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Sonochemically fabricated microelectrode arrays for biosensors – Part II. Modification with a Polysiloxane Coating - a short communication.

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Abstract

A polymer modified sonochemically fabricated glucose oxidase microelectrode array with microelectrode population densities of up to 2.5 x 10^5 microelectrodes cm^{-2} is reported. These microelectrode sensors were formed by first depositing an insulating film on commercial screen printed electrodes which was subsequently sonicated to form cavities of regular sizes in the film. Electropolymerisation of aniline at the microelectrode cavities formed polyaniline protrusions containing entrapped glucose oxidase. Chemical deposition of polysiloxane from dichlorodimethysilane was used to deposit a thin protective and diffusion mass transport controlling coating over the electrodes. The physical and electrochemical properties of these films were studied. The performance of the final glucose oxidase based microelectrode sensor array is reported.

Keywords. Sensors, Microelectrodes, Polyaniline, Polysiloxane, Glucose

1. Introduction

Biosensor technology has developed into an ever expanding and multidisciplinary field since the Clark enzyme electrode was first reported (Clark and Lyons 1962). Biosensors generically offer simplified reagentless analyses for a range of biomedical and industrial applications and for this reason this area has continued to develop into an ever expanding and multidisciplinary field during the last couple of decades. Since more than half of the sensors reported in the literature are based on electrochemical transducers (Meadows 1996) and at the time of writing approximately 85% of the world commercial market for biosensors is for blood glucose monitoring (Newman et al 2002).

We have already within our laboratory demonstrated the fabrication of enzymatic and affinity based sensors that lend themselves to interrogation by either (i) amperometric or (ii) impedimetric approaches. Enzymatic sensors have been developed for analytes including ethanol, oxalate and a series of pesticides. Affinity sensors have been developed using antibody/antigen and DNA hybridisation based approaches. These will all be reported in a series of further publications.

A number of generic problems with biosensor technology however remain to be overcome which if solved would allow greater practical expansion of the field. One such problem involves removing the fluctuations in sensor responses that are sometimes experienced with variations in convection caused by variable flow or stirring/agitation within an analyte sample. It has been understood for several decades that microelectrodes experience unique hemispherical diffusional profiles, and can impart stir-independent characteristics to electrochemical sensors employing microelectrodes as working electrodes as well as offering faster response times. Microelectrodes can moreover, often be used in high resistance media due to the low operational currents typically encountered.

Individual microelectrodes offer very small responses and one approach for overcoming this problem is to use many microelectrodes together in the form of an array to allow a cumulative and so larger response to be measured.

Microelectrode arrays may be fabricated by a number of approaches although techniques such as photolithography or laser ablation have to date proved cost prohibitive for the mass production of disposable sensor strips. We have previously described a novel sonochemical fabrication approach (Barton *et al* 2004) for the production of microelectrodes, that lends itself to the mass production of sensor arrays. These microelectrode arrays were used to entrap glucose oxidase and then used in the detection of glucose.

Many other workers have studied the immobilisation of enzymes (e.g. the oxidases and dehydrogenases) within conducting polymers such as polyaniline or polypyrrole for use within sensors (Foulds and Lowe 1986, Shaolin et al 1992). In our previous paper (Barton *et al* 2004) we described a technique that allows the co-deposition of glucose oxidase within the conducting polymer, polyaniline, at conducting microelectrode cavities to form “mushroom” shaped microelectrode protrusions.

One problem with this and many other sensor systems is a loss of signal linearity at high glucose concentrations (>10 mM). We describe in this paper the modification of a sonochemically fabricated microelectrode enzyme array via the deposition of a polysiloxane coating and the improvement of signal linearity.

2. Materials and Methods

Glucose oxidase from *Asperigillus niger* (80% protein, 132,000 units/g solid) was purchased from the Sigma Chemical Company (Poole, Dorset, UK). D-glucose, aniline, dichlorodimethylsiloxane, disodium hydrogen orthophosphate 12-hydrate, sodium dihydrogen orthophosphate 12-hydrate, sodium chloride and diaminobenzene dihydrochloride (all 'AnalaR' grade), were purchased from BDH (Poole, Dorset, UK). All chemicals were used without further purification.

The electrode substrates, Fig. 1(a), were purchased within the UK from Sycopel Scientific and were produced within the Czech Republic by Krecji Engineering Inc. The ceramic based substrate electrodes used within this study are designed so as to act as generic disposable templates that may be easily adapted for a range of differing applications. The electrodes possessed screen printed gold working and counter electrodes with an Ag/AgCl reference electrode.

2.1 Thin Film Deposition.

The deposition of microarrays of polyaniline in sonochemically ablated polydiaminobenzene films has been described in detail elsewhere (Barton *et al* 2004) and will only be described here briefly. Polydiaminobenzene was deposited from aqueous solution onto the electrodes and sonicated for 20 seconds. Polyaniline was grown electrochemically from a solution containing GOD, which led to the enzyme being entrapped in the polymer film.

Dimethyldichlorosilane, 0.5 ml, was placed in a watch glass at the bottom of a sealed reaction vessel kept at atmospheric humidity. A stage was designed to allow for the deposition of the polysiloxane directly at the working electrode surface, Fig. 1(b), thereby ensuring that the counter and reference electrodes, and all the electrode contacts were not exposed to the silane vapour. The electrode was held in the stage, exposing one side only, and was suspended 5 cm above the dichlorodimethylsiloxane liquid for pre-determined periods. The stage was then removed from the vessel and the electrode stored in a petri dish.

2.2 Electrochemical Characterisation.

All electrodes were connected to a potentiostat for electrochemical studies. A Sycopel PCI-100 MK3 Potentiostat computer interface was used in conjunction with a 'Ministat Potentiostat', H.B. Thompson and Associates, (Newcastle-upon-Tyne) for all electrochemical studies: current/charge transients were recorded using either an IBM compatible PC, or an Omniscribe X-T chart recorder, Houston Instruments, Belgium. AC impedance measurements were performed using a ACM Auto AC DSP frequency response analyser and potentiostat linked to an IBM compatible PC.

A Philips XL30 FEG SEM was used for all scanning electron microscopy.

3. Results and Discussion.

Sonochemical ablation of insulating films, followed by electropolymerisation of aniline gives a structure in which the insulating film serves as a support for an array of polyaniline microelectrodes (Barton *et al* 2004).

Previous work within this group has been focussed towards the development and exploitation of ultra-thin film composite membrane technology for the construction of microelectrodes. A technique for depositing polymethylsiloxane from dichlorodimethylsiloxane has been previously shown by us to give thin films which offer low diffusional resistance to glucose but which minimise surface biofouling (Myler *et al* 2002), offer protection against electrode passivation effects and allow the linearisation of sensor responses. Permittivity coefficients were measured for these films (Myler *et al* 2002) and showed a much higher permeability for oxygen than for glucose, this is important because oxygen is a co-factor in the glucose-glucose oxidase reaction and we do not wish it to become depleted at the electrode surface and cause the sensor to reach saturation.

We believed, that the performance of the disposable enzyme electrode could be developed further via the inclusion of a covering ultra-thin polymer film coating, and this forms the base of this paper. In this way it is possible that the response of the sensor may be linearised in order to extend the useful working range in which the sensor may operate whilst also imparting some enhanced surface biocompatibility. Polysiloxane was chosen as the most suitable ultra-thin polymer film coating since the condensation polymerisation method allows formation of a homogeneous thin-film across the entire exposed surface, without the need for a conductive host surface as previously demonstrated (Myler *et al* 2002).

An SEM image of the working electrode, Fig. 2(a), reveals a granulated surface structure at this magnification, characteristic of the deposition of the conducting gold particles within the screen printed inks used (Reimer 1988). Polydiaminobenzene was first electrochemically deposited onto the working electrode surface, Fig. 2(b), and then sonicated for 20s in a similar manner to

the procedure developed and described earlier (Barton et al 2004). SEM images of the polymer film following sonication, Fig. 2(c), again show evidence of the polymer cavitation and thus microelectrode formation.

Electrodes of this type were evaluated for stir dependant / independent characteristics for the reduction of $Fe(CN)_6^{3-}$ in a similar manner to earlier investigations (Barton *et al* 2004), by comparing the amperometric response under agitated (stirred) or quiet (non-stirred) solutions. These investigations again confirmed that under these conditions, the arrays formed upon the commercial substrates exhibited true stir independent and were therefore concordant with true microelectrode behaviour. This factor is important for sensors that would be used in 'real' situations such as *in vivo* where variations in blood flow might be anticipated.

Glucose oxidase / polyaniline microelectrodes were then electrochemically deposited via a cyclic voltammetric approach (Barton *et al* 2004) at the cavities resulting from the sonochemical ablation of the insulating polydiaminobenzene film. Voltammogram corresponding to this deposition polymerisation were obtainied similar to those in our previous paper (Barton *et al* 2004) showing steady deposition of polyaniline/glucose oxidase at the cavities in the polydiaminobenzene film.

An SEM of the working electrode, Fig. 3(a), clearly shows one of the resulting polymer / enzyme protrusions. SEM images of the siloxane coated enzyme microelectrodes are shown in Fig. 3(b).

A film can be seen to cover the entire electrode surface and previous work by this group has shown that polysiloxane films give enhanced biocompatibility (Myler *et al* 2002).

In order to assess the performance of the ultra-thin film as a coating for the linearisation of sensor responses, the ac response to glucose of an enzyme microelectrode array / ultra-thin polymer film was compared to that of a similar electrode lacking an ultra-thin polymer film coating.

AC impedimetric responses were determined over a range of frequencies from 0.1 Hz-10 kHz. Again the responses were verified to be free of stirring (enforced convectional) effects. The Bode and Nyquist plots were almost identical to those in our previous paper (Barton *et al* 2004) and therefore are not shown for brevity. Earlier work showed that the greatest variations in impedance occurred at 0.1 Hz (Barton *et al* 2004) and similar results were obtained for the siloxane coated membranes. When the differences in impedance at frequency of 0.1 Hz are plotted for microelectrodes exposed to varying glucose concentrations (compared to samples in pH 7.4 buffer containing no glucose), a calibration plot can be obtained, Fig. 3(c).

The instrumentation required for commercial exploitation of biosensors may be greatly simplified if the sensor gives a linear response with respect to analyte concentration. The resulting calibration graph, Fig. 3(c) shows that the siloxane coating lowers the magnitude of sensor response compared to uncoated microelectrodes whilst also linearising the sensor responses between 0-60 mM glucose ($r^2 = 0.671$ [uncoated], $r^2 = 0.995$ [silane coated]). We have determined both here and in previous work (Barton *et* al 2004) the reproducibility of sensor responses to be <5% variability in a series of trials.

4. Conclusions

This paper has successfully demonstrated how two disparate biosensor technologies may be combined to form a novel enzyme-based electrochemical sensor.

Initially we described the examination of a commercially available ceramic based disposable electrode as a suitable host for the fabrication of a microelectrode array. Upon coating the working electrode with the insulating polymer polydiaminobenzene, microelectrode arrays were fabricated via a sonochemical fabrication; these arrays again displayed true stir independent responses.

As the electrode substrate had displayed suitability for microelectrode fabrication, enzyme / polymer protrusions were then again electrochemically deposited (Barton *et al* 2004) at the host microelectrode templates so as to produce an enzyme (glucose oxidase) ultra-microelectrode array.

Enzyme microelectrode arrays were then coated with an ultra-thin film coating of polysiloxane (silicone) so as to provide a covering substrate diffusion limiting layer for the linearisation of sensor responses. This coating has been shown to extend the useful analytical concentration range for the sensor via linearisation of its response.

5. Acknowledgements

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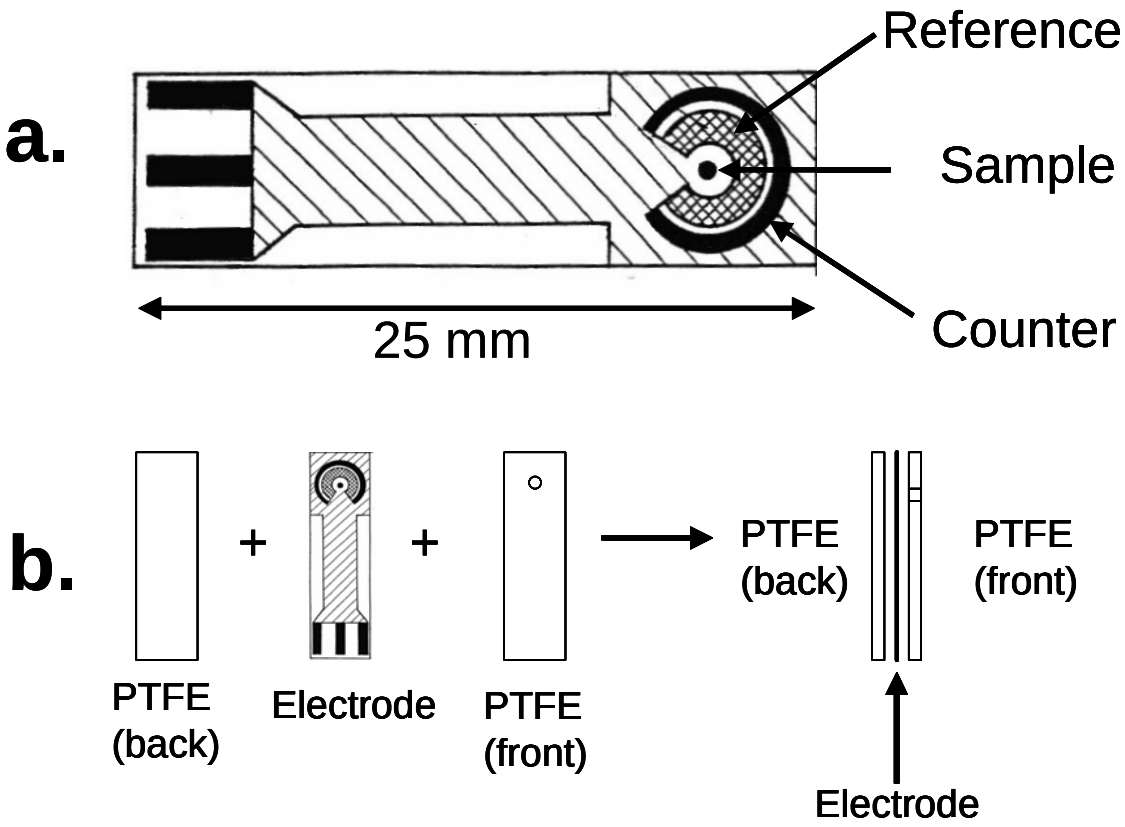
List of Figures

Fig. 1.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Sample
Counter
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b.
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PTFE
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PTFE
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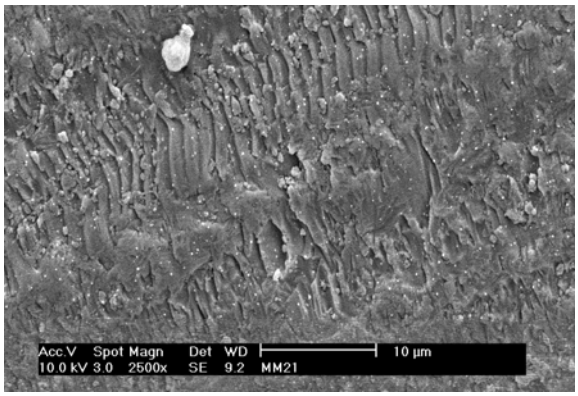
Electrode

Fig. 2.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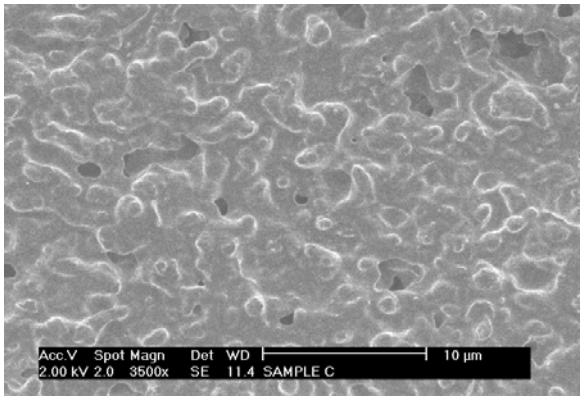
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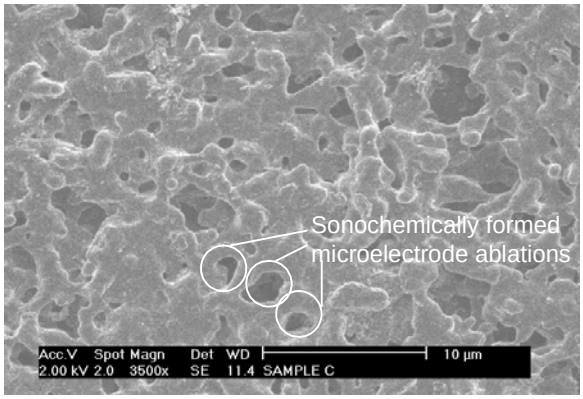
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microelectrode ablations
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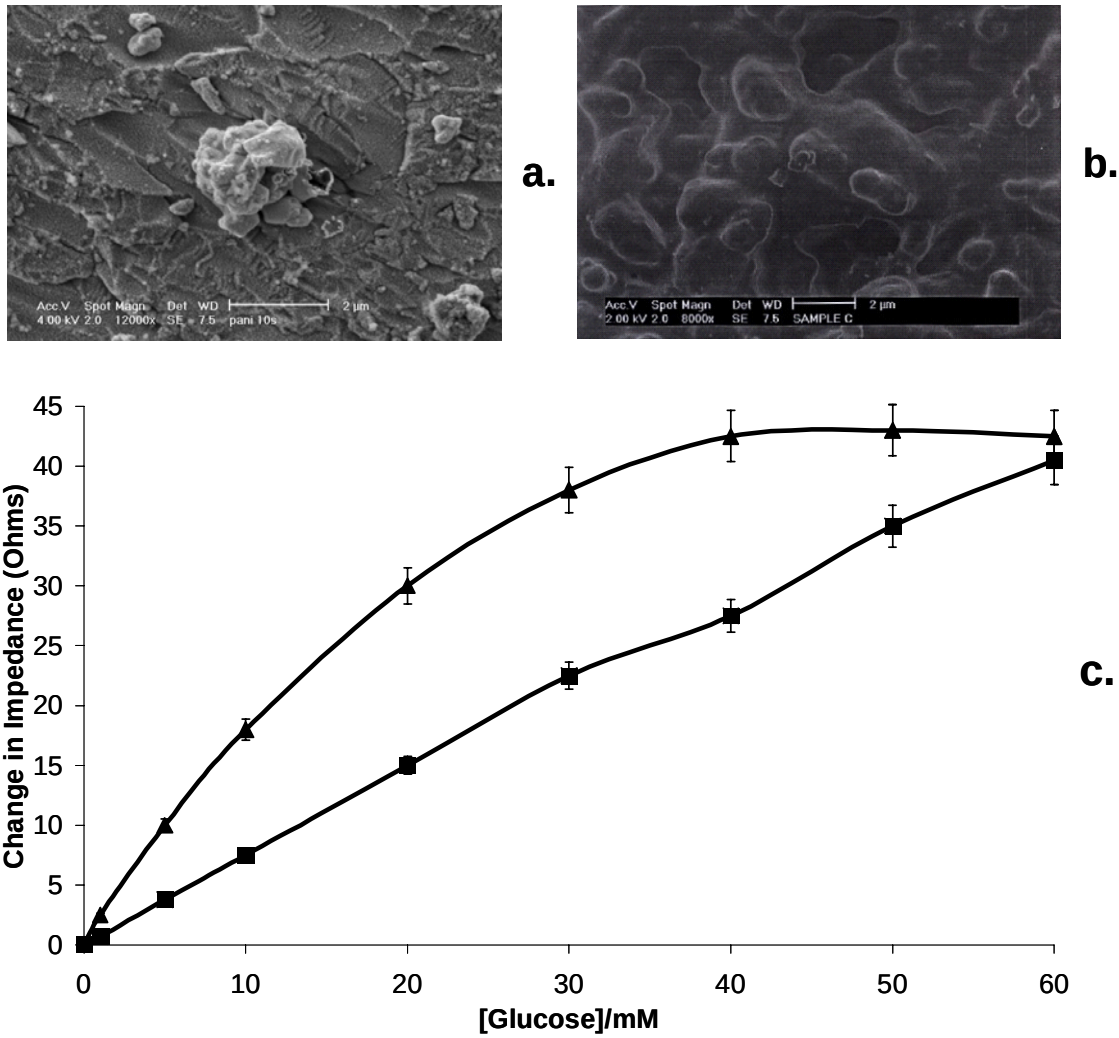
c.

Fig. 3


a.
b.
c.
Change in Impedance (Ohms)
[Glucose]/mM
0
5
10
15
20
25
30
35
40
45
0
10
20
30
40
50
60