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Microfabricated glucose biosensor for culture well operation ☆

R.M. Pemberton [a], T. Cox [b,1], R. Tuffin [b], I. Sage [b], G.A. Drago [c], N. Biddle [c], J. Griffiths [d], R. Pittson [e], G. Johnson [d], J. Xu [a], S.K. Jackson [a,2], G. Kenna [f], R. Luxton [g], J.P. Hart [a,*]

[a] Centre for Research in Biosciences, Faculty of Health and Life Sciences, University of the West of England, Bristol, UK
[b] QinetiQ Ltd., Malvern Technology Centre, Malvern, UK
[c] Applied Enzyme Technology Ltd., Monmouth House, Mamhilad Park, Pontypool, NP4 OHZ, UK
[d] Uniscan Instruments Ltd., Sigma House, Burlow Rd., Buxton, Derbyshire, SK17 9JB, UK
[e] Gwent Electronic Materials Ltd., Monmouth House, Mamhilad Park, Pontypool, NP4 OHZ, UK
[f] AstraZeneca R&D, Alderley Park, Macclesfield, UK
[g] Institute of Biosensing Technology, University of the West of England, Bristol, UK

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ABSTRACT

A water-based carbon screen-printing ink formulation, containing the redox mediator cobalt phthalocyanine (CoPC) and the enzyme glucose oxidase (GOx), was investigated for its suitability to fabricate glucose microbiosensors in a 96-well microplate format: (1) the biosensor ink was dip-coated onto a platinum (Pt) wire electrode, leading to satisfactory amperometric performance; (2) the ink was deposited onto the surface of a series of Pt microelectrodes (10–500 μm diameter) fabricated on a silicon substrate using MEMS (microelectromechanical systems) microfabrication techniques: capillary deposition proved to be successful; a Pt microdisc electrode of ≥ 100 μm was required for optimum biosensor performance; (3) MEMS processing was used to fabricate suitably sized metal (Pt) tracks and pads onto a silicon 96 well format base chip, and the glucose biosensor ink was screen-printed onto these pads to create glucose microbiosensors. When formed into microwells, using a 340 μl volume of buffer, the microbiosensors produced steady-state amperometric responses which showed linearity up to 5 mM glucose (CV=6% for $n=5$ biosensors). When coated, using an optimised protocol, with collagen in order to aid cell adhesion, the biosensors continued to show satisfactory performance in culture medium (linear range to 2 mM, dynamic range to 7 mM, CV=5.7% for $n=4$ biosensors). Finally, the operation of these collagen-coated microbiosensors, in 5-well 96-well format microwells, was tested using a 5-channel multipotentiostat. A relationship between amperometric response due to glucose, and cell number in the microwells, was observed. These results indicate that microphotolithography and screen-printing techniques can be combined successfully to produce microbiosensors capable of monitoring glucose metabolism in 96 well format cell cultures. The potential application areas for these microbiosensors are discussed.

1. Introduction

The development of miniaturised enzyme biosensors is desirable for the analysis of small sample volumes, for example in multiple array form, and for lab-on-chip applications in various formats. Several technological approaches have been used in attempts to integrate electrochemical glucose biosensors into small easy-to-use assay formats: Wang et al. (2007) have describeda miniaturised capillary electrophoresis microchip device for blood analysis which includes a 300 μm-wide screen-printed gold-coated carbon glucose biosensor suitable for use in the microfluidic system. Two recent publications from groups working on paper-based microfluidic electrochemical systems have described screen-printable biosensor devices capable of quantifying glucose in serum (Dungchai et al., 2009) and in aqueous solution (Nie et al., 2010) for low-cost batch-mode analysis at low cost. More recently, Li et al. (2010) described a lab-on-a-tube approach which used electrochemically-deposited Pt nanoparticles modified with Prussian Blue to form small electrochemical glucose biosensors which operated in chronoamperometric mode for serum samples.

Besides the advantage of small sample volume requirement seen with these flowing-stream, or single time-point devices,

Abbreviations: SPCE, Screen-printed carbon electrode; CoPC, Cobalt phthalocyanine; GOx, Glucose oxidase; Pt, Platinum; MEMS, Microelectromechanical systems

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* Corresponding author. Tel.: +44 117 3282469; fax: +44 1173282904.
E-mail address: John.Hart@uwe.ac.uk (J.P. Hart).
[1] Current address: Institute of Biosensing Technology, University of the West of England, Bristol, UK.
[2] Current address: Centre for Research in Translational Biomedicine, School of Biomedical and Biological Sciences, University of Plymouth, PL4 8AA, UK.

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there is a demand in cell culture assay applications (for instance, drug toxicity testing) for biosensors that can monitor metabolite levels continuously, even in unstirred solutions. These microbiosensors need to be of sufficiently small dimension that they exhibit radial diffusional behaviour and therefore produce a sustained steady-state current responses in the presence of a given analyte concentration; also they do not perturb the system by consuming the glucose in the culture medium. Our previous work on developing screen-printable water-based carbon inks containing both redox mediator (cobalt phthalocyanine; CoPC) and enzyme (glucose oxidase; GOx) showed that glucose biosensors could be screen-printed as thick-film macroelectrodes (Crouch et al., 2005a) and then cut transversely to expose a microband electrode surface (Pemberton et al., 2009a) which exhibited steady-state current behaviour. When suspended in 3 ml culture wells, these microband biosensors could be used to make continuous amperometric measurements in tissue culture medium (Pemberton et al., 2009b) and could monitor changes in the uptake of glucose by mammalian cells in the presence of selected toxins (Pemberton et al., 2011). This system was suitable for small-scale laboratory investigations but impractical for rapid, multiple well analyses. Therefore, with the aim of progressing towards a more integrated biosensor assay system, the work described in this paper addresses the challenge of fabricating glucose microbiosensors that exhibit stir-independent microelectrode behaviour and are compatible with 96-well plate single- or multi-well usage, to monitor metabolic events continuously over 24 h in cell culture.

The approach taken here was to use MEMS technology to create a microfabricated substrate with exposed Pt microelectrode areas and then to functionalise these areas to convert them into working (GOx enzyme+mediator), reference (Ag/AgCl) and counter (bare Pt) electrodes. The working electrode modification was based on screen-printing the previously demonstrated water-based carbon ink containing GOx and CoPC mediator. Since this would be screen-printed onto Pt, some preliminary tests for Pt/ink compatibility were carried out using a dip-coated Pt wire electrode, and Pt microdisks of various diameters, onto which the ink was deposited. The challenge of aligning the screen-printed inks onto microfabricated substrates and their formation into microwells was then addressed. The effect of applying a collagen surface coating to the sensors was investigated by testing the amperometric response of the sensors to glucose. Finally, the operation of these microbiosensors, in 96-well plate microwells, was performed amperometrically in the presence or absence of mammalian cells growing in glucose-containing culture medium.

2. Materials and methods

2.1. Reagents

All reagents were AnalaR Grade. Phosphate buffer supporting electrolyte was prepared in deionised water (Purite R200 system, Purite Ltd., UK) by mixing solutions of NaH_2PO_4 and Na_2HPO_4 to obtain the desired pH then diluting to a concentration of 0.05 M. Type I collagen, from rat tail tendon, was purchased from Sigma-Aldrich (Cat no. C7661). Dulbecco's Modified Eagles Medium (DMEM) containing high (25 mM) glucose (4.5 g L^{-1}) or low (5 mM) glucose (1.0 g L^{-1}), and Hepatocyte medium (glucose-free) were purchased from Sigma-Aldrich, as were foetal bovine serum (FBS), penicillin and streptomycin sulphate solution (pen+strep), and non-essential amino acids. L-glutamine was obtained from GibcoBRL. Standard solutions of β-D-glucose were prepared by mutorotation overnight, and filter sterilised as described previously (Pemberton et al., 2011).

2.2. Biosensor fabrication

The substrate material for preparing microfabricated structures was 0.5 mm-thick silicon, in the form of 11.5×11.5 mm^2 squares (for fabricating microdisks) or 16.5×71.0 mm^2 rectangles (for 5-well microelectrode strips). A process of chemical etching was used to fabricate tracks and pads of Pt onto these chips; a 10 μm-thick dielectric layer of SU8 polymer was then added as an insulating layer, leaving exposed microelectrode regions of Pt pads, suitable for use as counter electrodes, or as base pads for subsequent deposition of active layers to form working or reference electrodes.

Working electrodes were formed from a water-based carbon ink formulation (GEM product code C2041124D3) (Crouch et al., 2005b) which contained cobalt phthalocyanine as redox mediator, plus the enzyme GOx. This ink was dip-coated, drop-coated or screen-printed, as described below. Ag/AgCl reference electrode strips (macroelectrodes) were formed by screen-printing onto PVC substrate (see below). Ag/AgCl microelectrodes were formed by electroplating silver directly onto the Pt pads. The Ag surface was then chloridised by anodisation in constant current mode (20 mA/cm^2) for 30 s in 1 M HCl. Finally the electrodes were rinsed in deionised water.

2.3. Instrumentation

Cyclic voltammetry and amperometry at single working (Pt- or biosensor-) electrodes were conducted using a μAutolab Type II Potentiostat (Windsor Scientific UK) in 3-electrode system mode. Multiple 96-well-based glucose microbiosensors were tested individually or simultaneously in amperometric mode: for individual testing, contact between the three electrode tracks and a μAutolab potentiostat were made using gold alloy probes held in manual probe arms (Wentworth Labs Inc., Brookfield, CT, USA), whereas multiple simultaneous parallel measurements were made using a 5-channel PG580RM potentiostat (Uniscan Instruments Ltd., Buxton, UK) contacted via a single gold edge (zebra) connector to all five biosensors and their respective counter and reference electrodes; this was controlled using UiEChem software (Version 2.02) via a custom-made connector box (Uniscan Instruments Ltd.). In order to maintain well temperature at 37 °C, the strips were placed in a non-humidified incubator at 37 °C (culture medium experiments).

2.4. Culture well construction

Once the biosensors had been fabricated on the 5-sensor chip bases, wells were formed by attaching strips of 96-well bottomless 5-well strips (Greiner Bio-one) using 0.5 mm-thick press-to-seal 6.5-mm diameter hole silicone adhesive strips (Grace Bio-Labs, OR, USA).

2.5. Collagen coating of biosensor electrodes

Collagen was added to 0.02 N acetic acid, pH 2.65, to a concentration of 50, 100, 1000 or 2000 μg/ml and left on ice for 1 h to dissolve. Ten microlitre aliquots were then deposited onto the surface of 3×3 mm^2 glucose biosensor screen-printed macroelectrodes and their corresponding Ag/AgCl reference electrodes (Crouch et al., 2005b), or over the base of microwells containing screen-printed microbiosensors, Ag/AgCl reference and Pt counter electrodes. When these coatings were evaporated to dryness at room temperature (RT), and rinsed with phosphate buffer, SEM examination (not shown) revealed that detail of the underlying carbon electrode surface was visible at concentrations below