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A screen-printed microband glucose biosensor system for real-time monitoring of toxicity in cell culture

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ABSTRACT

Microband biosensors, screen-printed from a water-based carbon ink containing cobalt phthalocyanine redox mediator and glucose oxidase (GOD) enzyme, were used to monitor glucose levels continuously in buffer and culture medium. Five biosensors were operated amperometrically (E_{app} of +0.4 V), in a 12-well tissue culture plate system at 37 °C, using a multipotentiostat. After 24 h, a linear calibration plot was obtained from steady-state current responses for glucose concentrations up to 10 mM (dynamic range 30 mM). Within the linear region, a correlation coefficient (R^2) of 0.981 was obtained between biosensor and spectrophotometric assays. Over 24 h, an estimated 0.15% (89 nmol) of the starting glucose concentration (24 mM) was consumed by the microbiosensor. The sensitivity of the biosensor response in full culture medium was stable between pHs 7.3 and 8.4.

Amperometric responses for HepG2 monolayer cultures decreased with time in inverse proportionality to cell number (for 0 to 10^6 cell/ml), as glucose was being metabolised. HepG2 3D cultures (spheroids) were also shown to metabolise glucose, at a rate which was independent of spheroid age (between 6 and 15 days). Spheroids were used to assay the effect of a typical hepatotoxin, paracetamol. At 1 mM paracetamol, glucose uptake was inhibited by 95% after 6 h in culture; at 500 μM, around 15% inhibition was observed after 16 h. This microband biosensor culture system could form the basis for an *in vitro* toxicity testing system.

1. Introduction

In order to maximise process control and yield in industrial bioreactor and mammalian cell culture systems (Chu and Robinson, 2001), and in the development of technologies for *in vitro* toxicity testing (Baudoin et al., 2007), there is a great advantage in being able to monitor cell numbers (Guez et al., 2004), nutrient and metabolite levels in real time and with minimum intervention. Due to its important role in energy metabolism, one of the most commonly measured analytes is glucose.

In a collaborative project between the University of the West of England and Gwent Electronic Materials Ltd (GEM), a water-based carbon ink formulation (GEM product code: C2030901R2) was recently developed which incorporated cobalt phthalocyanine (CoPC) as an electrocatalyst together with the enzyme glucose oxidase (GOD) (Crouch et al., 2005a,b). The ink has been used to create sensors/biosensors having microelectrode dimensions and displaying microelectrode diffusional behaviour; these biosensor electrodes produced steady-state current responses at 25 °C under quiescent conditions and were applied to determine glucose in serum (Pemberton et al., 2009a) and at 37 °C to measure glucose metabolism by cells at the end of a period in culture (Pemberton et al., 2009b). This type of operation is valuable for batch-sampling, but requires off-line measurement and the associated likelihood of disturbing or stopping the culture.

The aim of the work described in the present paper was to investigate the ability of the same microband biosensors to monitor glucose levels continuously in cell culture. Observations in glucose-fortified buffer were followed by experiments in culture medium at 37 °C. Changes in glucose concentration were investigated using the human hepatocyte cell line HepG2. The cell number-dependence of changes in amperometric current was investigated over 24 h using single cells. The ability to monitor glucose metabolism was then investigated using three-dimensional agglomerates of cells or so-called "spheroids" which closely resemble "mini-livers" in their metabolic behaviour (Xu et al., 2003). Finally, the effect of the

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hepatotoxic compound paracetamol, on glucose uptake by HepG2 spheroids, was investigated.

2. Materials and methods

2.1. Chemicals and reagents

Foetal bovine serum (FBS), hepatocyte medium (Modified L-15), Dulbecco's Modified Eagle's Medium (DMEM) containing 4.5 g L^{-1} (25 mM) glucose, penicillin and streptomycin sulphate (pen + strep) and all other chemicals were of analytical reagent grade, purchased from Sigma–Aldrich. L-glutamine was obtained from GibcoBRL. 0.05 M phosphate buffer was prepared from 0.5 M stock solutions of sodium dihydrogen orthophosphate and disodium hydrogen orthophosphate, mixed to obtain the desired pH, then diluted with deionised water (dH_2O; Purite R200 system, Purite Ltd., Oxon, UK). In all buffer experiments, solutions were fortified with 50 mM NaCl. A 500 mM stock solution of β-D-glucose was prepared in water, allowed to mutorotate at room temperature overnight, then used to prepare standard solutions. The stock solution was sterilised by filtration (4.5 μm) where appropriate.

2.2. HepG2 cell culture

The HepG2 (Human Caucasian Hepatocyte Carcinoma) cell line (obtained from ECACC) was cultured as a monolayer in 75 cm^2 flasks, in a 5% CO_2-in-air atmosphere at 37 °C, at an initial density of 10^5 cells cm^{-2}. Cells were counted using a haemocytometer. The medium was DMEM containing 10% FBS, 1% non-essential amino acids, 200 nM L-glutamine, plus 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin (pen + strep). When confluent, cells were detached by trypsinisation, centrifuged at 150 × *g* and resuspended in DMEM containing glucose, with added 5% FBS, L-glutamine, and pen + strep. The final glucose concentration was 24 mM.

For monolayer assays, cells were plated at a density of 10^6 cells/ml, or otherwise, as stated, in 3 ml volumes into 12-well tissue culture well plates.

For spheroid formation, HepG2 cells were plated at a density of 10^6 ml^{-1} into 6-well plates, 3 ml per well, and shaken on a gyrorotatory shaker (Xu et al., 2003). Spheroids were ready for use after 6 or more days in culture, when they were transferred into 12-well plates, with fresh DMEM/5% FBS containing 24 mM glucose. Wells containing spheroids were then used for experimentation.

2.3. Biosensor fabrication

Microband biosensor working electrodes were fabricated as described previously (Pemberton et al., 2009a) from a water-based carbon ink formulation containing carbon, binder, CoPC and GOD (entire formulation is GEM code: C2041124D3)(Crouch et al., 2005b). Briefly, 3 mm × 3 mm working electrodes (thickness 20 μm) were screen-printed onto PVC, covered with insulating tape, then sectioned using a razor blade to expose a 3 mm × 20 μm microband edge. Reference electrodes were screen-printed separately onto 0.5-mm thick heat-shrunk PVC using a Ag/AgCl ink (GEM code: C61003P7) and used as 2-mm wide strips.

2.4. Apparatus

Experiments were performed using disposable 12-well tissue culture plates (Greiner bio-one). Microband biosensors, reference and Pt wire counter electrodes were held in home-made polypropylene 3-electrode sensor-heads which push-fitted into 12-well plate wells. These were connected to a PG580RM 5-channel potentiostat (Uniscan Instruments Ltd, Buxton, UK), controlled using UiEChem software (Version 2.02). To maintain well temperature at 37 °C, tissue culture plates were placed into a shallow water bath (Haake P5, Saddle Brook, USA) at 37 °C. Solution pHs were monitored using a pH meter (Fisherbrand Hydrus 400) and glass pH electrode (Russel, UK). For cell culture experiments, microband biosensors and Ag/AgCl reference electrodes were sterilised using gamma radiation (see Section 3.2.1). Sensor heads and Pt counter electrodes were sterilised with a 70% ethanol spray. The assembly of electrodes, sensor heads, media/cells and tissue culture plates was conducted in a sterile flow cabinet.

2.5. Extended real-time monitoring of glucose in buffer and in culture medium in the presence/absence of cells

Volumes (3 ml) of 0.05 M phosphate buffer, pH 7.5 (containing 50 mM NaCl or hepatocyte medium plus known glucose concentrations over the range 0–5 mM or 0–33 mM), or DMEM (containing 5% FBS, pen + strep, and 24 mM glucose, either alone, or in the presence of HepG2 single cells, or Day 6 spheroids) were pipetted into single wells of a 12-well tissue culture plate. A paracetamol (4-acetamidophenol) 100 mM stock solution was prepared in 20% ethanol, filter-sterilised and diluted in culture medium to obtain the required final concentrations. Sensor heads, carrying the electrodes, were push-fitted into each well. A small (1 mm diameter) hole in the top surface of each sensor head allowed air to escape and avoided pressure build-up during the push-fitting step. Each hole was plugged with a sterile filter-tip to allow exchange of air.

The electrode leads were connected to respective channels of the multichannel potentiostat. The plate was placed in a water-bath at 37 °C and covered with bubble-wrap in order to maintain the correct temperature. An operating potential of +0.4 V was applied to each working electrode, and the instrument was run in amperometric mode for the ensuing 17–24 h. Data were exported into Ms Excel and plotted as current-versus-time graphs.

At the end of the experiments, buffer or culture medium was harvested, stored at −20 °C, and subsequently assayed for glucose content using the spectrophotometric assay.

2.6. Spectrophotometric glucose assay

The glucose concentration in buffer and culture medium samples was determined using a method based on that of Trinder, as described elsewhere (Crouch et al., 2005b).

3. Results and discussion

An applied potential (E_{app}) of +0.4 V versus Ag/AgCl was selected for all experiments, based on voltammetric and amperometric experiments reported previously (Pemberton et al., 2009a). This represents the potential at which the CoPC-mediated electrocatalytic oxidation of H_2O_2, produced by the action of GOD on glucose (Gilmartin et al., 1995), occurs. The resulting anodic response is proportional to the bulk glucose concentration.

3.1. Continuous monitoring of glucose-containing phosphate buffer

Solutions of phosphate buffer fortified with glucose (0–33 mM) were interrogated simultaneously using the multipotentiostat/microband glucose biosensors over 24 h. Amperograms resulting from a single 5-well experiment are shown in Fig. 1. The time required to establish steady-state amperometric responses was around 2 h for 0, 5 and 10 mM glucose. A calibration plot of final current responses versus final glucose concentrations (determined

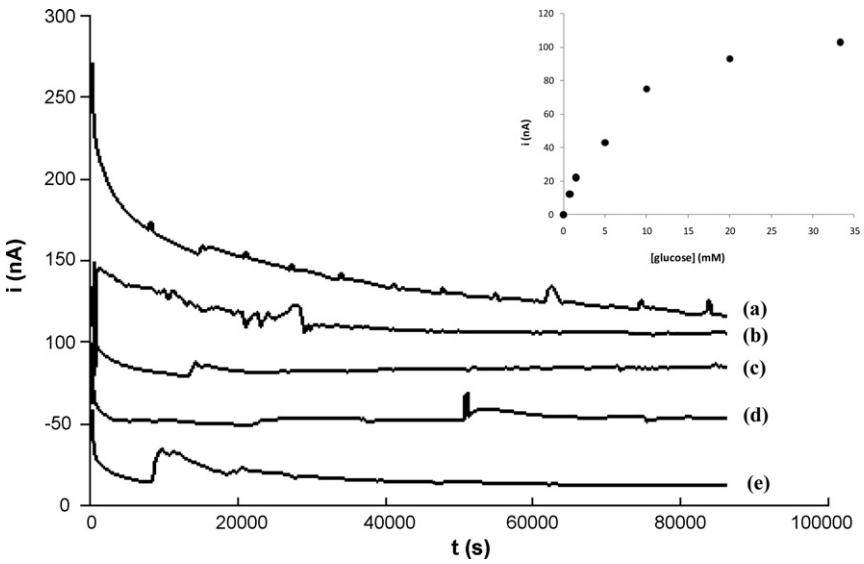

Fig. 1. Amperograms obtained using microband biosensors operated continuously for 24 h in phosphate buffer, pH 7.5/50 mM NaCl containing (a) 33 mM, (b) 20 mM, (c) 10.0 mM, (d) 5.0 mM and (e) 0 mM added glucose. E_{app} +0.4 V. Insert shows plot of 24 h current responses (baseline-subtracted) versus glucose concentration from two experiments.

spectrophotometrically) (Fig. 1, insert) was linear up to 10 mM, with an apparent k_m value estimated from a Lineweaver–Burke plot to be 12.5 mM. This value, and the shape of the plot, were similar to that obtained previously (Pemberton et al., 2009a) for short-term, batch-mode operation. This result showed biosensors were operationally stable over a 24 h period.

At higher glucose concentrations (20 and 33 mM), beyond the linear range, responses took longer to reach steady state (20 mM) or continued to decrease (33 mM). The ability of the biosensor to maintain a steady-state current is dependent on free diffusion of glucose to the electrode surface, the rate of glucose turnover by the GOD enzyme, and subsequent ability of the electrodes to oxidise the H_2O_2 product via the CoPC electrocatalyst. Linearity and continuous steady-state responses below the k_m value indicated that a radial-diffusion-dominated response was operating, whereas loss of steady-state with increasingly high glucose concentration above the k_m suggests a rather slow saturation of glucose binding sites and possibly some passivation of the electrode surface, possibly due to over-conversion of CoPC from its electrocatalytically active Co^{2+} to its oxidised Co^{3+} state. Any effect of pH can be ruled out since the original pH of the buffer (7.5) was preserved at the end of the experiment, even at 30 mM glucose.

Triplicate amperograms obtained in the absence of glucose generated low background values of 2.4, 4.5 and 11 nA, respectively (mean 6 nA). For calibration purposes it was important to run at least one blank well for each experiment and to subtract the blank current before plotting data values.

Spectrophotometric assay of supernatants revealed that glucose concentrations had not changed during the experiments. Based on an average current of 200 nA for 24 h, producing a total charge (Skoog et al., 1992) of 0.017C, an estimated 89 nmol of glucose were consumed by a microband biosensor; this is equivalent to <0.15% of the starting concentration.

The steady-state nature of the responses over the 0–10 mM concentration range indicated that a (blank-corrected) current response taken at any time-point over the 24 h period could be used directly to quantify glucose content. At concentrations above the k_m, however, the calibration plot was effectively changing with time, so experiments measuring glucose depletion would require internal control wells containing a constant glucose concentration, against which to compare any changes.

3.2. Continuous monitoring of glucose-containing culture medium

HepG2 cells are commonly cultured in DMEM containing high or low glucose, whereas for primary hepatocytes, a modification of L-15 medium (so-called "hepatocyte medium") is favoured (Xu et al., 2002). Since it does not contain any glucose, L-15 is suitable for fortification with added glucose to generate calibration standards and was chosen for initial study.

Initial experiments showed variations in the amperometric currents for replicate microbands/wells that were independent of any differences in pH (e.g. 160–240 nA at 15 min). The variation was attributed to differences in microband surface area/electrode thickness resulting from the fabrication procedure. By using a multiplier to normalise the data to a value of 234 nA at 15 min, extremely reproducible current responses and rates of decay were obtained for identical culture wells (see below).

3.2.1. Sterility considerations

In previous studies (Pemberton et al., 2009b), and buffer studies described above, no specific sterilisation procedures, besides inclusion of antibiotics, were adopted. However, after preliminary experiments, it became evident that in full culture medium rich in nutrients, additional sterility measures would be required to avoid contamination and to obtain reliable data over 24 h. Exposure to UV-light, or 70% ethanol solution proved inappropriate for survival of the ink and enzyme activity of the biosensors. However, gamma-irradiation was successful: biosensors and Ag/AgCl reference electrodes were packaged and exposed to 25–35 kGy of radiation in sealed trays. After exposure, glucose calibration studies revealed that ≥85% of the original sensitivity remained. The set-up of apparatus for experiments involving cell culture medium was conducted under sterile conditions (sterile tissue culture plates, medium and sterile-filtered glucose additive; 70% ethanol sterilisation of sensor heads).

3.2.2. Calibration in culture medium

Amperograms obtained in L-15 medium in the presence or absence of 5% FBS had similar profiles to those obtained in buffer and measurements of pH indicated an increase of no more than 0.2 U at the end of the experiment from the original value of 7.3.

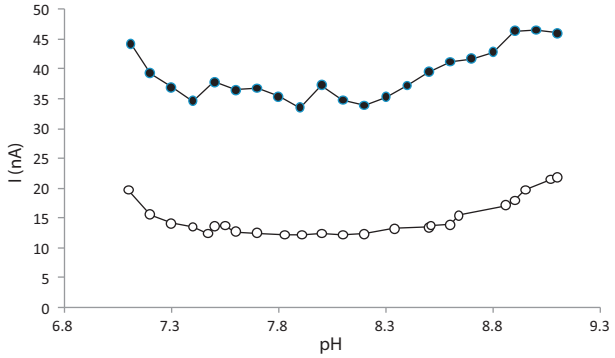

Fig. 2. Plot of current response versus pH for glucose microband biosensors in DMEM/FBS containing 24 mM glucose at 37 °C under stirred (●) and unstirred (○) conditions.

A calibration plot of 24 h current responses for biosensors incubated in the presence of L-15 medium containing added 0–24 mM glucose resembled closely that obtained in buffer. Sensitivities for the linear regions in buffer and culture medium were 7.84 nA mM^{-1} (R^2 = 0.957) and 8.43 nA mM^{-1} (R^2 = 0.993), respectively. A partial least squares statistical comparison (Minitab-15) of the L-15 medium and buffer data in the presence of 0, 1.5, 5, 10 and 20 mM glucose gave an R^2 value of 0.992, indicating good agreement. This result demonstrated that the ability of the microbiosensors to measure glucose was not impeded by the presence of culture medium or serum components.

3.3. Applications involving cells

3.3.1. Effect of pH

For experiments involving HepG2 cells, DMEM/5%FBS was the preferred medium. The studies reported here were conducted in air, since a humidified 5% CO_2 incubator posed problems of connector corrosion. In the absence of cells, an upward drift of medium pH from around 7.3–9.1 was observed after 24 h. It was important to determine the influence of such pH change on the response of microband glucose biosensors.

An initial background current was recorded in medium alone. Glucose was then added to give a concentration of 24 mM. Beginning at 7.1, medium pH was allowed to drift upwards, and current responses were monitored. The resulting responses, after background subtraction, were plotted versus pH (Fig. 2). Values were higher under stirred conditions, confirming our previous observations that the glucose microband biosensor response is not fully stir-independent.

Under both stirred and unstirred conditions (Fig. 2), currents remained constant between pHs 7.3 and 8.4, showing <5% change over this range. Above pH 8.4, an increase in response was observed, being similar under quiescent (1.5 nA/pH) and stirred (1.25 nA/pH) conditions. The apparent resistance to the effect of pH between 7.3 and 8.4 contrasts with the large increase in sensitivity observed previously in buffer (Pemberton et al., 2009a). This resistance may be attributable to a local buffering effect of serum proteins adsorbing onto the microband electrodes and controlling the local microenvironment pH. Further experiments in serum-free DMEM are planned in order to investigate this phenomenon.

3.3.2. Real-time monitoring of glucose metabolism in cell culture—single cells

In order to obtain information on the metabolic activity of cultured cells in real time, studies were initially performed using HepG2 single cells. Culture wells (24 mM glucose) were seeded at densities of 0, 0.125, 0.25, 0.5 and 1 × 10^6 cell/ml.

Without cells (Fig. 3), the 24 h current response was around 200 nA. The higher currents observed compared to those in buffer (Section 3.1) or L-15/FBS, may be attributed to electroactive component(s) in the DMEM or serum batch. The final solution pHs were 7.3, 7.5, 7.6, 8.6 and 9.1 for wells containing 1, 0.5, 0.25, 0.125 and 0 million cells/ml, respectively. The increase in pH from 7.3 to 9.1 would equate to an increase in current response of 33%, based on the pH data in Fig. 2. In fact, the response showed a 15% negative drift over the experimental period. A calculation of the number of moles of glucose consumed, based on the number of coulombs of charge passed during 24 h, revealed that <0.2 μm of glucose were consumed by the biosensor; this was confirmed by spectrophotometric determination which showed that no glucose depletion had occurred. Thus, the observed downward drift in current was due to gradual change in the double-layer at the electrode surface, to gradual depletion of Co^{2+} (see above), or to surface passivation.

In the presence of cells (Fig. 3), more rapid decreases in current were observed as cell numbers increased (see Supplementary Material, Fig. S1A). Only wells containing no cells or 0.125 million cells exhibited final pH values above 8.4 and could thus be subjected to minor correction factors of 0.745 and 0.896 respectively. Notwithstanding pH drift, the rank order of responses remained unchanged. For cells at the highest density (10^6 ml^{-1}), it appeared that glucose was depleted rapidly within 2 h, and that depletion

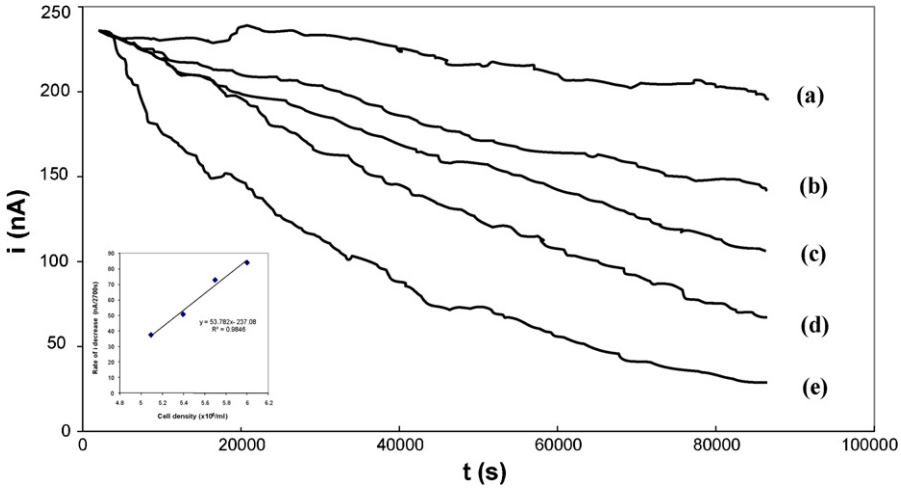

Fig. 3. Real-time monitoring of glucose concentrations in HepG2 cell cultures at different densities. Amperograms obtained for (a) 0 million cells/ml, (b) 0.125 million cells/ml, (c) 0.25 million cells/ml, (d) 0.5 million cells/ml and (e) 1.0 million cells/ml. Inset shows plot of rate of current decrease (nA 2700 s^{-1}) versus cell density (×10^6 ml^{-1}).

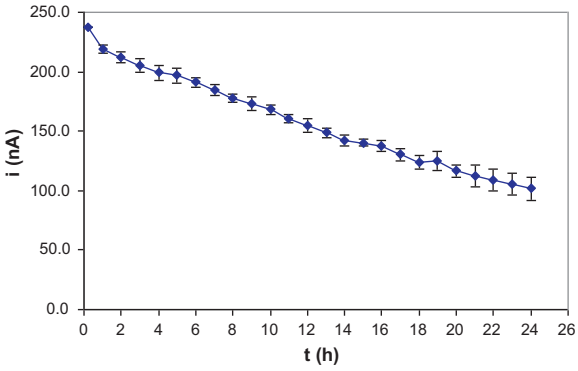

Fig. 4. Reproducibility of real-time monitoring of glucose biosensor response over 24 h in HepG2 cell cultures. Plot of mean + 1SD current responses from four replicate biosensors in wells containing 0.5 million cells/ml.

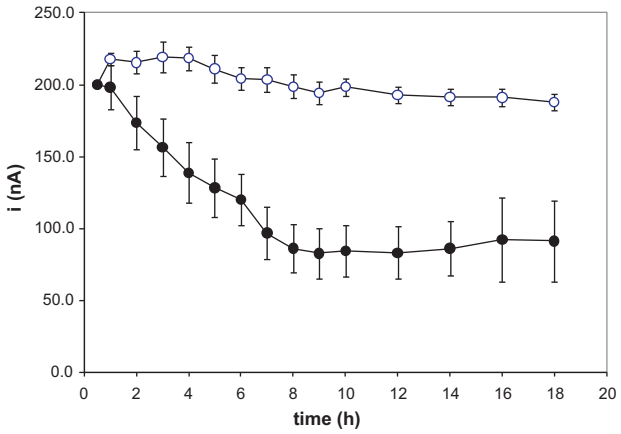

Fig. 5. Plots of mean + 1SD currents from ($n = 6$) amperograms obtained for DMEM/FBS containing 24 mM glucose in the absence (◯) or presence (●) of HepG2 spheroid cells (1 million cells/ml) at microband glucose biosensors.

continued over the ensuing 22 h. In wells containing fewer cells, depletion of glucose was less rapid. The glucose concentrations remaining in the wells were determined by spectrophotometric assay to be 23.4, 20.5, 18.0, 11.4 and 6.5 mM for 0, 0.125, 0.25, 0.5 and 1×10^6 cells/ml, respectively.

The rates of change of current with time were calculated for each cell number over the first 10 h of culture. During this time there was no change in the response in the absence of cells (Fig. 3). Rates of change (in nA 2700 s^{-1}) were plotted against cell density (Fig. 3, insert). The resulting linear relationship ($R^2 = 0.985$) suggests that rate of current decrease may be a good comparator for interpreting metabolic activity.

To obtain a calibration plot against which to read off glucose concentrations experimentally, the calibration plot obtained in L-15 medium was normalised for culture medium in the absence of cells. This was done by taking the 24 h current response and adjusting for the rise in pH from 7.3 to 9.1 using a multiplication factor of 0.745, based on Fig. 2 data. This gave a value of 148 nA for the top glucose standard of 33 mM. Glucose concentrations for the biosensor currents were then read off this new plot. The resulting correlation plot between spectrophotometric and electrochemical biosensor methods (Supplementary Material, Fig. S1B) indicated good agreement for concentrations up to 17 mM; a negative bias for the biosensor resulted in an underestimate of glucose concentrations. The downward drift in current response observed would appear to be a factor; further investigations are underway to study and attempt to ameliorate this effect.

In order to investigate reproducibility of the system, replicate wells were seeded with 0.5×10^6 cells/ml. Amperograms for four replicate wells/biosensors are shown in Supplementary Data, Fig. S2. The currents decreased from 237 nA ($t = 0$) to around 101 nA (24 h). Calculated mean hourly current responses ±SD are plotted in Fig. 4. The CV ranged from 1.4% at 1 h to 9.6% at 24 h.

The spectrophotometrically determined glucose concentrations after 24 h gave a mean value of 13.5 mM (CV = 12.9%), compared with the previous experimental value, for the same cell density, of 11.4 mM. The biosensor estimate, read from the previously obtained calibration plot, was 6 mM.

3.3.3. *Real-time monitoring of glucose metabolism in cell culture—HepG2 spheroids*

The metabolic behaviour of healthy spheroid cultures in terms of glucose metabolism was investigated. The number of Day 6 spheroid cells was based on the numbers of single cells (Fig. 3) capable of depleting the majority of the glucose in 18 h. The results (Fig. 5) demonstrated a steady current response of around 200 nA for biosensors in the absence of cells, but in the presence of cells, the response decreased gradually to a new value of around 90 nA. Spectrophotometric assay at the end of the experiment revealed that all the glucose had been depleted. The healthy spheroid cells had fully depleted the medium within 9 h (Fig. 5). This depletion was more complete than for single cells (see previous section), since cell division during the period of rotary shaking increases the final cell number (difficult to quantify) which become incorporated into spheroids.

HepG2 spheroids are ready to use after 6 days of gyrorotation, but are viable for up to 15 days if medium is changed regularly (every 3 days). It was therefore of interest to examine the relationship between age of the spheroids and their ability to metabolise glucose. Spheroids from the same batch were used after being gyrorotated for 6, 7, 8, 13 or 15 days. Current responses at 18 h in the presence of spheroids, subtracted from control values in the absence of cells, gave a mean change in current response of 129 nA, with a coefficient of variation of 11%; no decline in spheroid metabolism was observed. This result suggests that a single batch of spheroids will give consistent results over a 10-day period in a study involving several experiments on different days.

3.4. *Monitoring the toxic effect of paracetamol*

Fig. 6 shows the current responses over 18 h following incubation of Day 6 HepG2 spheroids in the presence or absence of 1 mM paracetamol. The depletion of glucose by healthy spheroid cells occurred most rapidly during the first 4 h and continued up to 16 h. In the presence of 1 mM paracetamol, a dramatic and sustained decrease in glucose depletion was observed within 1 h of

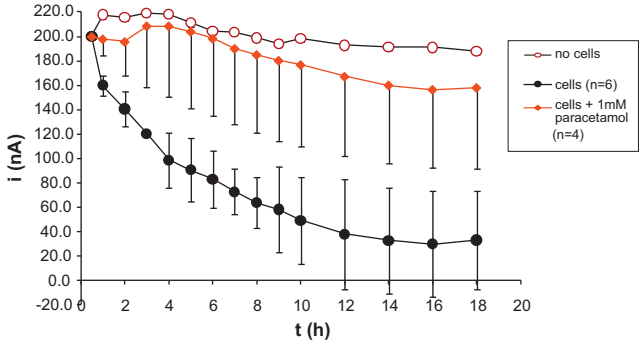

Fig. 6. (A) Plot of normalised current responses from amperograms obtained in DMEM/FBS/24 mM glucose in the absence of cells, or in the presence of 1 million/ml Day 6 HepG2 spheroids with or without 1 mM paracetamol. Points represent mean ± 1SD.

incubation. By comparison with the control healthy cells, the percentage inhibition was 65% after 1 h and a maximum of 95% after 6 h in the presence of 1 mM paracetamol (Supplementary Material, Fig. S3). Paracetamol toxicity appeared to be dose-dependent, since in the presence of 0.5 mM paracetamol, inhibition was not observed until 8 h, reaching a maximum of 15% after 16 h (Supplementary Data, Fig. S3). In three separate experiments, using Day 6, 10 and 11 spheroids, similar profiles for inhibition of glucose uptake were observed in the presence of 1 mM paracetamol; values for percentage inhibition of current response over 18 h did vary considerably between experiments (95%, 81% and 30%).

Regarding the validity of the paracetamol data, Xu et al. (2003) have demonstrated using colorimetric endpoint analysis after 24 h that paracetamol begins to exert an effect on glucose metabolism in fresh liver spheroids, without a change in total protein concentration (cell mass), at a concentration of between 0.5 and 5 mM. In terms of cell mass, HepG2 spheroids proved to be more sensitive to growth inhibition than fresh liver spheroids and showed a significant decrease in growth in the presence of 5 mM paracetamol, whereas fresh cells were unaffected until 50 mM of the drug was applied. The levels of glucose uptake inhibition observed for paracetamol on HepG2 spheroids using the electrochemical microbiosensors in the present study are therefore in the correct order of magnitude (0.5–1 mM). Furthermore, the continuous real-time nature of the electrochemical analysis provides additional metabolic information at earlier incubation times. Thus, it would appear that at the higher (1 mM) dose of paracetamol, cells show maximum inhibition of glucose consumption after 6 h; inhibition is visible as early as 1–2 h. The 6 h timescale may be related to the time taken for paracetamol (acetaminophen) to be oxidised to its cytotoxic imine form by cytochrome P450.

The data from the above experiments were obtained by sampling current every 8 s. This rate proved to be sufficiently frequent to produce relatively smooth amperograms. The form of data treatment required in order to best utilise the results of metabolism experiments will depend upon the interpretation required; in this case, current responses were either plotted at regular intervals (i.e. every 1 h over 16 h) or were used over an initial period (10 h) to obtain a rate of decrease with time. In the latter case, accuracy will be compromised by variations in the linearity of the decrease and further work will be required to obtain a reliable algorithm capable of coping with such variation. Notwithstanding these concerns, the results have shown that comparison with a control well/biosensor (unchanging glucose) is required and is sufficient to obtain a relative measure of the effect of a toxic compound on cell metabolism.

4. Conclusions

On the basis of the experimental findings presented above, it appears that continuous monitoring of mammalian cell cultures using screen-printed glucose microbiosensors can provide a means of assessing relative metabolic activity. Thus, cell number-dependent decreases in amperometric response correlated with numbers of actively metabolising individual (HepG2) cells, and inhibition of glucose uptake by HepG2 spheroids, in the presence and absence of paracetamol, was observed.

In repeat experiments, we observed that the percentage inhibition of glucose uptake due to the presence of paracetamol at 1 mM did vary between experiments (coefficient of variation of 40%). Such differences may be attributed to differential susceptibility/responsiveness of cell batches to the drug; variations in susceptibility may occur even if glucose metabolism in the absence of toxin appears similar. Other factors may be experimental setup variations (e.g. temperature, cell condition, culture medium/serum batch, exact concentration of glucose detected at the microbiosensor edge due to minor variation in distance between itself and the cells) and some of these are difficult to control under the existing experimental conditions. We are in the process of reviewing the experimental setup in order to standardise the temperature (by running inside a 37 °C incubator), the culture medium (including extra buffering capacity) and the electrode configuration (with respect to the cells).

The demonstration that electrochemical biosensor monitoring of glucose metabolism in real time can provide information on relatively early metabolic events provides an impetus to undertake further research in this area. A fully-developed array system incorporating screen-printed biosensor electrodes in a multi-well configuration would be an option worth considering for mammalian cell toxicity testing, with the potential for reduction or replacement of animal testing as an additional goal.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.10.030.

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