



Potentiometric biosensor for studying hydroquinone cytotoxicity *in vitro*

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ABSTRACT

Many processes in living cells have electrochemical characteristics that are suitable for measurement by potentiometric biosensors. Potentiometric biosensors allow non-invasive, real time monitoring of the extracellular environment changes by measuring the potential at cell/sensor interface. This can be used as an indicator for overall cell cytotoxicity. The present work employs a potentiometric sensor array to investigate the cytotoxicity of hydroquinone to cultured mammalian V79 cells. Various electrode substrates (Au, PPy-HQ and PPy-PS) used for cell growth were designed and characterized. The controllable release of hydroquinone from PPy substrates was studied. Our results showed that hydroquinone exposure affected cell proliferation and delayed cell growth and attachment in a dose-dependent manner. Additionally, we have shown that exposure of V79 cells to hydroquinone at low doses (i.e. 5 μ M) for more than 15 h allows V79 cells to gain enhanced adaptability to survive exposure to high toxic HQ doses afterwards. Compared with traditional methods, the potentiometric biosensor not only provides non-invasive and real time monitoring of the cellular reactions but also is more sensitive for *in vitro* cytotoxicity study. By real time and non-invasive monitoring of the extracellular potential *in vitro*, the potentiometric sensor system represents a promising biosensor system for drug discovery.

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1. Introduction

Human beings are exposed to thousands of naturally occurring and synthetic chemicals over a lifetime. Over 45,000 toxic chemicals were listed by the US National Institute of Safety and Health (NIOSH) in 1980. The traditional tools for *in vitro* cytotoxicity studies are microscopy (light and electron microscopy) and 96- or 386-well plates using either fluorescent markers or fixation procedures for cell viability (Jepras et al., 1995; Pike et al., 1993; Tsui et al., 1983). The multi-well plate format has several limitations including difficulty of (1) removal of reagents from the wells; (2) difficulty of subsequent washing of cell monolayers; (3) difficulty of addition of multi-reagents. It is also invasive due to the use of fluorescence labels or other reagents. Thus, non-invasive sensor based systems for on-line measurements of living cells *in vitro* represents a significant research direction in basic toxicology research as well as for reduction of animal experiments in drug screening applications (Andreescu and Sadik, 2005; Banerjee and Bhunia, 2009; Ziegler, 2000). Cultured cells transduce and transmit a variety of chemical and physical signals in response to extracellular stimulator, such as changes in pH, oxygen consumption, ion concentrations, impedance of cellular systems, membrane potentials, and release of proteins and metabolites. These signals are

usefully employed as parameters to obtain chemical information by using different transducers (Ehret et al., 1998; Haruyama, 2006). For example, electric cell-based substrate impedance sensing (ECIS) is achieved by measuring impedance change in response to cell behavior (Giaever and Keese, 1993; Male et al., 2008; Xiao et al., 2002). Light addressable potentiometric sensor (LAPS) monitors the extracellular acidification rate for detecting the cell physiological changes (Liu et al., 2007a,b; Owicki et al., 1994). Dielectrophoretic field-flow fractionation (dFFF) uses dielectrically detected changes in membrane capacitance and conductivity of cells (Pui-Ock et al., 2008). Open circuit potential (OCP) methods measure the potential change of cell adherent to bare gold electrode (Woolley et al., 2002a,b). In spite of these successful demonstrations, biophysical methods still need to be further developed and optimized for their implementation for applications in biomedical research to allow simple operation with accurate and sensitive measurement of cell activity.

It is well known that the unique properties of the cell membranes are responsible for the differences in the composition of not only the intracellular fluid but also the interstitial fluid. The cell surface can be properly described as an electrochemically dynamic system with electron generation and transfer on the interface (Grivell et al., 1995). The electrochemical signals are usually generated by redox reactions and changes in ionic composition derived from various cellular processes (Woolley et al., 2002b). Thus, these electrochemical signals are manifested at the surface of all living cells and the change of the total electrochemical signals can be an

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indicator for cell activities such as viability, proliferation, adhesion, and apoptosis (Arndt et al., 2004). When adherent cells are attached to the sensor surface, it would form a cell/sensor interface. The electrochemical signal change of the cell/sensor interface reflects the dynamics of the transport across cell membrane. Potentiometric sensors measure the potential of an electrode at equilibrium (i.e. in the absence of the appreciable currents) by measuring the electrochemical cell potential vs. a reference electrode potential (Koncki, 2007). Thus by measuring the electrochemical potential change of the sensor relative to the culture medium with a potentiometric sensor, the cytotoxicity of a chemical compound can be evaluated to support toxicological exposure assessment of a chemical. Recently, potentiometric biosensor array based simple cytotoxicity assay was demonstrated in our laboratory (Qiu and Zeng, 2008). Our results show that conductive polymers can be used as a porous ion-exchange membrane for controlled incorporation and release of ionic compounds (i.e. ionic liquid) for non-invasive *in vitro* potentiometric cell toxicity assay. The advantages of potentiometric sensors include sensitivity, non-invasion, real time, low cost and ease of miniaturization. These features are ideal for high throughput monitoring of cell properties using microarray format. Potentiometric sensors have been used to continually measure the rate of production of acidic metabolites of living cells, such as LAPS.

In this report, we intend to extend the methodology to a broad range of compounds, such as hydroquinone ([1,4-dihydroxybenzene, $C_6H_4(OH)_2$, HQ]), a widely used chemical that exists in cosmetics, medicines, cigarette smoke, the environment, the workplace, and human diet (Duarte-Davidson et al., 2001). It was reported that exposure of V79 (hamster lung fibroblast) cells to a non-toxic dose of N-methyl-N-nitrosourea, followed at intervals by exposure to toxic challenging doses of the same agent, resulted in increased survival of colony forming ability when these cells were compared with matched control cells that only received the challenging dose (Durrant et al., 1981). We were motivated to use conductive polymer based potentiometric sensor array in studying whether exposure of HQ to V79 cells can also lead to such enhanced survivability of V79 cells due to alterations in the ability to recover from cellular damage rather than by improved DNA repair.

HQ is a weak acid and has pKa of 11.56. It can be oxidized to benzoquinone. In cell, this oxidation can be enhanced by superoxide dismutase and results in the formation of H_2O_2 (Bolton et al., 2000a). HQ has long been known to cause myelotoxicity and leukemia in humans (Gowans et al., 2005; Winn, 2003). It is potentially a haematotoxic, genotoxic and carcinogenic compound (Andreoli et al., 1999; Bolton et al., 2000a; do Ceu Silva et al., 2003; Stockwin et al., 2007). Since HQ present in cigarette smoke induces chromosomal aberrations of lung fibroblast cells (do Ceu Silva et al., 2003), the V79 (hamster lung fibroblast) cells were selected as the model cell to study the toxicity of HQ. Conductive polymer polypyrrole (PPy) was used as the substrate for controlled incorporation and release of the HQ. PPy is biocompatible and is a suitable substrate for support of cell growth, function and processes (Barisci et al., 1996; Lange et al., 2008; Lee et al., 2006; Singh et al., 2006). PPy can be synthesized to contain a variety of (poly)anions. Thus, PPy provides a controlled microenvironment that allows the quantitative release of the anions of HQ into the cells and provides a versatile platform for a controlled cell toxicity study. In this work, both bare gold and polypyrrole (PPy) conductive polymers were studied as the substrate of the V79 cell growth and attachment upon exposure of HQ in cell culture and/or when HQ was doped in PPy matrix on the sensor surface. The real time extracellular potential changes were measured using a potentiometric sensor array. The potentiometric cytotoxicity assay described in this report is expected to facilitate assessing the potential health risk of the environmental chemical compounds in a much more quantitative and fast manner. The potentiometric cytotoxicity assay is non-invasive

and rapid, uses fewer cells and can be easily automated. Additionally, it can monitor complicated cell responses continuously and provide unique kinetic information throughout the entire course of the experiments.

2. Materials and methods

2.1. Materials

Chinese hamster lung fibroblast cell line (V79) was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). HQ, NaCl and Trypan blue were purchased from Sigma Inc. Poly(styrene sulfonic acid) sodium salt (PS) (MW 70,000) and pyrrole were obtained from Alfa Aesar (Karlsruhe, Germany). Pyrrole was doubly distilled before use. Solid 24K gold plates were purchased from Hoover & Strong Inc. (Richmond, VA, USA). Ag/AgCl reference electrodes were purchased from CH Instrument Co. (Austin, TX, USA). Biological grade water (resistance greater than 18 M Ω , and further radiated by UV light and filtered with a 0.2 μ m filter) was used in all of this experiment.

2.2. Cell culture and treatments

V79 cells were cultured in Dulbecco's modification of Eagle's Medium (DMEM, ATCC) plus 5% fetal bovine serum (FBS, ATCC) and 1% penicillin/streptomycin at 37 °C under 5% CO₂. Cells were removed by trypsin (ATCC) and then 1.4×10^5 cells were seeded onto the gold indicator electrodes or in six-well culture plates. HQ was dissolved in culture medium yield the desired concentrations. Photographs were taken using Nikon Microscope with a digital camera (SPOT, 1600 $\times$ 1200 pixels, Diagnostic Instrument Inc. USA).

2.3. Potentiometric cell sensor set-up

The set-up for the potentiometric cell sensor array that allows parallel evaluation of cell toxicity was described in our early report (Qiu and Zeng, 2008). Briefly, a 16-channel potentiometric sensor array using gold indicator electrodes vs. Ag/AgCl reference electrode was immersed in the culture medium. A Lawson EMF-16 precision electrochemistry EMF interface instrument (Malvern, PA, USA) was used to monitor potential change simultaneously in real time. All the electrodes were autoclaved and then 1 ml of the stock cell culture (containing 1.4×10^5 cells) and 1.5 ml of culture media were added to the working electrodes. Cells were allowed to grow for 48 h in a CO₂ incubator and the potential change was monitored in real time.

2.4. Conductive polymer biomembrane preparation

PPy membrane preparations were carried out with a Potentiostat–Galvanostat (EG&G PARC Model 283 with a software M270) and a three-electrode system with a modified gold working electrode (area 0.22 cm²), a platinum wire counter electrode and an Ag/AgCl reference electrode (saturated KCl) was employed. Before the polymerization of pyrrole, the working electrodes were polished on microcloths with alumina powder and cleaned with Piranha solution (H_2SO_4/H_2O_2 1:3). The doped PPy thin films were made using 0.01 M pyrrole monomer in different electrolytes by electrochemical oxidation of pyrrole monomer under a constant current of 10 μ A and time of 500 s. PPy-HQ was prepared in different concentration of HQ with 0.01 M NaCl as the electrolyte. PPy-PS was formed using 0.01 M poly(styrene sulfonic acid) sodium salt (PS) as the electrolyte. Cyclic voltammetry were used to characterize each PPy film made.

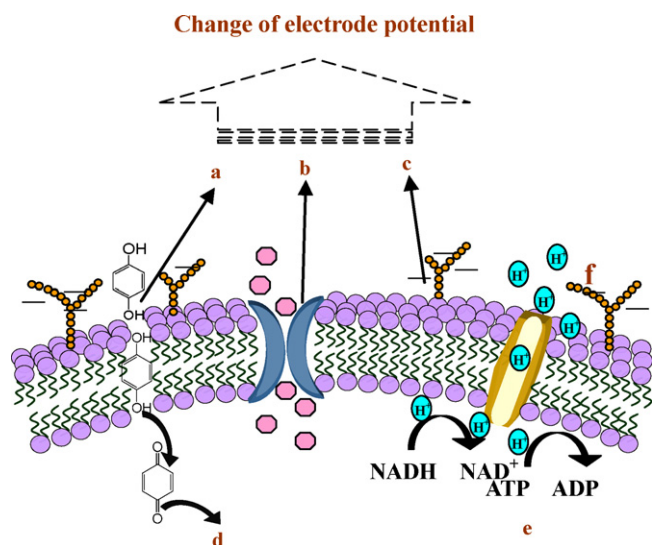


Fig. 1. The proposed mechanisms of potentiometric changes due to various cellular responses upon exposure to HQ. (a) Cellular metabolism changes. (b) Ion transport changes. (c) Cell morphology and growth changes. (d) Alteration of cellular redox states. (e) Redox reactions. (f) Glycoprotein array (negative).

3. Results and discussion

Many processes in living cells have electrochemical characteristics. For example, membrane potential, the potential difference across the membranes, exists in most if not all cells, showing that the inside of the cells is negative to the exterior (Tekle et al., 1990). Redox reactions and ionic composition exchanges derived from various cellular processes can lead to a potential change. Additionally, most cells have an outermost electrically negative zone created by the negatively charged sialic acid molecules that cap the tips of glycoproteins and glycolipids that extend outward from the cell membrane (Kitchen, 1996). This outermost zone of steady negativity makes each cell act as a negatively charged body. This means that every cell creates a negatively charged field around itself, so that when the cell attaches on the electrode surface, it will give a negative potential signal. Furthermore, intracellular HQ undergoes slow auto-oxidation to produce semiquinone radical and subsequently benzoquinone. The auto-oxidation of HQ can be enhanced by superoxide dismutase and results in the formation of H_2O_2 (Bolton et al., 2000a). This process will change the cellular redox environment. As shown in Fig. 1, the control of intracellular redox homeostasis plays a pivotal role in the regulation of cell death induced by HQ. Potentiometric sensors provide an overall observation of the cellular potential changes. They are very sensitive and can give quantitative information of the relationship between dosage and toxicity. Additionally, real time potentiometric sensors could be used to study and obtain the information of the critical non-toxic dose of HQ for V79 cells in which adaptive response can be reverse. In the experimental results discussed below, we used potentiometric sensors to characterize the cellular response induced by HQ.

3.1. Evaluation of HQ cell toxicity using different electrode substrates

The cell and electrode substrate interactions that allow or promote the cell attachment and growth are important for the potentiometric cytotoxicity assays shown in this report. We studied three-electrode substrates (Au, PPy-HQ and PPy-PS) for cell growth and attachment with and without exposure to the HQ. PPy substrates were studied to incorporate HQ by two methods: (1) electrochemical doping and (2) physical absorption. The rate of the

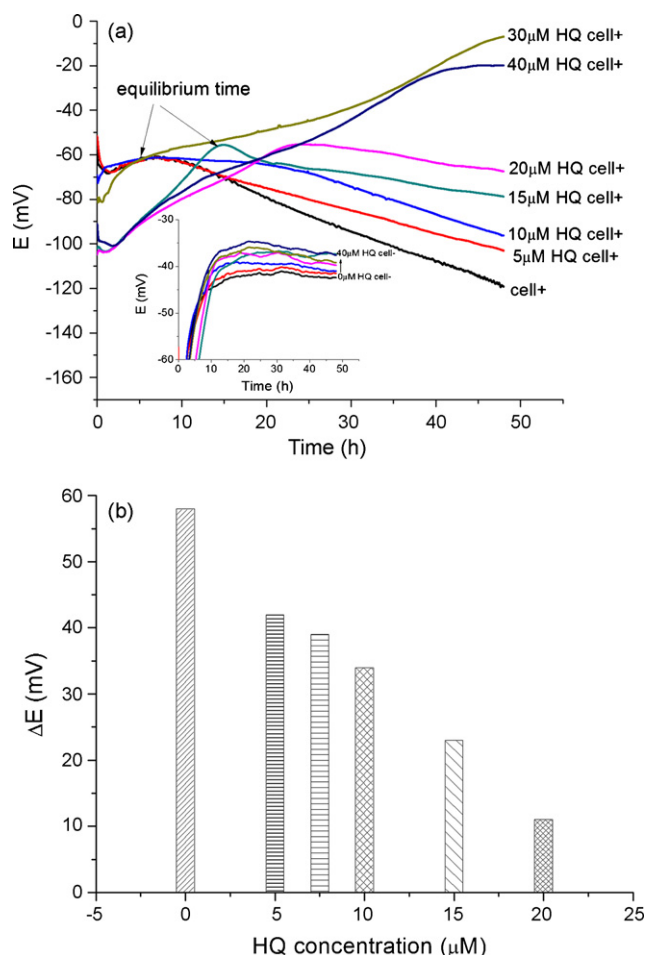


Fig. 2. (a) Potential vs. time curves of bare Au electrodes treated with different amounts of HQ (0, 5, 10, 15, 20, 30, 40 μM) with (cell+) for 48 h. Inset: curves of bare Au electrodes treated with different amounts of HQ (0, 5, 10, 15, 20, 30, 40 μM) without cultured cells (cell-) for 48 h. (b) Potential change vs. HQ concentration.

release of HQ from PPy substrate to the cells was controlled by the thermodynamics and kinetics of the ion-exchange processes or desorption.

3.1.1. Bare Au electrode

Au electrode was used since Au is inert and very stable. Au also has low toxicity to the cells. In order to see whether cells can attach and grow on the bare Au electrode surface, signal of the sensor with or without V79 cell was monitored. As shown in Fig. 2a, the electrode potentials quickly reached a constant when they were in the culture medium and hydroquinone only. However, the gold electrode potential shifted toward to a more negative direction when the cells were present. This implies that the formation of the dynamic cell sensor interface on the electrode will produce electrochemical signals and give rise to the negative potential change. It validates that the gold electrode provides a biocompatible surface for cell growth and adhesion. An important part of this investigation was to determine the dosage concentration of HQ exposed to V79 cell that can give rise to toxicity. To do this, different concentrations of HQ were added to the culture medium with or without cells and the potentiometric response of the sensor due to HQ exposure was measured. Fig. 2a shows that the slope of the negative potential shift decreased in the presence of HQ. This means that V79 cell is very sensitive to the toxicity of HQ. As HQ is added, the cell activity will be changed likely due to damaged transport dynamics across the cell membrane. Little negative potential shift was

observed when 30 μM HQ was added to the culture medium and the electrode potential shifted toward a more positive direction. As the microscope photos shown (Fig. 5F), when cell were exposed to 30 μM HQ, majority of the cells floated in the culture medium with only a few of the cells attached on the culture plate as a dot. The few cells attached were also significantly different from the normal cells. They could not grow and spread. It was rationalized that a positive potential shift indicated that the cells exposed to 30 μM HQ changed their normal cellular dynamics. Thus, the potentiometric sensor is very sensitive to monitor the cellular reactions. When cells were exposed to 40 μM HQ, none of the cells were able to attach and grow on the culture plate and as expected, an equilibrium potential was obtained (Fig. 2a) suggesting the discontinuing of cellular processes of the cells.

Results in Fig. 2a showed that it took several hours before the signals of the negative potential shift were observed in cell culture. In the potentiometric method, the potential measured as the living cells attached to the electrode could be the result of overall changes of extracellular environment. However, the signals will be observed only when the cells have attached on the electrode surface and formed a unique cell-electrode interface. It typically took several hours for the cells to attach and grow on the electrode surface. So the initial time scales reflect more about the cell attachment and growth rather than the ionic transport between cell membranes and electrode interface. With addition of 5, 10, 15, 20 μM HQ, the equilibrium time (t) prolonged from normal 6.5 to 7.2, 8, 15, 20 h. Thus, the delay of the time to reach equilibrium potential is due to the delay of cell growth and attachment and can be used as an indicator of the HQ toxicity. After the equilibrium potential is reached, the potential shifted toward the negative direction. The negative potential shift indicates the cell is still viable and the cell maintains a normal dynamic transport across the cell membrane and membrane potential. Fig. 2b summarizes the absolute change of the negative potential shift ΔE (i.e. the potential change from equilibrium time (t) to the end of the experiment) when the cells were grown in the culture medium with various concentration of HQ (5, 7.5, 10, 15, 20 μM). It was shown that the higher concentrations of HQ added, the lower the negative potential shift. This means that the cell toxicity of HQ is dose-dependent. The slope ($\Delta E/48-t$) indicates the rate of the cell proliferation on the electrode substrate. The slope increased (-1.4 , -1.0 , -0.9 , -0.6 , -0.5 mV/h, respectively) as 0, 5, 10, 15, 20 μM HQ added (Fig. 2a). This indicates that HQ reduces the rate of cell proliferation and changes the redox environment of the cell.

3.1.2. PPY-HQ conductive polymer substrates

Cation, anion and neutral molecules can be doped into or released from PPY films since PPY can be “switched” between a cation-exchanger state of the electrode at negative electrode potentials and an anion-exchanger state at more positive potentials (Hepel, 1996). In this work, HQ was doped into PPY film as anion molecules during the electropolymerization of pyrrole under galvanostatic condition. For characterizing the electrochemical behaviors of the modified electrodes coated with conductive polymer and V79 cells, the cyclic voltammograms were recorded (Fig. S1). After coating with PPY-HQ films, the characteristic redox peak of PPY was observed, and oxidation peaks of bare Au electrode disappeared. This means PPY-HQ was immobilized on the gold electrode successfully. The PPY redox peak vanished after the V79 cells were seeded, confirming that V79 cells grew on the PPY-HQ modified electrode and the redox behavior of PPY was inhibited by the layer of V79 cells. Fig. 3 gives the potential change of cell growth on the PPY-HQ conductive films with various concentrations of HQ doped (0, 5, 7.5, 10, 15, 30 μM). The negative shift of the electrode potential was observed as expected. The rate of the negative potential shift decreased with increasing HQ dopant con-

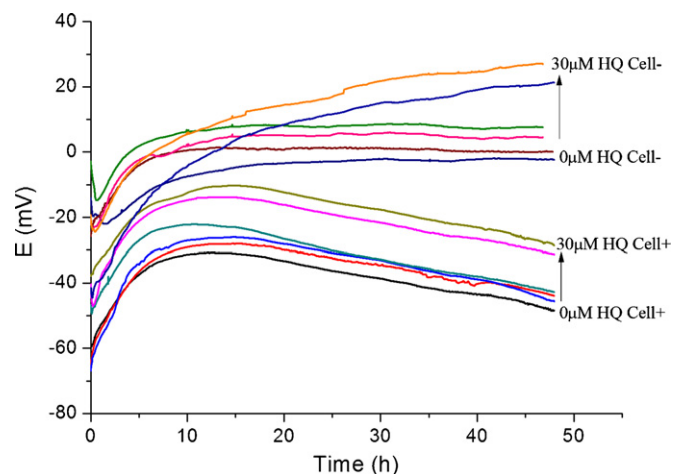


Fig. 3. Curves of potential vs. time for PPY-HQ substrate treated with different amounts of HQ (0, 5, 7.5, 10, 15, 30 μM) with (cell+) and without (cell-) cultured cells for 48 h.

centration, indicating that some doped HQ anions (benzoquinone) were released from the PPY-HQ films and interacted with the cells. This process was further studied by quartz crystal microbalance (QCM), a mass sensor. When the PPY-HQ coated Au quartz crystal was immersed in culture medium, frequency increases were observed, indicating the loss of mass due to HQ desorption (Fig. S2). Compared to the results using bare Au substrate in which 30 μM is lethal dose to V79 cells, the potential still shifts toward the negative direction indicating some cell viability when 30 μM HQ was doped in the PPY substrates. It can be rationalized that a limited amount of hydroquinone had been incorporated into PPY during its electropolymerization. Additionally, strong electrostatic interactions between PPY and doped HQ anions reduce the rate of HQ released from the PPY films. Thus, conductive polymer substrates can provide the control of toxic chemical release rates by fine tuning the ion-exchange processes compared to those methods in which the toxic chemicals under study were added to the culture medium directly.

3.1.3. PPY-PS conductive polymer substrates

PPY-PS was made to absorb HQ directly in comparison with the PPY-HQ film. The PPY-PS substrate is non-toxic to the V79 cells. The HQ was doped into PPY-PS film by adding different concentrations of HQ on the PPY-PS films and allowing them to absorb overnight. Then the modified PPY-PS-HQ substrate potentials with or without the cultured cells were monitored. The PPY-PS film thickness was varied by varying the time of electrochemical polymerization PPY-PS film (from 200 to 2000 s). Our results showed that the PPY-PS films made with 500 s electrochemical polymerization time were the best for cell growth and attachment. The PPY-PS uptake and release of hydroquinone anion were monitored by QCM (Fig. S3). The frequency decreased as HQ was absorbed in the PPY-PS film. When putting these films in the culture medium, the frequency would increase. These frequency changes were ascribed to the mass of HQ desorbed or released from PPY-PS film. As shown in Fig. 4, when various concentrations of the HQ (0, 0.1, 0.5, 3, 5, 7.5, 10 μM) were absorbed into the PPY-PS substrates without cultured cells, there was almost no negative potential change. A higher amount of HQ absorption results in a more positive shift in the PPY-PS substrate potential. The physical absorption of HQ onto PPY-PS substrate seems to have a larger effect on cell growth and attachment, since when HQ concentration was more than 5 μM , no negative potential shift was observed. Most likely, the physically absorbed HQ is released easily in the PPY-PS film compared with that PPY-HQ

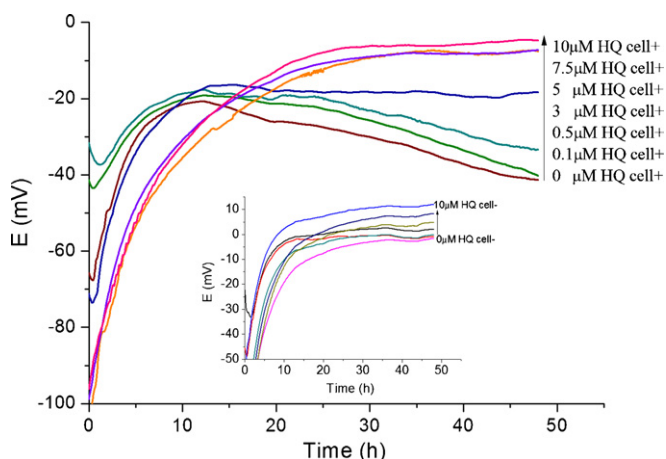


Fig. 4. Curves of potential vs. time for PPY-PS substrate treated with different amounts of HQ (0, 0.1, 0.5, 3, 5, 7.5, 10 μM) with cultured cells for 48 h. Inset: curves of potential vs. time for PPY-PS substrate treated with HQ without cultured cells for 48 h.

in which HQ was directly electrochemically doped in PPy film and was bound by electrostatic interaction to cationic PPy membranes.

3.2. Evaluation of HQ cell toxicity using microscope

In order to confirm our potentiometric results, parallel microscope photos and counting assay were performed to study the HQ dose dependant cytotoxicity on V79 cells using identical experimental conditions, i.e. the same concentrations of hydroquinone on the same number of the V79 cell cultured under the same culture conditions. V79 cells morphological change upon exposure to HQ in cell culture was studied using optical microscope. Six-well cell culture plates were used for cell culture. Each well bottom had an area of 9.5 cm^2 . A cross mark (“+”) was drawn on the bottom of each well to mark where photos should be taken. HQ solutions of 0, 5, 7.5, 10, 15, 30 μM were added into the six wells, respectively, from $t=0\text{ h}$ and then at about every 2 h interval, each well was put under the inverse microscope with a digital camera to take photos. Fig. 5 gives the images of V79 cells after 24 h of culture. The normal V79 cells were distributed evenly over the entire surface and grew healthily in the culture medium. They are usually triangular or rhombic in shape. When 5 μM HQ was added, cells were

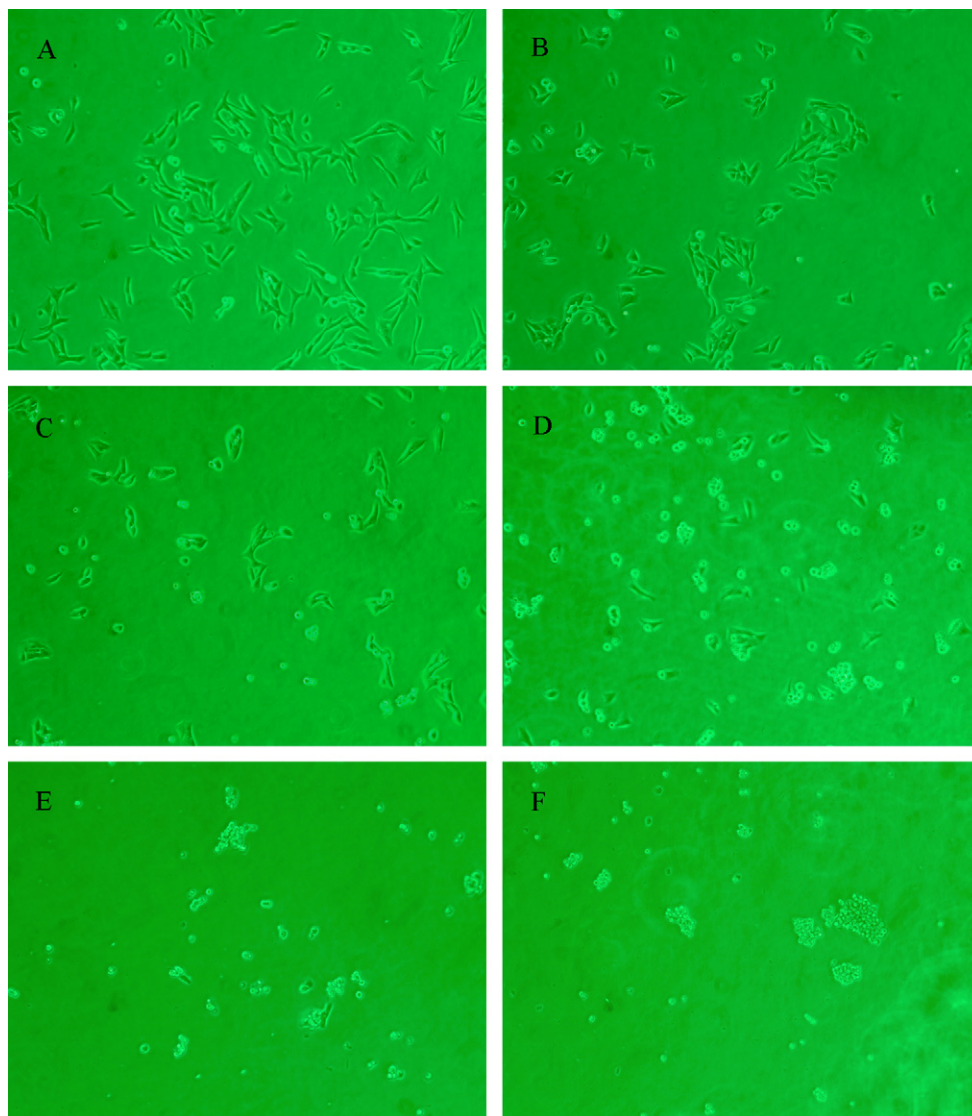


Fig. 5. Images of V79 cells treated with different amounts of HQ after 24 h (the photographs were taken under $100\times$ magnification). (A–F) 0, 5, 7.5, 10, 15, 30 μM HQ.

not as big as the normal cell. This indicates that the attachment and growth of cell were delayed. The number of the cells attached decreased with increasing HQ concentration. More and more cells were observed floating in the culture medium as dots and some clustered together when higher concentrations of HQ were added. There were also some cells spreaded on the substrate when exposed to 5, 7.5, 10, 15 μM HQ indicating some cell viability. Almost all the cells floated in the culture medium when 30 μM of HQ was added; few cells were attached on the surface as a dot.

Cell viability was also determined by the treatment of Trypan blue, which stains non-viable cells in a dark blue color, whereas viable cells do not absorb the dye. The number of cells was counted by hemacytometer under a microscope after 48 h, and fractional viability was calculated by dividing the number of clear cells by the total number of cells. Considering the cell viability for control cell as 100%, the results for the effect of hydroquinone on the viability of V79 cell obtained from the Trypan blue exclusion assay showed that as the hydroquinone concentration increased the cell viability decreased (Fig. S4). These results are similar to what we obtained by using potentiometric method. Compared with these traditional methods, potentiometric methods not only detect the cell viability but also observe the cell physiological change in real time. It is rapid and has high sensitivity and stability for *in vitro* cytotoxicity studies.

3.3. Cell adaptive response by HQ

It is well known that microbes have evolved to adapt to the environmental conditions of their particular ecosystem and respond to a myriad of changes and challenges in order to survive. Over more than two decades the existence of an adaptive response has been reported and there is growing evidence of its importance in cellular reactions to against toxic injury (Frosina and Abbondandolo, 1985; Leonard, 2007). It was reported that pre-treatment of Chinese hamster V79 cells with MNU increases survival without affecting DNA repair or mutagenicity (Durrant et al., 1981). We studied V79 cells adaptive response under sustained exposure to low-level dose of HQ. From our early studies, we show that V79 cells exposed to 5 μM of HQ in culture medium are able to grown and attach. This study suggests that 5 μM of HQ concentration was not the lethal dose to V79 cells but when V79 cells exposed to 30 μM HQ in culture medium, the cells were unable to grow, indicating that the concentration of 30 μM of HQ was the lethal dose to V79 cells. In this experiment, we used 5 μM as the non-toxic dose and 30 μM HQ as the lethal dose. 30 μM HQ was added into the culture medium after V79 cells were pre-treated with 5 μM HQ for 4, 15 or 24 h. From Fig. 6, it was observed that when the V79 cells were exposed to 30 μM HQ after growth for 24 h, or after pre-treated with 5 μM HQ for only 4 h, the potential shifted towards positive direction, which was the same as exposed to 30 μM alone. This means the cell did not have the adaptive response yet. But if V79 cells were pre-treated with 5 μM for 15 or 24 h, a much longer exposure time, the potential shifted in a negative direction, and pre-treatment for 24 h gave a sharper negative potential drift. This result was significantly different from those when the cells were exposed to 30 μM alone. This result demonstrates that low dosage of HQ can induce adaptive response in V79 cells, e.g. pre-treatment with 5 μM HQ for certain time. It also confirmed that cells can adapt to the presence of the toxic chemical rendering subsequent treatment with high doses of the same agent less effective; the longer pre-treatment time, the better adaptive response. This result is similar to those reported by Li et al. (2006) that low levels of HQ can induce an adaptive response in MRC-5 cells using alamarBlue assay. Compared with alamarBlue assay, our potentiometric method shows great promises as a tool for drug resistance study as it is real time and gives more visualized results of the cell adaptive response.

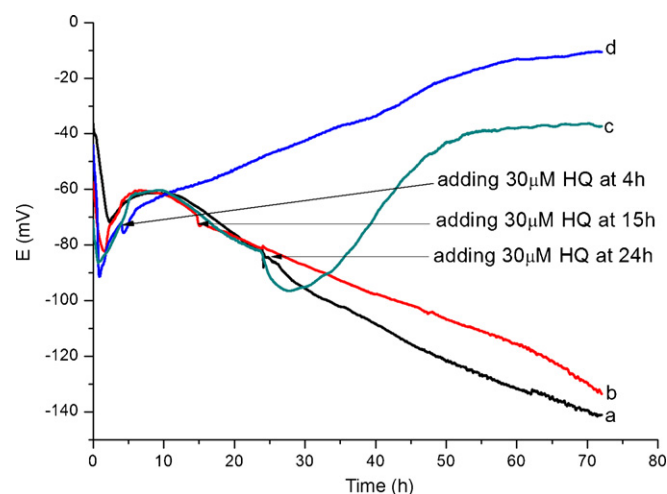


Fig. 6. HQ induced adaptive response of V79 cells. (a) Cells were exposed to 5 μM HQ for 24 h followed by treatment with 30 μM HQ; (b) cells were exposed to 5 μM HQ for 15 h followed by treatment with 30 μM HQ; (c) cells were exposed to 30 μM HQ after 24 h without pre-treatment; (d) cells were exposed to 5 μM HQ for 4 h followed by treatment with 30 μM HQ.

4. Conclusion

Our results in this report have further validated that a potentiometric based biosensor can be used for *in vitro* cytotoxicity assay by measuring the potential changes caused by the changes of various cellular processes upon exposure to a toxic chemical, HQ. The toxicity of HQ to various cell activities such as cell viability, recovery and proliferation can be detected by monitoring the potential changes when cells were cultured in various electrode substrates. Conductive PPy films were further tested as a controlled substrate for HQ incorporation and release via physical absorption or ion-exchange processes. We studied the controllable release of hydroquinone from PPy substrates as well as the reversibility of drug effects by evaluating cell adaptive response by the real time potentiometric biosensor. It was shown that physical absorption released HQ much faster than that of electrochemically doped HQ in PPy films. Our result was confirmed by the parallel study using traditional microscope and counting cell methods. Compared with traditional methods, the conductive polymer based potentiometric biosensor system allows the ease of controlled doping and release of various reagents from the substrates without the need for the subsequent washing of cell monolayers. It can monitor complex cell responses continuously and provide unique kinetic information throughout the entire course of the experiment. Due to the ease of operation, high-through measurement and real time detection, this technique may hold great promise for cell-based assays that enable drug toxicity detection and screening. However, work still needs to be done to increase the specificity of this biosensor system so that the chemical and mechanical complexity of the cellular systems can be understood. In order to measure more specific ionic transport, we plan to incorporate an ion selective electrode in our potentiometric biosensor, in future work, to measure dynamically the cell ionic transport or fluxes. This will allow us to measure one single parameter of cell-substrate contacts in a more specific manner so that the mechanical complexity of the cellular systems can be more greatly understood.

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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.2009.10.027](https://doi.org/10.1016/j.bios.2009.10.027).

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