Sensors and Actuators B 156 (2011) 416–422

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Correlation between cell growth rate and glucose consumption determined by electrochemical monitoring

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ARTICLE INFO

Article history:
Received 17 February 2011
Received in revised form 20 April 2011
Accepted 20 April 2011
Available online 30 April 2011

Keywords:
Micro-sensors
Electrochemistry
Cell growth rate
Glucose consumption

ABSTRACT

The electrochemical monitoring of glucose consumption is relevant for cell biology studies because of its wide detection range, high sensitivity and easy implementation. Whereas the glucose consumption and cell growth rate can be tightly correlated, they should also be cell population density dependent. In this work, we fabricated high sensitive enzyme electrodes for accurate monitoring of glucose consumption of cells in different growth stages. The performance of the fabricated device was firstly evaluated by cyclic voltammetry (CV) with *p*-benzoquinone (PBQ) as redox mediator, showing a linear response over a wide detection range (0.3–60 mM), a high sensitivity ($1.61 \pm 0.10\ \mu A\ mM^{-1}\ mm^{-2}$ ($n = 5$)) and a low detection limit (80 μM). Then, daily glucose consumptions of NIH 3T3 cells in 24-well plates were determined for a period of 7 days. The results could be compared to the cell population growth curve, showing a close correlation but different behavior. We found that the increase of the glucose consumption took place prior the cell number increase but the glucose consumption per cell decreases linearly in the exponential growth stage of cells.

1. Introduction

Glucose consumption is one of the key indicators of metabolic activities of cells so that it can be tightly correlated to the cell growth rate or the cell population dynamics. Previously, different techniques including electrochemistry, infrared adsorption spectroscopy, Raman spectroscopy, fluorescence, impedance spectroscopy, etc., have been used for the glucose concentration monitoring [1–9]. Among them, electrochemical methods have shown clear advantages such as high sensitivity and high selectivity by proper choice of detection potential and/or electrode material [10–15]. Moreover, the electrochemical sensing elements can be miniaturized for portable devices and they are now routinely used for blood and food sample analyses and bioreactor control. Finally, the electrochemical methods are also useful for analysing many other biological and biochemical factors [16–21] or multiple analytes in real-time [22–25].

The electrochemical methods are based on rather complex reactions at the electrode surfaces which can be changed rapidly due to adsorption or desorption of reagents, surface catalyzing, diffusion and migration as well as convection of species. Accordingly, a large amount of investigations have been developed by using different detection methods (amperometry, cyclic voltammetry (CV), impedance techniques and chrono-amperometry, etc.), different electrode geometry, different material types (Pt, Au, etc.), and different surface coating techniques (self-assembled monolayer, mediators, enzyme, etc.). In the case of high precision glucose detection, both glucose oxidase (GOx) and electrocatalytic molecules based enzyme electrodes are now widely used. With GOx coated electrodes, the detection relies on the quantity of H_2O_2 generated by the GOx-catalyzed reaction when oxygen is the electron mediator between GOx and electrode surface. Since the detection of H_2O_2 requires a relatively high oxidation potential, it may cause severe interference from readily oxidizable species presented in physiological samples. In addition, Prussian-Blue [26,27] can be added to reduce the applied potentials (ca. 0.0 V versus Ag/AgCl). Moreover, when electrocatalytic molecules such as benzoquinone, ferric hexacyanoferrate, are used as mediators, the over-potential can be reduced which is favorable for fast electron transfer kinetics [28–30]. Consequently, the measurements will be independent of the oxygen concentration and the reaction interference between coexisting electroactive species can be minimized.

The electrochemical monitoring of glucose consumption is relevant for cell biology studies because of its wide detection range, high sensitivity and easy implementation. In general, the cell

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doi:10.1016/j.snb.2011.04.067

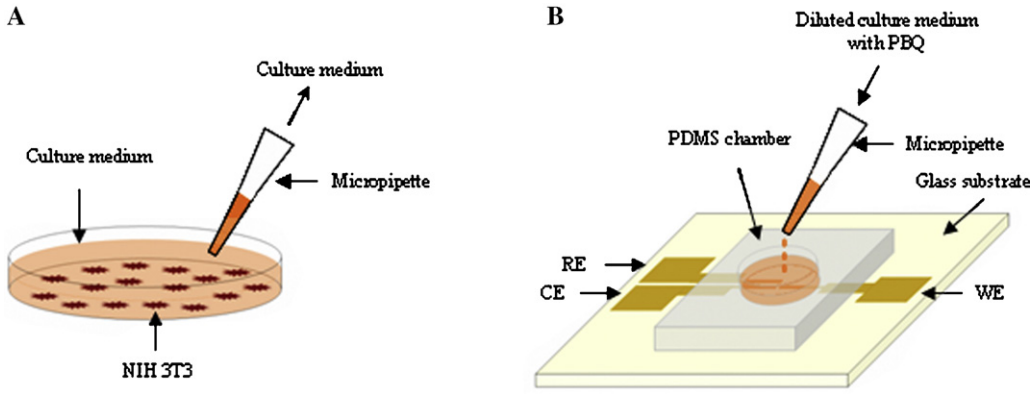

Fig. 1. Schematic diagram of glucose consumption monitoring of cells cultured in 24-well plate: (A) 50 μl of culture medium was taken and mixed with 50 μl of 10 mM PBQ; (B) the glucose concentration of the solution was electrochemically analyzed using a fabricated micro-sensor which consists of a PDMS chamber and three patterned electrodes: WE: working electrode (enzyme electrode); RE: reference electrode (Au); CE: counter electrode (Au).

growth rates are determined by direct methods such as hemacytometer cell counting, whereas indirect methods based on the measurement of nutrient consumption offer complementary information on the cell culture processes. The combination of the two allows a better understanding of metabolic activities of cells so that the cell culture processes can be controlled more accurately. In this work, we are particularly interested in correlation between population dynamics and glucose consumption of cells at different cell growth stages. We firstly describe the fabrication method of a mediator-based glucose micro-sensor and show its linear electrochemical response in the range of 0.3–60 mM with a monitoring sensitivity of 0.383 ± 0.025 μA/mM ($n = 5$) and a low detection limit of 80 μM. Then, we compare the results obtained by counting cell number and glucose consumption measurements on cell population increases over a period of 7 days and deduce the glucose consumption of single cell. We believe that our method is robust and versatile for the determination cell metabolic activities on chip.

2. Materials and methods

2.1. Materials

Glucose oxidase (GOx, 100,000 units/g, EC 1.1.3.4, Type VII, from Aspergillus Niger), D-(+)-glucose, bovine serum albumin (BSA), glutaraldehyde (GA, 25% aqueous solution), sodium chloride (NaCl), sodium phosphate monobasic (NaH_2PO_4), sodium phosphate dibasic (Na_2HPO_4), sulfuric acid (H_2SO_4, 98%), hydrogen peroxide (H_2O_2), potassium iodide (KI), iodine (I_2), *n*-hexane, trypan-blue, penicillin/streptomycin (P/S) and trypsin–EDTA were purchased from Sigma–Aldrich and used without further purification. *p*-Benzoquinone (PBQ) was purchased from Fluka and recrystallized from *n*-hexane. 2-Mercaptoethanol (ME) was purchased from Acros Organics. AZ5214E photoresist and AZ726MIF developer were obtained from AZ Electronic Materials. Dulbecco's minimum essential medium (DMEM), fetal bovine serum (FBS), L-glutamine and fungizone were purchased from Gibco. Base polymer and cross-linker (methylhydrosiloxane dimethylsiloxane copolymers) were purchased from GE RTV 615 kit. Glasses, 24-well plates and hemacytometer were purchased from Fisher Scientific.

All solutions were prepared with deionized water (DI, 18.2 MΩ cm^{-1}). 0.1 M phosphate buffer saline (PBS) with two pH values (pH 7.3 and pH 5.6) was prepared by mixing 0.05 M NaCl with 0.05 M NaH_2PO_4/Na_2HPO_4. 0.1 M stock glucose solution was prepared in 0.1 M PBS (pH 7.3) and stored at 4 °C to allow an overnight equilibration before use.

2.2. Device design and fabrication

The operation process and electrochemical micro-sensor are schematically shown in Fig. 1. NIH 3T3 cells were cultured in 24-well plate and 50 μl of culture medium was taken out every 24 h (A). After culture medium was mixed with 50 μl of 10 mM PBQ, the glucose concentrations in culture media were measured in the electrochemical micro-sensor (B). We have fabricated different types of electrochemical micro-sensors by using conventional photolithography, lift-off and soft lithography techniques, including open and close chambers as well as microfluidic circuits made of poly(dimethylsiloxane) (PDMS). For the sake of simplicity, only sensor device with open PDMS chamber were used in this work with 0.24 mm^2 active area on working electrode.

The glass substrates were cleaned in the piranha solution (H_2SO_4:H_2O_2 = 3:1). Firstly, 8 nm titanium (Ti) adhesion layer and 30 nm gold (Au) layer were deposited on the clear glass substrates by an electron beam evaporator (Edward Auto 500). Then, a thin layer of AZ5214E photoresist was spin-coated at 3000 rpm for 30 s. After soft baking for 1 min 45 s at 125 °C on the hotplate, it was exposed to ultraviolet light (Hamamatsu Lighting Cure LC5) at exposure energy of 75 mJ/cm^2 for 6 s and developed in AZ726MIF developer. Afterward, the open area of Ti/Au film was removed by

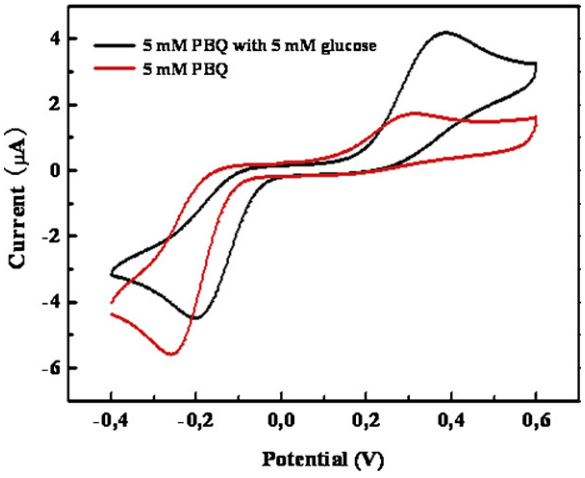

Fig. 2. Cyclic voltammograms of 5 mM PBQ (red line) and 5 mM PBQ containing 5 mM glucose (black line) in 0.1 M PBS solution (pH 7.3) at GOx electrode (0.24 mm^2) with scan rate of 0.1 V/s. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 1
Electrode material, mediator, immobilization method of enzyme, sensitivity, linear range and limit of detection of partial literatures and our work for electrochemical glucose micro-sensors.

Electrode	Mediator	Immobilization method of GOx	Sensitivity ($\mu A\,mM^{-1}\,mm^{-2}$)	Linear range (mM)	Limit of detection (mM)	Reference
Pt	O_2	Entrapment	0.008	1–20	0.8	[34]
Pt	O_2	Entrapment	0.005–0.020	0.1–35	–	[35]
Pt	O_2	Crosslinking	0.860	0.1–1	–	[36]
Au	$K_3Fe(CN)_6$	Adsorption	0.690	0–44.5	–	[29]
Au	O_2	Adsorption	0.003	2–10	0.12	[38]
Paper	O_2	Adsorption	0.049	0–100	0.21	[37]
Au	PBQ	Crosslinking	1.61	0.3–60	0.08	This work

wet etching of Au in a KI/I_2 solution ($KI:I_2:H_2O$ = 115 g:65 g:100 ml) and reactive ion etch of Ti in a plasma chamber (Nextral N100) of SF_6 (30 mTorr, 10.0 sccm and 50 W).

To reduce the interfering signals between oxygen species in particular, a self-assembled monolayer of ME was formed on the fabricated working electrode by liquid phase deposition in a ME aqueous solution (20 mM) over night at room temperature. Then, the ME modified electrodes were rinsed with DI water and dried with nitrogen stream. A 5 μl mixture of 15 mg/ml GOx, 15 mg/ml BSA and 0.1 M PBS (pH 5.6) was finally dropped onto the surface of working electrode. After evaporation, a yellow film appeared on the surface of the electrode. For GOx cross-linking, about 1 ml of 25 wt.% GA was dropped on a glass slide and the sample with electrodes was flipped 1 cm above the glass slide for evaporation. To ensure a saturated GA atmosphere, both glass slide and sample were placed in a sealed device. After 1 h exposed to GA, the electrodes were dried at room temperature for 15 min and then immersed into 0.1 M PBS (pH 7.3) for 30 min for the equilibration of system. Finally, the fabricated glucose micro-sensors were immersed into 0.1 M PBS solution and stored at 4 °C.

PDMS chambers were prepared by mixing PDMS base polymer and cross-linker (methylhydrosiloxane dimethylsiloxane copolymers) at the ratio of 10:1 with a mixer (UNIX Cyclone MM-103S). After removing all bubbles, the mixture was poured onto the silicon wafer and cured thermally for 2 h at 80 °C. Then, the PDMS was peeled off and drilled with punch.

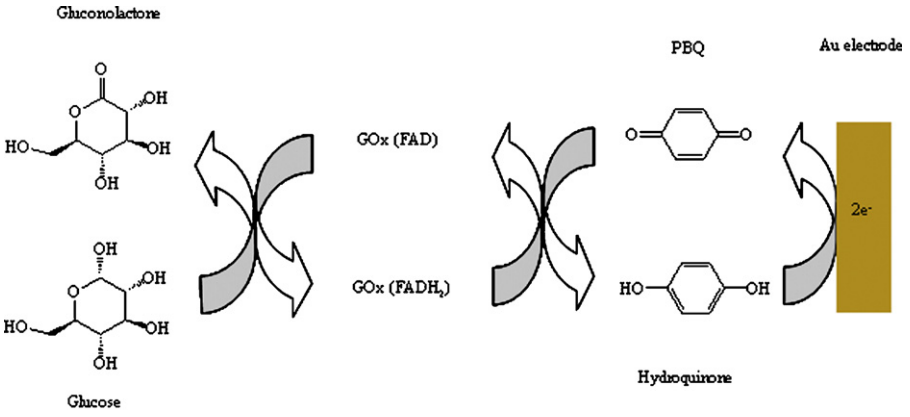

Fig. 3. Schematic of glucose reaction on micro-sensor electrode when the technique of CV is applied, including enzyme reactions and electrochemical reaction at working electrode.

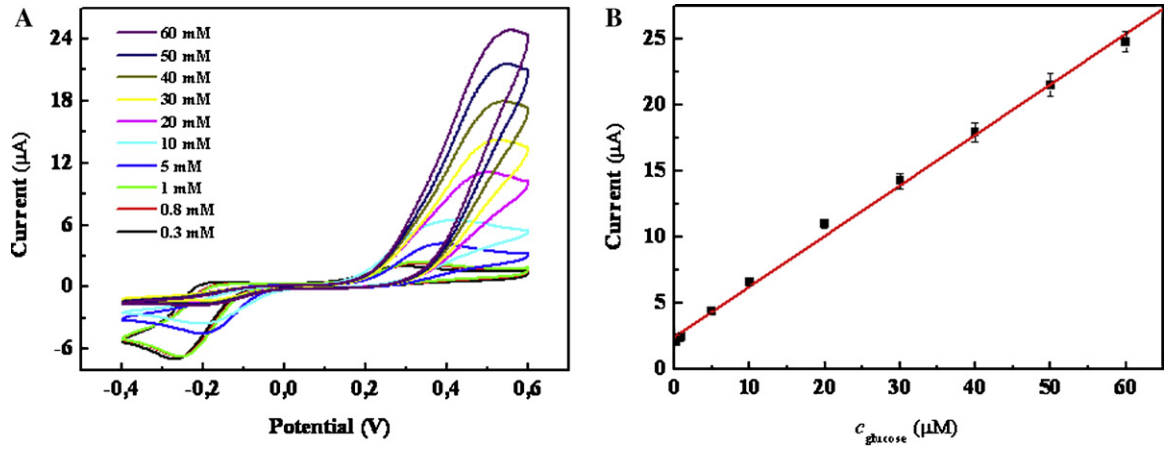

Fig. 4. (A) Cyclic voltammogrames obtained with different glucose concentrations (from 0.3 mM to 60 mM) in 0.1 M PBS solution (pH 7.3) at GOx electrode (0.24 mm^2). (B) Calibration curve deduced from cyclic voltammogrames of (A).

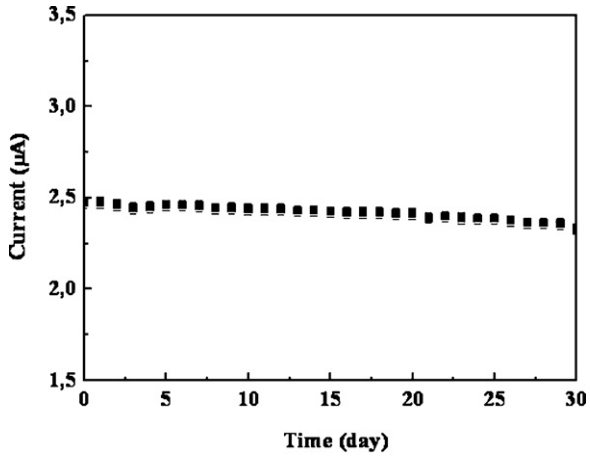

Fig. 5. Long-term stability of the glucose sensor used in this work. The measurement of CV was executed in 1 mM glucose containing 5 mM PBQ. The micro-sensor was stored in 0.1 M PBS solution (pH 7.3) at 4 °C while not in use.

2.3. Cell culture and growth rate monitoring

NIH 3T3 cells were cultured at 37 °C in 5% CO_2 incubator (Sanyo, Japan) with a culture medium of 88% DMEM (containing 4.5 g/l glucose), 10% FBS, 1% L-glutamine, 1% P/S, 0.01% fungizone until confluence. After dissociation in a 0.25% trypsin–EDTA solution and centrifugation, cells were resuspended at a density of 1×10^4 cells/cm^2 and seeded into 24-well plate with 1 ml culture medium in each well. The media were renewed everyday. Three wells were assayed every 24 h. Cell numbers were determined by the trypan blue using a hemacytometer. The photos of NIH 3T3 were imaged using microscope (Zeiss Axiovert 200) with CCD camera.

Glucose solutions for micro-sensor calibrations and glucose concentrations in the culture media (diluted by 10 mM PBQ in 0.1 M PBS (pH 7.3) with the same volume) were measured with the electrochemical micro-sensor in the presence of 5 mM PBQ as a mediator at room temperature. CVs were proceeded in the potential range of −0.4 V to 0.6 V (vs. Au pseudo-reference electrode) with scan rate of 0.1 V/s (CH Instrument 660C).

3. Results and discussion

3.1. Calibration of glucose micro-sensor

It is well known that the working electrodes modified with self-assembled monolayer of appropriate molecules can be used to reduce both non-Faradaic background currents and interfering Faradaic currents [31,32]. In our case, the working electrodes were treated in ME solution for the reduction of the background current and the inhibition of the electrochemical reactivity of O_2 due to its potential barrier. Whereas PBQ is an effective mediator, its redox reaction is much less affected by the presence of the ME monolayers [33]. It allows shuttling charges back and forth for a more efficient electric communication between the immobilized GOx and the electrode. Fig. 2 shows the cyclic voltammograms acquired with (black line) and without (red line) the addition of 5 mM glucose. Clearly, the presence of glucose leads to a much increased oxidative current and a decreased reduction current. Such a difference can be understood by considering the fact that the electro-generated hydroquinone was consumed in the reaction that regenerates PBQ, i.e.,

$$\text{Glucose} + \text{GOx(FAD)} \rightarrow \text{Gluconolactone} + \text{GOx(FADH}_2\text{)} \quad (1)$$

$$\text{GOx(FADH}_2\text{)} + \text{PBQ} \rightarrow \text{GOx(FAD)} + \text{Hydroquinone} + 2\text{H}^+ \quad (2)$$

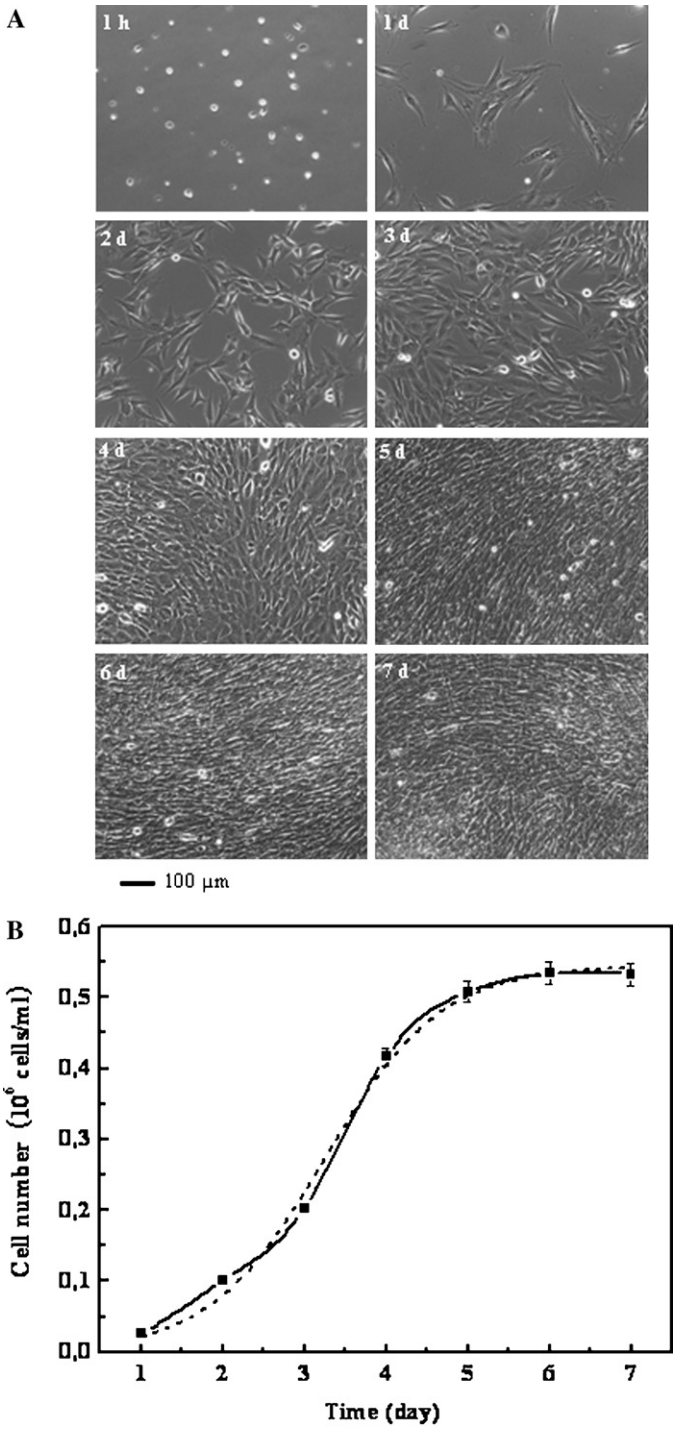

Fig. 6. (A) Microphotographs of NIH 3T3 cells cultured in a 24-well plate during 7 days (the scale bar is 100 μm). (B) Variation of the cell density as a function of time with an initial seeding density of 1×10^4 cells/cm^2. The dashed line is a fitting curve obtained using the standard logistic growth function.

for the *enzymatic reaction* (FAD: Flavin Adenine Dinucleotide) and

$$\text{Hydroquinone} \rightarrow \text{PBQ} + 2\text{e}^- \quad (3)$$

for the *electrochemical reaction* at working electrode, respectively. Therefore, the glucose detection of the present work is based on the electrochemical oxidation of hydroquinone generated by the GOx catalyzed oxidation of glucose at anodic potential. These reactions are schematically illustrated in Fig. 3. Glucose is oxidized to gluconolactone. Simultaneously, PBQ, as the electronic mediator,

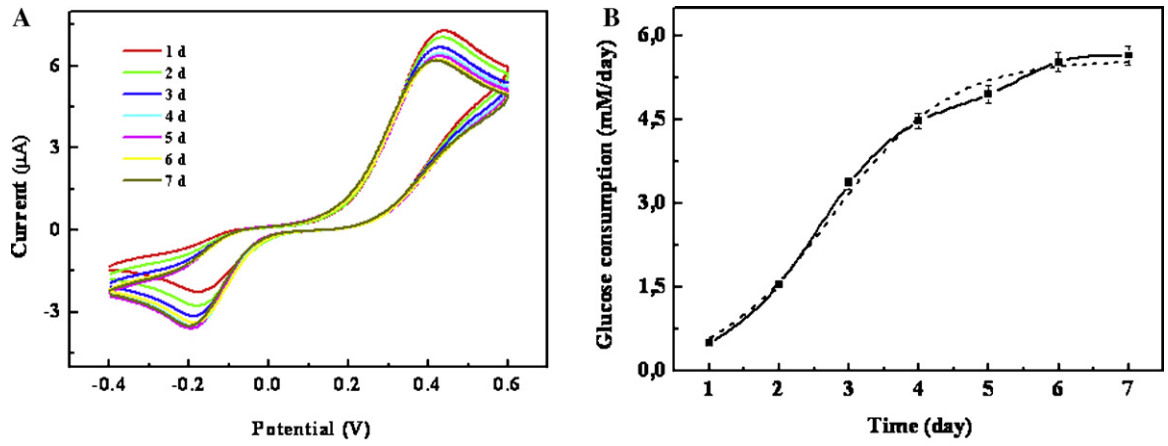

Fig. 7. (A) Cyclic voltammogrames of the culture media diluted by 10 mM PBQ, for a glucose consumption survey during 7 days. (B) Daily glucose consumption of NIH 3T3 cells over 7 days of culture with an initial seeding density of 1×10^4 cells/cm^2. The dashed line is a fitting curve obtained using the standard logistic growth function.

receives electrons to become hydroquinone. The redox reaction is catalyzed by GOx. Ultimately, the current is detected by the Au electrode and hydroquinone changed to PBQ.

Fig. 4(A) displays a set of cyclic voltammograms for different glucose concentrations ranging from 0.3 mM to 60 mM. This wide concentration range could be adapted for the study of glucose consumption during the whole cell culture processes. The commonly used cell culture media include DMEM, roswell park-memorial institute 1640 (RPMI 1640), DMEM/F12 and minimum essential medium (MEM). The concentration levels of glucose in these media are 25 mM, 11.1 mM, 17.5 mM and 5.56 mM, respectively. Obviously, the normal levels of glucose in culture media are well within our linear detection range. We then obtained a calibration curve, as shown in Fig. 4(B), with a linear regression coefficient $r = 0.9978$. Accordingly, we deduced a detection sensitivity of $1.61 \pm 0.10\ \mu A\ mM^{-1}\ mm^{-2}$ ($n = 5$) and a detection limit of about 80 μM. Table 1 shows the comparison of our results with the previous reports on electrochemical monitoring of glucose, including electrode material, mediator, immobilization method of enzyme, sensitivity, linear range and limit of detection [29,34–38]. Clearly, our method has the advantage of a wider linear range, a higher sensitivity and a lower detection limit, indicating a better suitability for the monitoring of glucose consumption in cell culture systems.

We also studied the long-term stability of the fabricated micro-sensors by measuring the peak current of 1 mM glucose containing 5 mM PBQ everyday over 30 days, the sensor being stored in 0.1 M PBS (pH 7.3) at 4 °C after each measurement. As shown in Fig. 5, the monitoring current slightly decreased over time but the sensitivity of the sensor still remained about 94% of the initial value after 30 days, indicating this micro-sensor has high stability.

Finally, we checked the suitability of our sensors for the glucose consumption monitoring by using diluted culture medium (containing 12.5 mM glucose and 5 mM PBQ). No significant difference could be observed in CV response curves comparing to the case of pure glucose solution (data not shown). Apparently, the presence of other cell factors in culture medium does not disturb the detection of glucose concentration.

3.2. Glucose consumption monitoring of NIH 3T3 cells

The cultured NIH 3T3 cell samples were taken daily for both cell number counting and glucose consumption analyses. Fig. 6(A) shows the attachment and the growth behaviors of NIH 3T3 cells between day 0 (1 h after cell seeding) and day 7. Clearly, after 1 h of cell seeding, most cells adhered but only a few of them began to spread. From day 1, the cell number increases quickly until its confluence stage on day 4. Subsequently, the cell growth slowed down with a little change in cell numbers. Fig. 6(B) shows the growth curve of NIH 3T3 cells cultured in 24-well plate, demonstrating a perfect logistic population growth: exponential growth phase (days 1–4) and stagnating phase (days 4–7). The observed cell growth curve could then be fitted by using well-known logistic growth function in Bartlett form, i.e.

$$N = \frac{\bar{N}}{1 + e^{-r(t-t_0)}} \tag{4}$$

where N is the cell density, $\bar{N}$ is the saturation density and r is a rate parameter. When $t = t_0$, $N = \bar{N}/2$. The best fitting curve was obtained with the following parameters $\bar{N} = 0.54$, $t_0 = 3.2$ and $r = 1.4$.

At the same time, the daily glucose consumption of NIH 3T3 cells in culture medium has been determined by measuring CV with our micro-sensor. The results obtained over 7 days are reported in Fig. 7(A), indicating the suitability of our sensors for the cell culture monitoring in terms of both detection range and detection sensitivity. Referring the calibration curve Fig. 4(B), the daily glucose consumption could be deduced (Fig. 7(B)). At the beginning of the culture, the daily glucose consumption increased fast, indicating an exponential growth phase. Then, the increase of the daily glucose consumption was slowed when cell growth entering a stagnating phase. In Fig. 7(B), a fitting curve was added by using the same logistic growth function in Bartlett form (Eq. (4)) but different fitting parameters ($\bar{N}' = 5.6$, $t_0' = 2.8$ and $r' = 1.2$, $\bar{N}'$ being now the saturated glucose consumption). Obviously, the increase of the glucose consumption does not follow exactly the cell growth function, but roughly speaking they have a quite similar behavior with limited differences for the most important two parameters $t_0(t_0')$ and $r(r')$. However, the measurable time delay between the point at half of the maximum consumption ($t_0' = 2.8$) and half of the maximum cell density ($t_0 = 3.2$) is meaningful which reflects a phase shift between the glucose consumption and the cell growth regimes.

In general, the glucose consumption of a cell population increases with the increase of the cell number. But this does not mean that the consumption of individual cells of the population also increases. In fact, when the cell density becomes sufficiently high, the available habitat becomes small so that the increase of the population size will slow down and the cell uptaking rate will be reduced (Fig. 8). When the cell population increase rapidly (till day 3), a large portion of glucose was consumed for cell proliferation. From day 4, the cell population came into stagnating phase so that

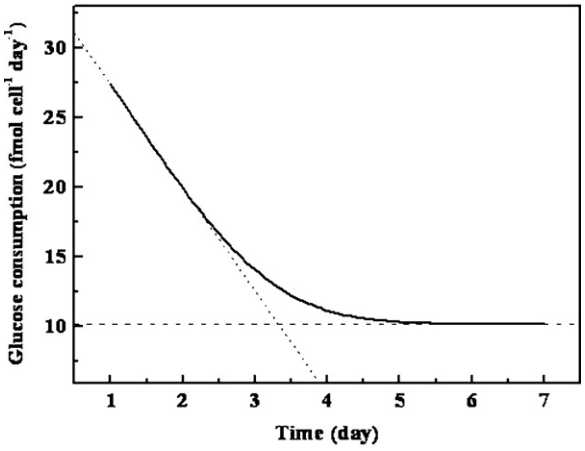

Fig. 8. Variation of glucose consumption per cell over 7 days, deduced from the fitting curves of Figs. 6(B) and 7(B). It shows that a liner decrease of the glucose consumption per cell even in the cell active growing phase and a constant consumption during the stagnating cell phase.

the glucose was mainly consumed for cell survival. Accordingly, the glucose consumption per cell remains almost the same as shown by the dotted line ($c = 10.2$).

4. Conclusions

We fabricated a mediator-based glucose micro-sensor for electrochemical analyses of cell growth rate and metabolic activities. The device showed a linear response for the glucose concentrations in the range of 0.3–60 mM, a higher sensitivity and a lower detection limit comparing to the previously studies. It was then applied for the determination of daily glucose consumption of NIH 3T3 cells cultured in 24-well plate during 7 days. The results obtained in this way could be compared with cell population growth curve, showing a close and logical correlation between the cell growth and glucose consumption behaviors. Although the daily glucose consumption of cells showed a similar increase in cell numbers, the increase of glucose consumption should take place prior of cell number increase. Moreover, we deduced a linear decrease of the glucose consumption per cell during the active growing phase but a constant consumption when the culture entered into the stagnating phase. To conclude this work, we have significantly improved the performance of electrochemical micro-sensors which are suited for real-time monitoring of glucose consumption of cells as well as for the systematic study of metabolic activities of cells and bacteria under different stresses or perturbations. Finally, we would like mention that our method is fully compatible to the microfluidics based on-line monitoring which can be used for the glucose detection in cells, bacteria, blood and food. More systematic studies are underway including automatic sampling and data collection.

Acknowledgements

This work was supported by European Commission through project contract CP-FP 214566-2 (Nanoscales), and the French National Research Agency through project contract ANR-09-PIRI-0030-03 (PAGDEG), ANR-156-06-BLAN-0029-02 (mPhyChemBio) and ANR-06-PCVI-0006-03 157 (DynaCell 3D). F.Z. and L.W. are grateful to the Chinese Scholar Council for Ph.D. grants. The authors are also grateful to Prof. C. Amatore of Ecole Normale Supérieure (Paris), and Prof. Z.Q. Tian of Xiamen University (China) for useful discussions and supports. The content of this work is the sole responsibility of the authors.

References

[1] S.-R. Lee, Y.-T. Lee, K. Sawada, H. Takao, M. Ishida, Development of a disposable glucose biosensor using electroless-plated Au/Ni/copper low electrical resistance electrodes, Biosens. Bioelectron. 24 (2008) 410–414.
[2] N. Saiga, C. Hamada, J. Ikeda, Near infrared spectroscopy assessment of the glucose solution processed by ultrasonic cavitation, Ultrasonics 44 (2006) e101–e104.
[3] J.A. Dieringer, A.D. McFarland, N.C. Shah, D.A. Stuart, A.V. Whitney, C.R. Yonzon, M.A. Young, X.Y. Zhang, R.P. Van Duyne, Surface enhanced Raman spectroscopy: new materials, concepts, characterization tools, and applications, Faraday Discuss. 132 (2006) 9–26.
[4] F. Hussain, D.J.S. Birch, J.C. Pickup, Glucose sensing based on the intrinsic fluorescence of sol–gel immobilized yeast hexokinase, Anal. Biochem. 339 (2005) 137–143.
[5] J.H. DeVries, I.M.E. Wentholt, A. Zwart, J.B. Hoekstra, Pendra goes Dutch; lessons for the CE mark in Europe, Diabetes Res. Clin. Pract. 74 (2006) S93–S96.
[6] S.S. Ozturk, J.C. Thrift, J.D. Blackie, D. Naveh, Real-time monitoring and control of glucose and lactate concentrations in a mammalian cell perfusion reactor, Biotechnol. Bioeng. 53 (1997) 372–378.
[7] J.E. Dowd, I. Weber, B. Rodriguez, J.M. Piret, K.E. Kwok, Predictive control of hollow-fiber bioreactors for the production of monoclonal antibodies, Biotechnol. Bioeng. 63 (1999) 484–492.
[8] P.W. Sauer, J.E. Burky, M.C. Wesson, H.D. Sternard, L. Qu, A high-yielding, generic fed-batch cell culture process for production of recombinant antibodies, Biotechnol. Bioeng. 67 (2000) 585–597.
[9] C. Altamirano, C. Paredes, A. Illanes, J.J. Cair, F. Godia, Strategies for fed-batch cultivation of t-PA producing CHO cells: substitution of glucose and glutamine and rational design of culture medium, J. Biotechnol. 110 (2004) 171–179.
[10] L. Jiang, Y. Lu, Z.P. Dai, M.H. Xie, B.C. Lin, Mini-electrochemical detector for microchip electrophoresis, Lab Chip 5 (2005) 930–934.
[11] J. Wang, Electrochemical biosensors: towards point-of-care cancer diagnostics, Biosens. Bioelectron. 21 (2006) 1887–1892.
[12] R.E. Holcomb, J.R. Kraly, C.S. Henry, Electrode array detector for microchip capillary electrophoresis, Analyst 134 (2009) 486–492.
[13] W.R. Matson, P. Langlais, L. Volicer, P.H. Gamache, E. Bird, K.A. Mark, *n*-Electrode three-dimensional liquid chromatography with electrochemical detection for determination of neurotransmitters, Clin. Chem. 30 (1984) 1477–1488.
[14] M. Alvarez-Icaza, U. Bilitewski, Mass production of biosensors, Anal. Chem. 65 (1993) 525A–533A.
[15] R.S. Martin, A.J. Gawron, S.M. Lunte, C.S. Henry, Dual-electrode electrochemical detection for poly(dimethylsiloxane)-fabricated capillary electrophoresis microchips, Anal. Chem. 72 (2000) 3196–3202.
[16] C. Amatore, S. Arbault, Y. Chen, C. Crozatier, F. Lemaitre, Y. Verchier, Coupling of electrochemistry and fluorescence microscopy at indium tin oxide microelectrodes for the analysis of single exocytotic events, Angew. Chem. Int. Ed. 45 (2006) 4000–4003.
[17] C. Amatore, S. Arbault, Y. Chen, C. Crozatier, I. Tapsoba, Electrochemical detection in a microfluidic device of oxidative stress generated by macrophage cells, Lab Chip 7 (2007) 233–238.
[18] N.C. Tansil, F. Xie, H. Xie, Z.Q. Gao, An ultrasensitive nucleic acid biosensor based on the catalytic oxidation of guanine by a novel redox threading intercalator, Chem. Commun. (2005) 1064–1066.
[19] S.Y. Zheng, M. Liu, Y.C. Tai, Micro coulter counters with platinum black electroplated electrodes for human blood cell sensing, Biomed. Microdev. 10 (2008) 221–231.
[20] M. Cretich, V. Sedini, F. Damin, G. Di Carlo, C. Oldani, M. Chiari, Functionalization of poly(dimethylsiloxane) by chemisorption of copolymers: DNA microarrays for pathogen detection, Sens. Actuators B 132 (2008) 258–264.
[21] M. Varshney, Y. Li, Interdigitated array microelectrode based impedance biosensor coupled with magnetic nanoparticle-antibody conjugates for detection of *Escherichia coli* O157:H7 in food samples, Biosens. Bioelectron. 22 (2007) 2408–2414.
[22] S.S. Park, C.E. Tatum, Y. Lee, Dual electrochemical microsensor for simultaneous measurements of nitric oxide and oxygen: Fabrication and characterization, Electrochem. Commun. 11 (2009) 2040–2043.
[23] S.Y. Ly, J.K. Kim, Simultaneous real-time assay of copper and cadmium ions by infrared photo diode electrode implanted in the muscle of live fish, J. Biochem. Mol. Toxicol. 23 (2009) 256–262.
[24] A. Morales-Villagran, L. Medina-Ceja, S.J. Lopez-Perez, Simultaneous glutamate and EEG activity measurements during seizures in rat hippocampal region with the use of an electrochemical biosensor, J. Neurosci. Methods 168 (2008) 48–53.
[25] Y. Lee, J. Kim, Simultaneous electrochemical detection of nitric oxide and carbon monoxide generated from mouse kidney organ tissues, Anal. Chem. 79 (2007) 7669–7675.
[26] F. Ricci, A. Amine, C.S. Tuta, A.A. Ciucu, F. Lucarelli, G. Palleschi, D. Moscone, Prussian Blue and enzyme bulk-modified screen-printed electrodes for hydrogen peroxide and glucose determination with improved storage and operational stability, Anal. Chim. Acta 485 (2003) 111–120.
[27] F. Ricci, D. Moscone, C.S. Tuta, G. Palleschi, A. Amine, A. Poscia, F. Valgimigli, D. Messeri, Novel planar glucose biosensors for continuous monitoring use, Biosens. Bioelectron. 20 (2005) 1993–2000.
[28] M.A.A. Lomillo, J.G. Ruiz, F.J.M. Pascual, Biosensor based on platinum chips for glucose determination, Anal. Chim. Acta 547 (2005) 209–214.

[29] C.T. Hsu, H.H. Chung, D.M. Tsai, M.Y. Fang, H.C. Hsiao, J.M. Zen, Fabrication of a glucose biosensor based on inserted barrel plating gold electrodes, Anal. Chem. 81 (2009) 515–518.
[30] S. Kaimori, T. Kitamura, M. Ichino, T. Hosoya, F. Kurusu, T. Ishikawa, H. Nakamura, M. Gotoh, I. Karube, Structural development of a minimally invasive sensor chip for blood glucose monitoring, Anal. Chim. Acta 573 (2006) 104–109.
[31] S.E. Creager, K.G. Olsen, Self-assembled monolayers and enzyme electrodes: progress, problems and prospects, Anal Chim. Acta 307 (1995) 277–289.
[32] A.S.N. Murthy, J. Sharma, Glucose oxidase bound to self-assembled monolayers of bis(4-pyridyl) disulfide at a gold electrode: amperometric determination of glucose, Anal. Chim. Acta 363 (1998) 215–220.
[33] R.K. Shervedani, A. Hatefi-Mehrjardi, Comparative electrochemical behavior of glucose oxidase covalently immobilized on mono-, di- and tetra-carboxylic acid functional Au-thiol SAMs via anhydride-derivatization route, Sens. Actuators B 137 (2009) 195–204.
[34] P.C. Nien, M.C. Huang, F.Y. Chang, K.C. Ho, Integrating an enzyme-entrapped conducting polymer electrode and a prereactor in a microfluidic system for sensing glucose, Electroanalysis 20 (2008) 635–642.
[35] I. Moser, G. Jobst, G.A. Urban, Biosensor arrays for simultaneous measurement of glucose, lactate, glutamate, and glutamine, Biosens. Bioelectron. 17 (2002) 297–302.
[36] S. Gaspar, X.W. Wang, H. Suzuki, E. Csoregi, Amperometric biosensor-based flow-through microdetector for microdialysis applications, Anal. Chim. Acta 525 (2004) 75–82.
[37] W. Dungchai, O. Chailapakul, C.S. Henry, Electrochemical detection for paper-based microfluidics, Anal. Chem. 81 (2009) 5821–5826.
[38] N.P. Rodrigues, Y. Sakai, T. Fujii, Cell-based microfluidic biochip for the electrochemical real-time monitoring of glucose and oxygen, Sens. Actuators B 132 (2008) 608–613.

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