched down to remove unwanted rGO, leaving PVA pillars protecting the rGO active layer array. (d) PMMA layer is spin-coated on top of the patterned and etched PVA layer. (e) PMMA layer etched to expose PVA layer. (f) PVA layer is dissolved in DI water to reveal underlying rGO active layer. The final product consists of exposed rGO disk array surrounded by PMMA, the latter passivates the unexposed area from the electrolyte.