Graphene biosensors for disease biomarkers

Owen J. Guy, Z. Tehrani, G. Burwell, S. Teixeira, S. Doak

College of Engineering, Swansea University, Singleton Park, Swansea, UK
o.j.guy@swansea.ac.uk

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The development of miniaturized systems for detection of disease biomarkers, at clinically relevant concentrations in biological samples, is key in the early diagnosis and monitoring of diseases. This paper presents the development of novel antibody functionalized epitaxial graphene devices for bio-sensing applications.

This is the first reported example of an epitaxial graphene biosensor. A generic biosensor technology has been developed that is capable of detecting sub-nano molar concentrations of biological molecules. Growth of multilayer epitaxial graphene, on silicon carbide (SiC) substrates, has been performed under ultra high vacuum (UHV) conditions and under high-temperature / high vacuum growth condition. The graphene layers grown in this work have high structural integrity and exist as a continuous layer, extending over the terraced SiC substrate. In monolayer epitaxial graphene, the electron transport properties are dominated by the graphene-SiC interface layer. Multilayer graphene is less influenced by the substrate and has therefore been used in the fabrication of these sensors. The process for multilayer graphene grown has been investigated using X-ray Photoelectron Spectroscopy (XPS).

Silicon Carbide (SiC) has been discovered to be a suitable substrate for graphene growth [1, 2]. During annealing at temperatures of between 1100ºC and 1700ºC the SiC surface reconstructs itself, with silicon atoms subliming and leaving behind a layer, or multiple layers, of epitaxial graphene [3]. Epitaxial graphene's superb electronic properties (high carrier mobilities), and reported biocompatibility [4], and substrate-inferred processability make it ideal for fabrication of nano-scale electronics and sensors.

Few-layer epitaxial graphene (FLEG) has been grown on silicon carbide substrates and patterned into resistor channel devices (Fig. 1). The channels have subsequently been electrochemically functionalised with antibody bio-receptors.

A generic electrochemical surface functionalisation chemistry, which can be used to attach a variety of "bio-receptors" to graphitic surfaces, has been developed. The novel electrochemical method for attachment of antibodies to epitaxial graphene/SiC surfaces using chemical functionalisation of graphene with nitro groups and subsequent reduction to an amine, has been monitored using X-ray Photoelectron Spectroscopy (XPS) (Fig. 2). Subsequent attachment of a fluorescently labeled antibody to the graphene surface has been confirmed using fluorescence microscopy, in the first known bio-functionalisation of epitaxial graphene on SiC (Fig. 3).

This change can be detected as an electrical signal from the biosensor, enabling highly sensitive detection of biomarker analytes. The electrochemical functionalisation technology reported in this paper is a generic platform for the attachment of any number of antibodies or other bioreceptor molecules. Several antibodies including those targeted against Beta-actin, 8-OHdG and troponin have been covalently attached to graphite and to graphene.

Attachment has been verified using laser scanning confocal fluorescence microscopy (LSCM) and atomic force microscopy (AFM). AFM shows an increase in surface roughness from 1nm before functionalisation to around 2nm after antibody attachment. Fluorescence measurements were conducted by using a labeled secondary antibody to the surface bound primary antibody. The

functionalisation technology has been integrated with a graphene electronic device to fabricate a prototype biosensor which has been used to detect nM concentrations of the oxidative stress biomarker 8-OHdG.

The biosensors work on the principle of a target disease biomarker, binding with a “bio-receptor” attached to the graphene surface, yielding a change in the surface charge density. This change can be detected as an electrical signal from the biosensor device. The device itself consists of a conductive graphene channel – functionalised with the “bioreceptor”. Graphene nano-channel sensors have the potential for much greater sensitivity to biomarkers than traditional bioassays because of their high signal-to-noise ratios (S:N).

The results from a specific sensor, fabricated by functionalising a graphene nanochannel surface with an antibody bioreceptor, indicative of oxidative stress and prostate cancer risk, will be presented.

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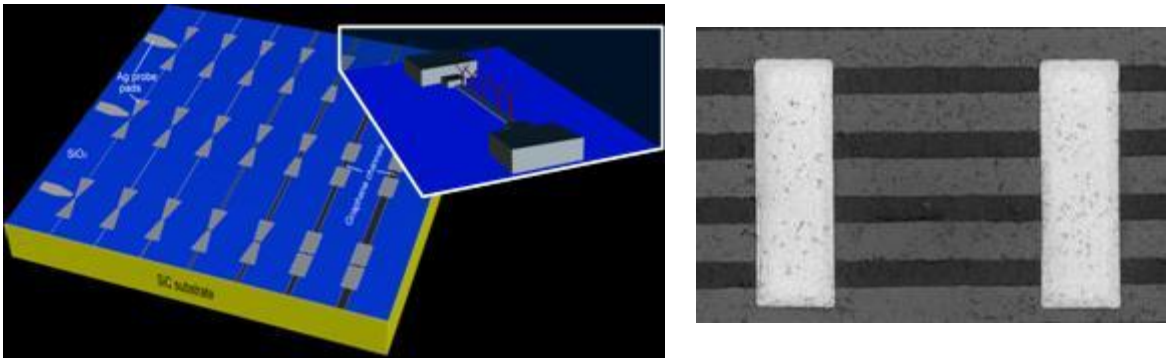

Fig. 1: (a) Schematic of graphene devices and (b) SEM image of epitaxial graphene microchannel device.

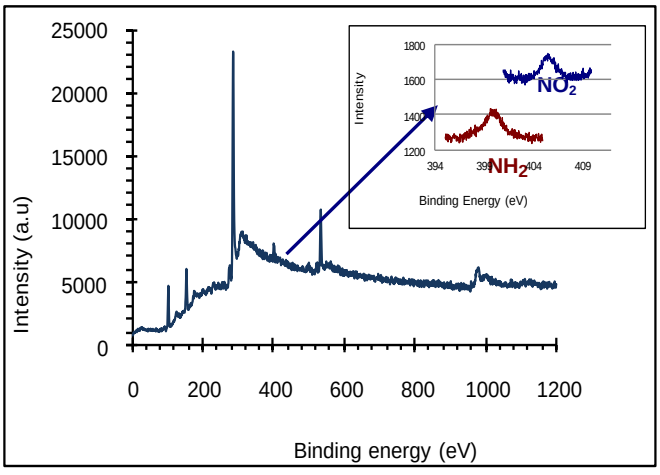

Fig. 2: XPS spectrum of nitrobenzene functionalized graphene surface. Inset: N 1s peak, conversion of nitro to amino group upon subsequent electrochemical reduction to aniline.

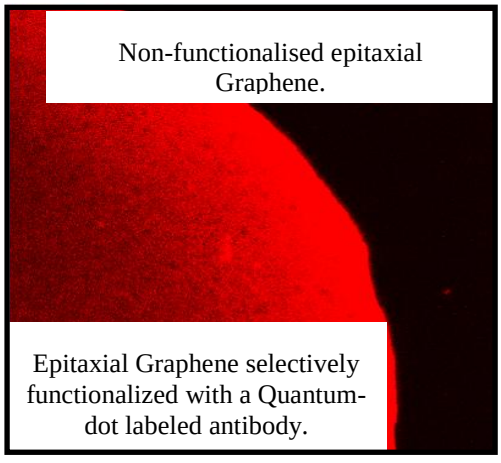

Fig. 3: Laser scanning confocal fluorescence micrograph of epitaxial Graphene selectively functionalized with a Quantum-dot labeled antibody.