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Fabrication of Electrical Cell Chip for the Detection of Anticancer Drugs and Environmental Toxicants Effect

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Abstract

The effect of anticancer drugs and toxins on the viability of HepG2 cells were examined by the cyclic voltammetry (CV) and the potentiometric stripping analysis (PSA) methods. The cells were immobilized on gold patterned silicon substrate. The voltammetric behaviors of HepG2 cells showed a quasi-reversible process and the peak current showed a linear relationship with cell number. The attached living cells were treated with different concentrations of anticancer drugs and toxin. As the exposed concentrations of anticancer drugs and toxins were increased, we observed that the peak current in CV assay and the area under the peak in PSA assay were decreased. Trypan blue dyeing experiment was performed to confirm the results of the effects of anticancer drugs on the cell viability which were obtained from CV assay and PSA assay. These results indicate that the proposed direct cell immobilization method technique can be applied to construct the cell chip for the diagnosis, drug detection, and on-site monitoring depended on the voltammetric and PSA methods.

Keywords: Cell chip, HepG2 cell, Cyclic voltammetry, Potentiometric stripping analysis, Anticancer drugs effect

Introduction

The understanding of modeling cell behavior based on only RNA or protein expression levels is difficult, because a cell is a much more complicated system than the sum of its components[1]. Cell-based sensor arrays[2] and electrical sensing devices have been used for signal-frequency patterns in cell growth media[3], making a cell-based assay an attractive method for the aforementioned investigations. There are currently two types of cell-based assay. One comprises a microfluidic device for the analysis of living cells, of which the example is a microfluidic device fabricated with polydimethylsiloxane (PDMS) consisting of an array of micro-injectors integrated in a base flow channel. This device allows controlled application of drugs to cell cultures[4]. The second design of microfluidic device uses electrical fields for cell immobilization and analyzes single cell ion channel using the patching clamping technique. Both microfluidic device designs electrically measured cell viability by detecting changed electrical resistance of a cell membrane within milliseconds when the cells are exposed to a toxic agent[5]. Cell-based sensor arrays are potentially useful for studying effects of drug and cell-external stimuli interactions[6,7]. In vitro immobilization of living cell is an important process in the fabrication of cell-based chip[8], and the interaction between cell-cell interactions and cell-substratum interactions are highly regulated processes that range in time from transient to long-lasting, and these interactions plays a crucial role in most fundamental cellular functions including motility, proliferation, differentiation, apoptosis and also it can be a reliable candidate for cellular attachment without loss of viability[9,10]. Cell based biosensor can be classified as the type of a secondary transduction module to monitor the change of cell reaction into (1) Resistance based biosensor, (2) Metabolism based biosensor, (3) Optical biosensor and (4) Electrical biosensor. Cells attached to the electrode can produce electrochemical signals[11], which have received considerable attention in the development of biosensors[12]. A living cell can properly be described as an electrochemical dynamic system with electron generation and electron transfer on the interface[13]. Action potential of cell is a good measurable indicator to detect cell characteristics[7]. The recording of cell membrane potential requires sophisticated processes using electrodes of patch clamp or pierce. This unconventional process is not appropriate to develop cell based biosensor. Therefore, various methods for the measurement of extracellular action potential using microelectrode

have been researched widely because action potential can generate the current out of cell which can be detected by electrodes, not penetrated into the cell. First recording of action potential was achieved by Tomas *et al*. in 1972[14]. They explained in situ extrapotential action poteintial from chick myocardial cell cluster using micro electrode array. PSA is a technique that is concerned mainly with the determination of metals that accumulate on a mercury electrode. PSA monitors the change of potential with time during chemical oxidation of the accumulated metal[15]. Gold electrodes also can be used in PSA experiment[16].

Presently, we used immobilized living HepG2 cells on cell chip to study electrochemically determined viability. Living HepG2 cells immobilized on gold patterned silicon substrate exhibited a quasi-reversible voltammetric response. PSA was used to study the effect of anticancer drugs which evident as a change in the area under the peak by increasing concentration of anticancer drugs. Anti-cancer drugs and toxin affects to the growth of HepG2 cells, which is significant to the study the action of anti-cancer drugs. The anticancer drugs (hydroxyurea and cyclophosphamide) and phenol were selected to study the ability of cell chip to detect the effect on the cell viability by using the voltammetric and potentiometric methods. The CV and PSA results were verified with comparison of counter assay (trypan blue exclusion) test. The results demonstrate that, the cell chip design which is quick and easy to do, is useful not only as a good substrate for the culture of HepG2 cells but also as an electrode for measuring cellular electrochemical properties, and permits the assessment of cell viability. The CV and PSA methods may provide a simple way to study the cell viability, cell growth and the effect of anticancer drugs on the cells viability.

Results and Discussion

Cyclic Voltammetry of HepG2 Cells at a Gold Electrode

HepG2 cells immobilized on working electrode were allowed to grow for two days before measuring the

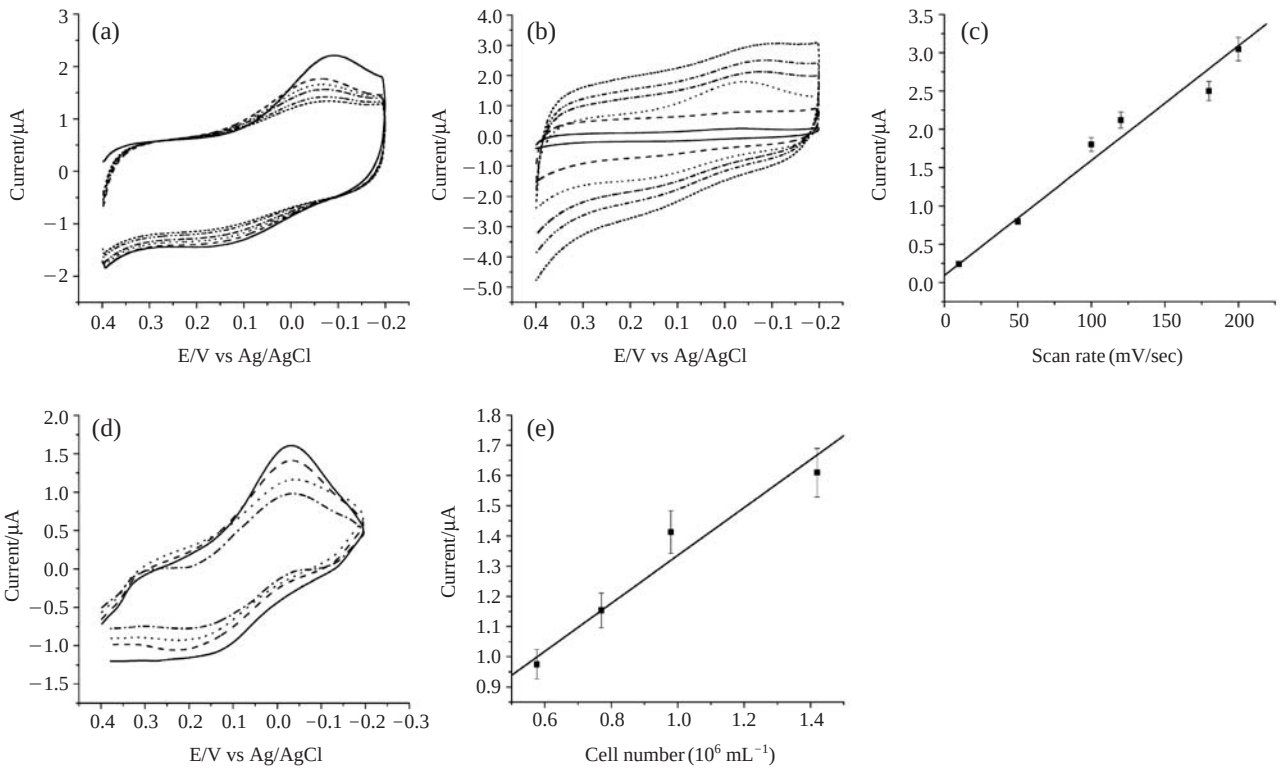

Figure 1. Electrochemical response (a) of HepG2 cells at different cycles number (—) 1, (– –) 10, (· · ·) 20, (–·–·) 30, (–··–) 40, (– – –) 50, the scan rate was 100 mVs^{-1}, and the cell number was 1.6×10^6 mL^{-1}, (b) Cyclic voltammograms of HepG2 cell at different scan rate (—) 10, (– –) 50, (· · ·) 100, (–·–·) 120, (–··–) 150, (– – –) 200 mVs^{-1} and the cell number was 1.31×10^6 mL^{-1}, (c) a linear plot of reduction current peak as a function of scan rate (d) Relationship of peak current with the cell number (—) 5.76×10^5, (– –) 7.7×10^5, (· · ·) 9.8×10^5, (–·–·) 1.42×10^6 cells/mL. The scan rate was 100 mVs^{-1}. (e) a linear plot of reduction current peak as a function of cell number, Data are means S.D. of three different experiments.

voltammetric behavior of the cells. The cyclic voltammogram of HepG2 cells in the potential range from −0.2 to +0.4 V (versus Ag/AgCl) at different cycles number until 50 cycles is shown in Figure 1a. A quasireversible process with cathodic peak at −0.094 V and anodic peak at +0.154 V were observed on the first scan. The subsequent potential scans after the initial scan move the peaks to the more positive potential, cathodic peak appeared at −0.056 V and anodic peak appeared at +0.174 V. A slight peak separation was observed in second scan as compared to the first. The peaks of the rest cycles showed no change in potential peaks but there was a slight decrease in peak current. The change in potential between first and rest of the cycles was related to the high scan rate, but when we applied 50 mVs^{-1} there is no change in potential peaks between first and second cycle. This cyclic voltammetry measurement shows the stable behavior of HepG2 cells. Cyclic voltammograms at different scan rates of the HepG2 cells on gold electrode are also shown in Figure 1b; with the increase of the scan rate from 10 to 200 mVs^{-1}, the potential peaks move positively and the peak current increases. Figure 1c shows a linear plot of reduction current peak as a function of scan rate. These results showed the advantage of gold electrode, which offer fast electron-transfer kinetic more than none metal electrodes i.e., the rate of electron transfer between cell and electrode is more fast; also the advantage of using immobilized living cell on electrode rather than the using of cell suspension in buffer.

Relationship between the Peak Current and the Cell Number

In order to investigate the relationship between the peak current and the cell number, HepG2 cells with the different density were incubated under the same conditions in many cell chips for 48 h. Figure 1d shows the cyclic voltammogers for HepG2 cells with different numbers. The peak current found to be increased

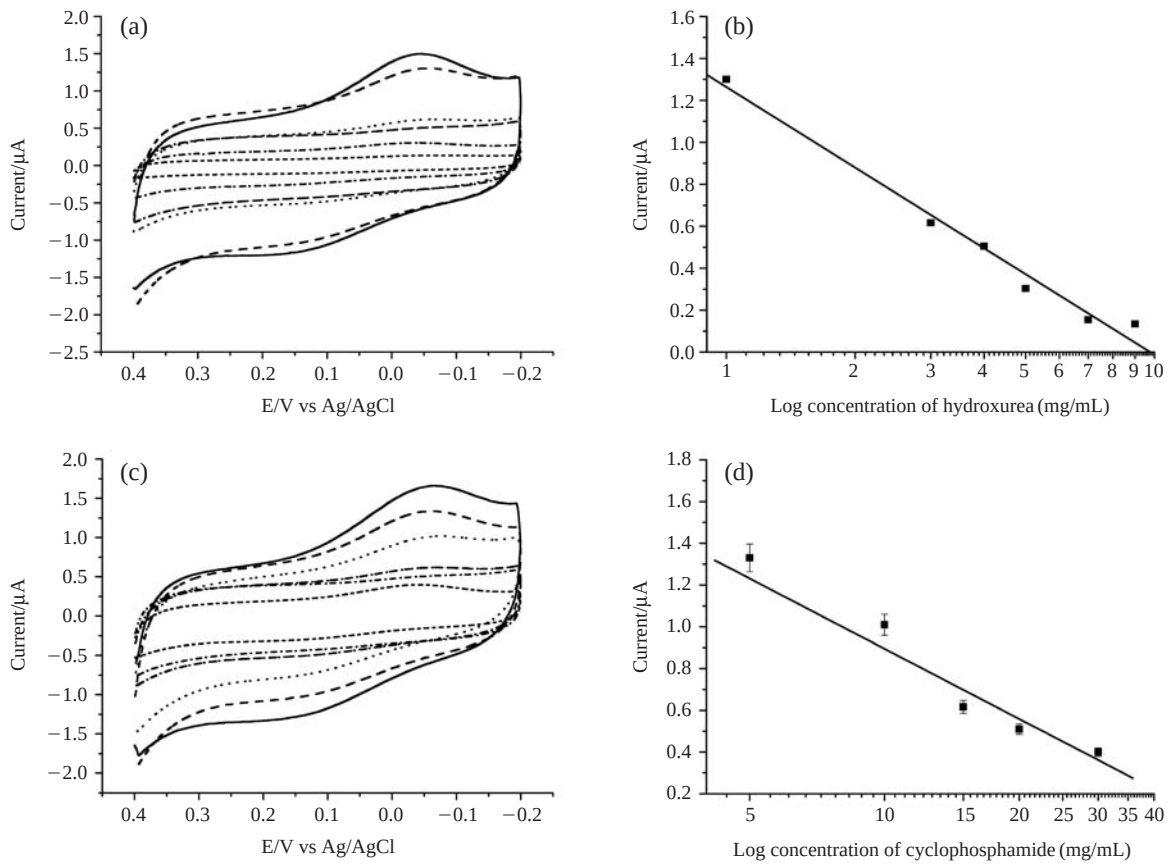

Figure 2. Cyclic voltammetry of HepG2 cells treated with varying concentration of (a) Hydroxyurea (—) 0, (– –) 1, (· · ·) 3, (–·–·) 4 and (–··–) 5 mg/mL. (b) a linear plot of reduction current peak as a function of log concentration of hydroxyurea, (c) Cyclophosphamide concentration, (—) 0, (– –) 5, (· · ·) 10, (–·–·) 15, (–··–) 20 and (– – –) 30 mg/mL. (d) a linear plot of reduction current peak as a function of anticancer concentration of cyclophosphamide. The cell number was 1.4×10^6 mL^{-1} and the scan rate was 100 mVs^{-1}. Data are means S.D. of three different experiments.

with the increasing cell number. Figure 1e shows a linear plot of reduction current peak as a function of cell number. This result indicated that the peak current had a positive relationship with the cell number, so under the same conditions we can use the cyclic voltammetry to determine the cell number by measuring its peak current.

Voltammetric Study of the Effect of Anticancer Drugs on HepG2 Cell

Hydroxyurea is an effective inhibitor for DNA synthesis in cancer cells, and the continued synthesis of RNA and protein in the presence of hydroxyurea leads to a state of unbalanced growth[17]. Cyclophosphamide is a cytotoxic nitrogen mustard derivative widely used in cancer chemotherapy; it cross-links with DNA leads to strand breakage, and induces mutations[18]. Its clinical activity is associated with a decrease in aldehyde dehydrogenase 1 (ALDH1) activity[19,20].

To investigate the effect of anticancer drugs on the cyclic voltammetric response of HepG2 cells, the inoculated HepG2 cells with the same number of cells were allowed to attach and grow for 24 h, then fresh culture medium containing different concentrations of anticancer drugs was supplied and the CV signals were detected after 24 h. Figure 2a shows the effect of different concentrations of hydroxyurea on cyclic voltammetry response of HepG2 cells, it was observed that as the concentration of hydroxyurea increases the peak current decreases drastically. Figure 2b shows the corresponding linear plot between the reduction current peak and the log of hydroxyurea concentration. The effect of varying concentrations cyclophosphamide on CV response of HepG2 cells was also similar results to those obtained with hydroxyurea were evident (Figure 2c). The corresponding linear plot between the reduction current peak and the log of cyclophosphamide concentration is shown in Figure 2d. These results indicated that the decrease of current peak depended on the concentration of the anticancer drugs. The reduction in peak current was related to the decrease in the viability and the proliferation of the HepG2 cells, by considering the cell-cell interactions that affect drug sensitivity have been found in vivo and in vitro for cells grown under specific experimental conditions. Therefore, the voltammetric response of immobilized living cancer cells could be used to monitor the change of cell physiological viability, which can provide a simple way to study the function of anti-cancer drugs in cancer cell growth. These results indicated that the decrease of current peak was anticancer drugs concentration dependent, which indicates that the CV can be used as anticancer drug sensitivity test.

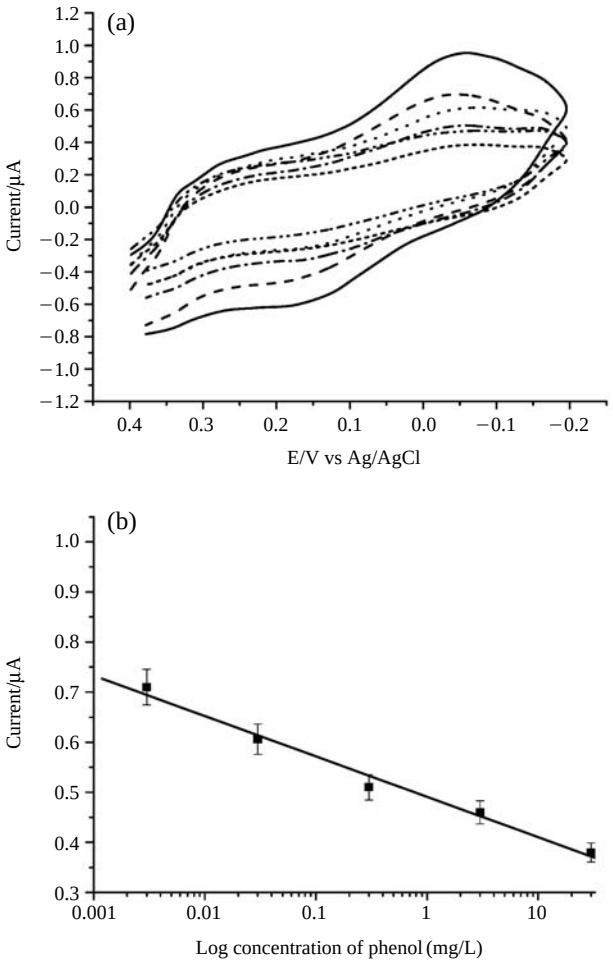

Figure 3. (a) CV of HepG2 cells treated with varying concentration of phenol (—) 0, (– –) 0.003, (· · ·) 0.03, (–·–·) 0.3, (–··–) 3, (– – –) 30 mL/L and (b) a linear plot of reduction current peak as a function of phenol concentration. The cell number was 1.4×10^6 mL^{-1} and the scan rate was 100 mVs^{-1}. Data are means S.D. of three different experiments.

Voltammetric Study of the Effect of Phenol on HepG2 Cell

Phenol has the potential to be genotoxic for DNA synthesis and induced DNA damage in human fibroblasts and cells[21]. To investigate the effect of phenol on the cyclic voltammetric response of HepG2 cells, the inoculated HepG2 cells were allowed to attach and grow for 24 h, and then fresh culture medium containing different concentrations of phenol was supplied. The cytotoxic effect of phenol on HepG2 cells was confirmed by morphological observation. Figure 3a shows the effect of different concentrations of phenol on cyclic voltammetry response of HepG2 cells. Figure 3b shows the corresponding linear plot between the reduction current peak and the log of

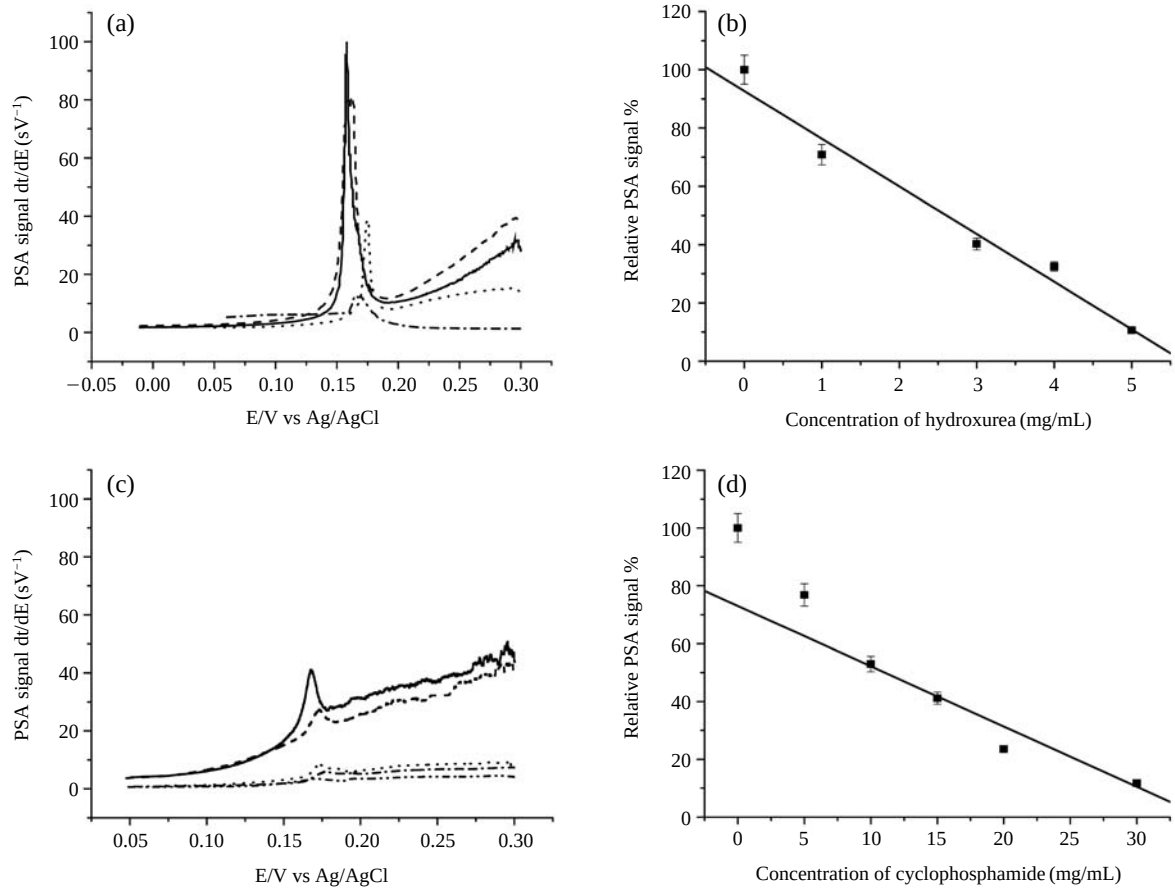

Figure 4. PSA study of HepG2 cells treated with varying concentration of (a) Hydroxyurea (—) 0, (– –) 1, (· · ·) 3, (–·–·) 4 and (–··–) 5 mg/mL. (b) linear plot of relative area under the PSA peak as a function of hydroxyurea. (c) Cyclophosphamide concentration (—) 0 , (– –) 5, (· · ·) 10, (–·–·) 15 and (–··–) 20 mg/mL. (d) linear plot of relative area under the PSA peak as a function of cyclophosphamide.

phenol concentration. We observed that as the concentration of phenol increased the peak current decreases drastically. These results indicated that the decrease of current peak was phenol concentration dependent, which indicates that the cyclic voltammetry can be used as toxin sensitivity test.

PSA Study of the Effect of Anticancer Drugs on HepG2 Cell

PSA assay parameters were optimized at 400 s as deposition time, 6 μA as the optimal stripping current and 0.0 V as deposition potential. HepG2 cells with the same density were incubated under the same conditions in many flasks. After 24 h, fresh culture medium containing different concentrations of anticancer drugs was supplied and after 24 h the PSA were measured. Figure 4a shows the effect of different concentrations of hydroxyurea on PSA response of HepG2 cells. We observed that as the hydroxyurea concentration increases, the area under the peak decrease. Figure 4b shows the corresponding linear plot between the area under the peak and hydroxyurea concentration. The effect of varying concentrations cyclophosphamide on PSA response of HepG2 cells was also studied and showed similar behavior as in the case of hydroxyurea as shown in Figure 4c. The corresponding linear plot between the area under the peak and cyclophosphamide concentration was shown in Figure 4d. So the decrease of PSA signal in is related to the decrease of cell viability. These results indicate that the baseline corrected PSA signal can represent cell viability and be used for monitoring the effect of anticancer drugs on the cells.

Counting Assay and Comparison with the Proposed Electrochemical Anticancer Drug Sensitivity Test

The effect of anticancer drugs on the cell viability

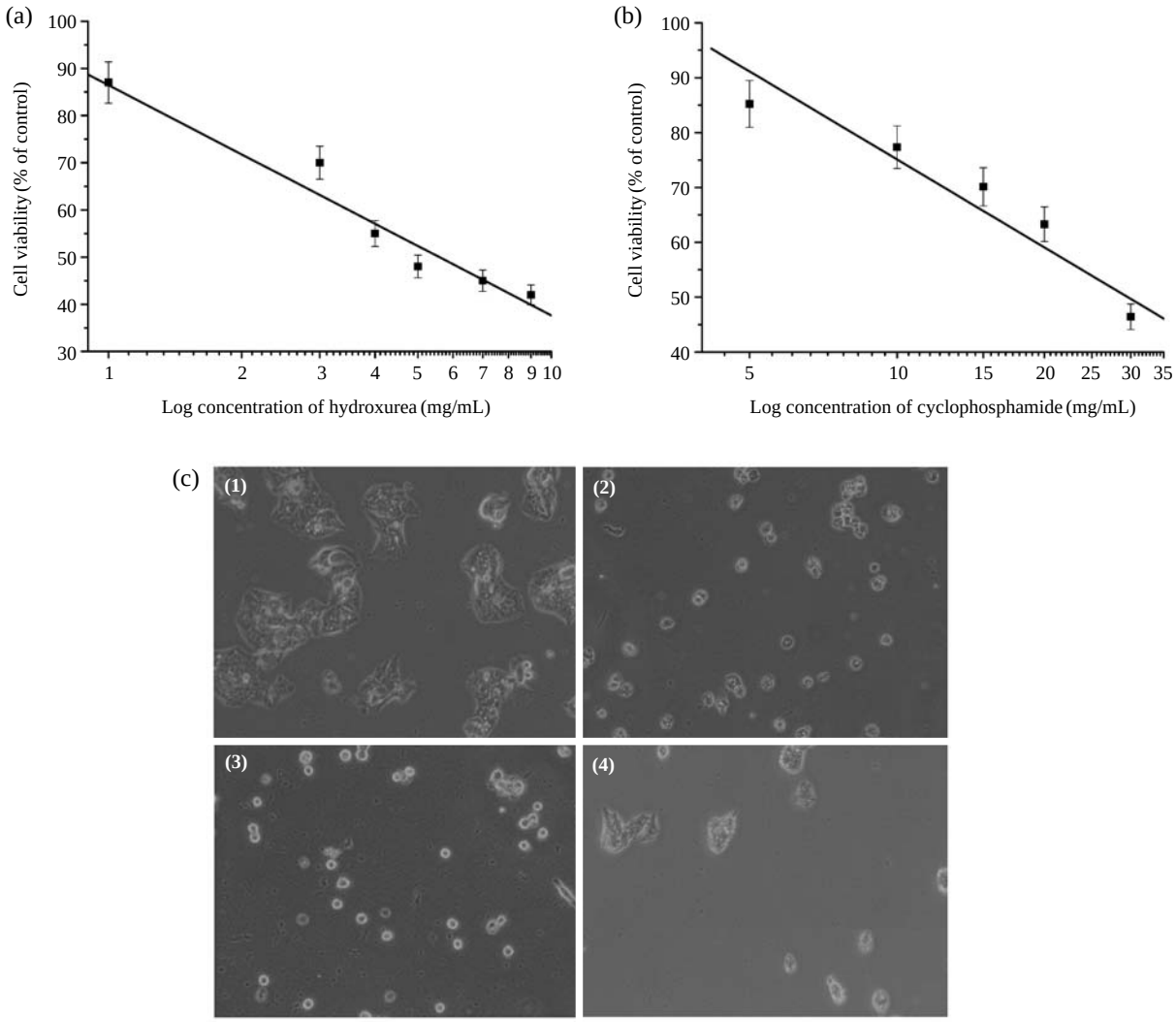

Figure 5. Variance of cell viability with concentration of (a) Hydroxyurea. (b) Cyclophosphamide. The cell number was 1.4×10^6 mL^{-1}. Data are means S.D. of three different experiments (c) Microscopy image of (1) control HepG2 cells, (2) HepG2 cells treated with hydroxyurea, (3) HepG2 cells treated with cyclophosphamide, (4) HepG2 cells treated with phenol.

was determined by the exclusion of Trypan blue dyeing experiment. The cells that exclude the dye are viable[22]. Considering that the cell viability for control cell as 100% the results for the effect of anticancer on the viability of HepG2 cells which was obtained from the cell counter assay Figure 5a and Figure 5b it was observed that, as the drug concentration increases the cell viability decreased and we obtained the effective concentration (EC_{50}). EC_{50} value for hydroxyurea is 4.355 mg/mL, and EC_{50} value for cyclophosphamide is 28.75 mg/mL. These findings verify the cytotoxicity effects of the two drugs when compared with CV and PSA method, were in good accordance. Due to their accuracy and stability, these methods can be used as an in vitro test for anticancer drugs sensitivity.

Conclusions

The present study shows the feasibility of cell chip to monitor the extracellular potential for the detection of anticancer drugs effect. Also, the results establish the generality of CV and PSA for use as direct electrochemical detection techniques to monitor cell growth, viability and the effect of anticancer drugs on the cell viability. In this work the advantageous of immobilization of cancer cells and its application to cell chip platform were developed. The immobilized living cells exhibit a quasi-reversible voltammetric response and the peak current had a positive relationship with the cell number. The cytotoxicity of two anticancer

drugs has been investigated by using CV and PSA techniques, which shows that anticancer drugs display significant influence on the electrochemical response of immobilized living HepG2 cells. It is significant to the study on the characterization of the physiological function of living cells and the advice for chemotherapy. The electrochemical results also agree with the counting assay test. The proposed direct cell immobilization method technique may be applicable to diagnosis, drug detection, and on-site monitoring.

Materials and Methods

Materials

Hydroxyurea and Cyclophosphamide were purchased from Calbiochem (Germany). Phosphate buffered saline (PBS, pH 7.4, 10 mM) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All Other chemicals that are used in this study were obtained commercially as reagent grade.

Cell Culture

HepG2 cell was collected from the liver of human. The histopathology is hepatoma and its growth pattern is monolayer. Cell line was cultured in DMEM supplemented with 10% heat inactivated fetal bovine serum (FBS) (from Gibco); antibiotics ((from Gibco), 1%). Cells were maintained under standard cell culture at 37˚C in an atmosphere of 5% CO_2. The medium was changed every three days.

Cell Chip Design and Fabrication

The chip contained three gold (Au) working electrodes the area of each electrode is 5 mm^2, separated by 2 mm, creating an exposure area for cell attachment of approximately 2.6 mm^2. Gold electrodes 150 nm in thicknesses was patterned on silicon substrate by DC magnetron sputtering. Prior to this, a 50 nm thick layer of titanium (Ti) was established by sputtering to promote the adhesion of Au on silicon. The chamber created had dimensions of was about 2 cm × 2 cm × 0.5 cm (width × length × height). PDMS was used to affix substrates to the chamber. The cells were transferred into the chip at a known cell density by infusion of new culture medium.

Electrochemical Behavior of HepG2 Cells by Cyclic Voltammetry

The CV and PSA studies were performed using potentiostat (CHI-660, CHI, USA) controlled by the general propose electrochemical system software. A homemade three-electrode system comprised a cell-based chip as the working electrode, a platinum wire as auxiliary electrode, and Ag/AgCl as reference electrode. PBS (10 mM, pH 7.4) was used as an electrolyte at a scan rate of 0.1 V/s.

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References

1. Choi, J.W., Park, K.W., Lee, D.B., Lee, W. & Lee, W.H. Cell immobilization using self-assembled synthetic oligopeptide and its application to biological toxicity detection using surface plasmon resonance. *Biosens. Bioelectron.* **20**, 2300-2305 (2005).
2. Brogan, K.L. & Walt D.R. Optical fiber-based sensors: application to chemical biology. *Curr. Opin. Chem. Biol.* **9**, 494-500 (2005).
3. May, K.M., Wang, Y., Bachas, L.G. & Anderson, K.W. Development of a whole-cell-based biosensor for detecting histamine as a model toxin. *Anal. Chem.* **76**, 4156-4161(2004).
4. Thiébaud, P., Lauer, L., Knoll, W. & Offenhäusser, A. PDMS device for patterned application of microfluids to neuronal cells arranged by microcontact printing. *Biosens. Bioelectron.* **17**, 87-93 (2002).
5. Huang, Y., Sekhon, N.S., Borninski, J., Chen, N. & Rubinsky, B. Instantaneous, quantitative single-cell viability assessment by electrical evaluation of cell membrane integrity with microfabricated devices. *Sens. Actuators A* **105**, 31-39 (2003).
6. Choi, J.W. Cell-based biochip to analyze the effect of anticancer drug. *Biotechnol. Bioprocess. Eng.* **9**, 12-20 (2005).
7. Yea, C.H., Min, J. & Choi, J.W. The fabrication of cell chip for use as bio-sensors. *Biochip J.* **1**, 219-227 (2007).
8. Choi, J.W., Nam, Y.S. & Fujihira, M. Nanoscale fabrication of biomolecular layer and its application to biodevices. *Biotechnol. Bioprocess. Eng.* **9**, 76-85 (2004).
9. Blau, H.M. & Baltimore, D.J. Differentiation requires continuous regulation. *J. Cell Biol.* **112**, 781-783 (1991).
10. Ruoslahti, E. & Obrink, B. Common principles in cell adhesion. *Exp. Cell Res.* **227**, 1-11 (1996).
11. Woolley, D.E., Tetlow, L.C., Adlam, D.J., Gearey, D. & Eden, R.D. Electrochemical monitoring of cell behaviour in vitro: A new technology. *Biotechnol. Bioeng.* **77**, 725-733 (2002).

12. Burlage, R. & Kuo, C.T. Living biosensors for the management and manipulation of microbial consortia. *Ann. Rev. Microbiol.* **48**, 291-309 (1994).
13. Bery, M.N. & Grivell, M.B. An electrochemical description of metabolism. In: Walz, D., Berry, H. & Milazzo, G. Editors, Bioelectrochemistry of cells and tissues, Birkhauser, Basel, Verlag, pp. 134-158 (1995).
14. Thomas, C.A., Jr., Springer, P.A., Loeb, G.E., Berwald-Netter, Y. & Okun, L.M. A miniature microelectrode array to monitor the bioelectric activity of cultured cells. *Exp. Cell Res.* **74**, 61-66 (1972).
15. Jagner, D. & Graneli, A. Potentiometric stripping analysis. *Anal. Chim. Acta* **83**, 19-26 (1976).
16. Wang, J. *et al*. Remote electrochemical sensor for trace metal contaminants. *Anal. Chem.* **67**, 1481-1485 (1995).
17. Hatse, S., De Clercq, E. & Balzarini, J. Role of antimetabolites of purine and pyrimidine nucleotide metabolism in tumor cell differentiation. *Biochem. Pharmacol.* **58**, 539-555 (1999).
18. Ji, Y.W., Greg, P. & William, P.V. Cytotoxicity, DNA cross-linking, and DNA single-strand breaks induced by cyclophosphamide in a rat leukemia in vivo. *Cancer Chemother. Pharmacol.* **31**, 381-386 (1993).
19. Ren, S., Kalhorn, T.F. & Slattery, J.T. Inhibition of human aldehyde dehydrogenase 1 by the 4-hydroxycyclophosphamide degradation product acrolein. *Drug Metab. Dispos.* **27**, 133-137 (1999).
20. Ru, Q.H., Luo, G.A., Liao, J.J. & Liu, Y. Capillary electrophoretic determination of apoptosis of HeLa cells induced by trichosanthin. *J. Chromatogr. A* **894**, 165-170 (2000).
21. Poirier, M.C., DeCicco, B.T. & Lieberman, M.W. Nonspecific inhibition of DNA repair synthesis by tumor promoters in human diploid fibroblasts damaged with N-acetoxy-2-acetylaminofluorene. *Cancer Res.* **35,** 1392-1397 (1975).
22. Freshney, R. Culture of Animal Cells: A Manual of Basic Technique, p. 117, Alan R. Liss, Inc., New York (1987).