Detection of monoclonal antibodies using chemically modified graphite substrates

[*]**Tehrani Z.,** Guy O.J., Castaing A., Burwell G., [+]Doak S.
College of Engineering, Swansea University, Swansea, UK,Z.Tehrani.449409@swansea.ac.uk,
o.j.guy@swansea.ac.uk [+] College of Medicine, Swansea University, Swansea, UK

This paper presents a novel sensor device based on chemically modified Highly Ordered Pyrolytic Graphite (HOPG). Biosensor diagnostics based on bio-functionalsised semiconductor devices are an important development in ultrasensitive sensors for early detection of disease biomarkers.

Electrochemical devices using chemically modified graphite (CMG) channels are excellent candidates for nano-biosensors[1] By attaching aniline to HOPG, via coupling with an aryl diazonium salt, the amino group of the aniline molecule has been used graft antibodies - (1) targeted against beta-actin and (2) targeted against 8-hydroxydeoxyguanosine (8-OHdG) - onto the HOPG surface.

Antibody attachment to graphitic surfaces has been verified using Laser Scanning Confocal Microscopy (LSCM) to detect attached quantum-dot labeled antibodies.

The current-voltage characteristics of virgin and chemically modified HOPG surfaces have been used to detect the presence of antibodies at nm concentrations.

This paper reports on a novel electrochemical method for chemical functionalisation of (HOPG) surfaces for the development of a generic biosensor technology. Following chemical functionalisation with an aryl amine linking molecule (see Fig. 1 and Fig. 2), any biomolecule with a carboxyl group can be attached to the HOPG surface.
The binding reaction has been validated using two antibodies i) an antibody targeted against the commonly expressed beta-actin molecule and ii) an antibody targeted against the prostate cancer biomarker, 8-hydroxydeoxyguanosine (8-OHdG). Detection of this biomarker using enzyme-linked immunosorbant assays (ELISAs) has been used to successfully differentiate patients with bladder and prostate cancer from healthy patients [2].
However, biomarkers are often present at very low concentrations and ELISAs have limited sensitivity. Detection of 8-OHdG using highly sensitive, label-free, electrochemical, graphene nano-biochips is an important development in early detection methods for cancer risk. Bio-functionalisation of surfaces with “bio-receptor” molecules capable of specific and selective binding with disease biomarkers such as 8-OHdG is an important enabling technology in the development of nanoscale diagnostic sensors.

Electrical characterization of the chemically modified HOPG surfaces shows that a decrease in current can be detected upon functionanalisation (Fig. 3). The amino group of the aniline molecule has been used graft antibodies – (1) targeted against beta-actin and (2) targeted against 8-hydroxydeoxyguanosine (8-OHdG) - onto the HOPG surface. Antibody attachment to graphitic surfaces has been verified using LSCM to detect quantum-dot labeled antibodies bound to the surface (Fig. 4). The method has been successfully verified using X-ray photoelectron spectroscopy (XPS), Atomic Force Microscopy. The effect of electrochemical functionalisation on the current-voltage characteristics of the HOPG device shows a reduction in the current upon chemical functionalisation of the HOPG (Fig. 3).

References:

[1] A.-M. Chiorcea, A.M. Oliveira Brett .Bioelectrochemistry, vol.63, 2004. p229–232.
[2] C.-C. Chiou et al. Clinica Chimica Acta, 334, 2003, pp87–94

Figures

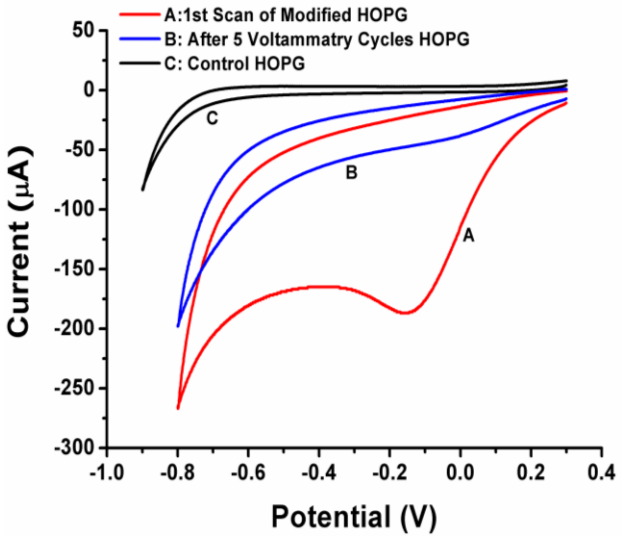
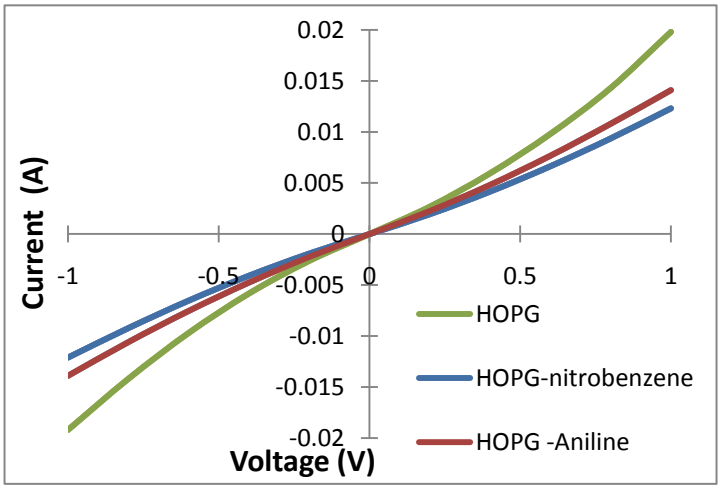


Fig. 1: *Cyclic voltammograms of a (A) during functionalisation of the HOPG electrode with the diazonium salt; (B) after 5 voltammatry cycles, the functionalisation is complete and the HOPG electrode is saturated (C) control HOPG electrode in non-aqueous electrolyte;.*



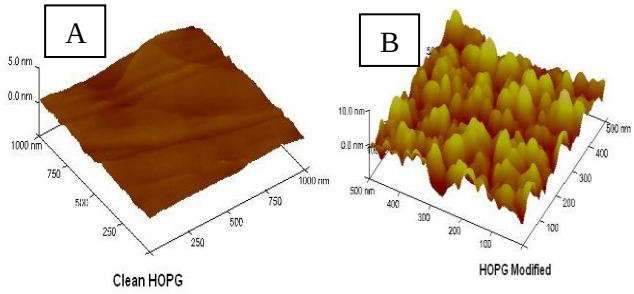


Fig. 2: AFM images of (A) control HOPG surface and (B) amine modified HOPG surface.

Fig. 3: I–V curves for blank HOPG and chemically modified HOPG substrates.

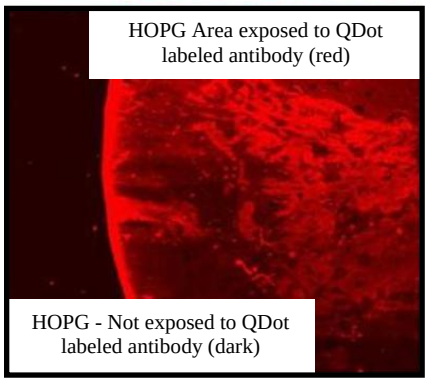


Fig. 4: Confocal microscopy images of aniline-modified HOPG, exposed to QDot labeled antibody in selected areas (red). Areas not exposed to the QDot antibody appear dark.