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Cell chip composed of nanostructured layers for diagnosis and sensing environmental toxicity

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Outline:

Introduction

Cell-based research has been applied in a wide variety of fields, such as pharmacology, medicine, cell biology, toxicology, basic neuroscience, and environmental monitoring. In vitro assays are popular methods for drug screening or assessment of chemical toxicity because they can monitor effects of chemicals more easily and readily than any other method, including animal-based research. It is well known that cell is a basic building block of all kinds of living organisms. Therefore, useful informations obtained from a living cell reflects information of the respective tissue, organ or even whole living organism. Hence, using cells, effects of drugs, toxins, or functional particles can be easily and accurately monitored. This is not possible in protein/DNA analysis or in animal-based tests. Many techniques incorporate optical or fluorescence methods, which may cause unwanted signal errors or variations due to light interference or photo-bleaching effects. They can cause critical errors in determining the cellular responses, whereas, the cell chip consisting of a conducting surface with a chamber for cell immobilization has been developed to improve accuracy and compatibility by detecting redox or electrical reactions via electron generation and transfer on the cell-electrode interface [1]. A variety of electrochemical sensing techniques have been developed to detect the cellular signal, such as open circuit potential at the cell/sensor interface, electric cell-substrate impedance sensing (ECIS), and electrochemical impedance spectroscopy (EIS) [2]. These electrochemical tools have been used to assess the effects of anticancer drugs, histamine toxicity, cell viability, and cell proliferation [3,4,5,6]. Each of these methods detected cellular behavior sensitively; however, they also detect voltammetric signals, which are strongly dependent on cell adhesion to electrode surfaces. These findings are very important to the field of electrical detection of cellular response because most cells anchor weakly on the artificial electrode surface due to insufficient amounts of positively charged extracellular matrix (ECM) proteins [3]. Hence, modifications of chip surfaces using cell adhesion motifs are of great interest in the fabrication of a cell-based chip.

Cellular behaviors (e.g., adhesion, migration, proliferation, and differentiation) are known to be sensitive to the bioactivity, interspacing, and density of surface RGD ligands on artificial ECM materials. Cell surface receptors play a major role in establishing links between cell and artificial surface. Several ECM proteins, such as fibronectin, collagen, laminin, and their components (RGD, PLL, etc.), possess excellent ability to immobilize cells on metal surfaces via integrin receptor-based linking [7,8]. The cell adhesion process involves complex mechanisms; however, most are related to integrin-mediated cell adhesion because integrin connects cytoskeleton to the ECM components that provide strong attachments [1,8]. Consequently, homogenously structured $C(RGD)_4$, RGD-MAP-C, and collagen produced by self-assembly techniques have been used to attach living cells to chip surfaces. The RGD motifs successfully linked the $\alpha_v\beta_3$ domain of integrin to the Au surface, but the large portion of the motif not used for cell attachment blocked electron transfer around the cell surface and decreased the sensitivity of electrochemical signals [1,3]. Therefore, the spatial organization of the integrin-specific domain of ECM components on artificial substrates has been investigated as an approach to improve cell adhesion and maintain high electrical sensitivity; the nano-scale RGD ligand patterns increased cell adhesion more effectively than monolayer peptides [1,9].

Cell-artificial surface interactions have attracted considerable attention due to the difficulty of cell immobilization on artificial surfaces whilst maintaining *in vivo*-like conditions, which is the most important factor in *in vitro* research. In this chapter the details of cell based chip fabrication for electrochemical analysis of living cell based on electrochemical dynamics at cell-electrode interface have been discussed. Cysteine terminated $C(RGD)_4$ peptide film was fabricated on a gold electrode for improving the attachment of cells [1,3,9]. The comparative efficacy of several biomaterials, such as synthetic $C(RGD)_4$, RGD-MAP-C peptide, and poly-L-lysine on cell adhesion, proliferation and

electrochemical signal transmission were studied. RGD-MAP-C provided the strongest voltammetric signals when the chip was subjected to cyclic voltammetry and differential pulse voltammetry [1]. It was also observed that nanopatterned peptides are more suitable than nonpatterned monolayers. Amongst the nanopatterned peptides three dimensional RGD nanopillars arrays were found to be more suitable than RGD nanodots and RGD nanorods [9]. Recently, a newly fabricated RGD nanopillar array was applied as a novel platform for the electrochemical determination of cell-cycle-arrest, where a cell-based chip has been employed for the assay of electrochemical redox property from cell at different phases of growth cycle [10]. In addition, phase specific cytotoxicity of BPA and PCB were analyzed using the cell-chips completely synchronized at G1/S and G2/M phase, respectively [11]. This newly developed chip-based living cell detection system can be a useful tool for diagnostic applications. The current disease diagnosis methods commonly based on conventional cell culturing process are costly, laborious and susceptible to contamination. Cell chip based methods may overcome above limitations due to their simple fabrication process and rapid detection techniques. Moreover, the accuracy and high sensitivity prove the potential of cell based chip for biological and clinical testing and disease diagnosis in the near future.

Cell-based Chip design and fabrication

Cell chip chamber design and fabrication is the first step of the cell chip based research. Size and shape of a chip chamber greatly influences the cumulative signal intensity arise from the numbers of cells cultured on it. The cell numbers vary from single to millions depending on exposure arrears of the chip. The chip designed with micro scale exposure area for cell attachment is known as microchip [12-18]. Integration of several microchips on a single silicon support has been used for obtaining cumulative signal intensities since the last decade [1,3,9-11]. Recently single chip with centimeter scale exposure area has been simply fabricated on silicon support [1,3,9-11]. According to Choi's group cell chip chamber (Lab-Tek®, Thermo fisher scientific, USA) of 2 cm × 2 cm × 0.5 cm (width × length × height) dimensions created on freshly prepared Au working electrodes with an area of 3 cm^2 is the most suitable design for appropriate electrode positioning to achieve maximum signal intensities [1,3,9-11]. In most cases chip chamber are established on a silicon based Au working electrode [3, 19]. A 50-nm thick titanium (Ti) layer is established on the silicon substrate and then a 150-nm thick gold (Au) layer patterned by DC magnetron sputtering [3,19]. The Au surface is cleaned with piranha solution previously described elsewhere [1,3-6,9]. It is then polished carefully by sonication in absolute alcohol and double-distilled water for 5 min, respectively. Finally, the electrode is electrochemically cleaned in 0.5 M H_2SO_4 until a stable cyclic voltammogram is obtained and dried with purified nitrogen [10]. To develop an adhesion molecule (AM) layer on the Au surface, a freshly cleaned Au substrate is incubated in desired concentrations of AM solution diluted in distilled water at 37^0C for 24 h. for the formation of cell immobilization platform [1,3, 9-11]. Finally, the substrate is washed with deionized distilled water and dried under N_2 gas. Schematic of the fabricated cell based chip is shown in figure 6.1.

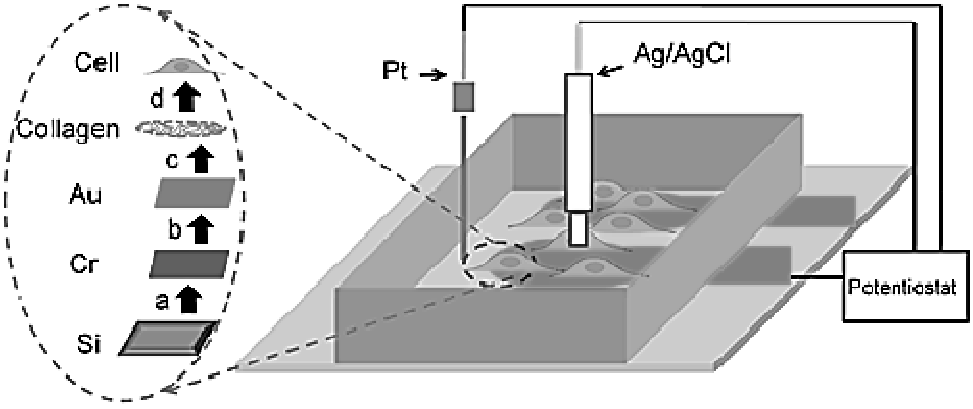

FIGURE 6.1
Schematic of a cell-chip: the dotted circle shows the steps of fabrication, (a) sputtering of 50nm titanium on silicon, (b) establishment of 150nm Au, (c) collagen coating and (d) cell seeding. Figure reproduced with permission from: ref. 42, © 2011ASP.

Establishment of Nanostructured bio-ligand molecule on chip surface

Surface engineering of a bio-platform creates materials which elicit controlled cellular adhesion and plays an important role in the transmission of intracellular signals to extracellular surfaces [2]. Some extracellular matrix (ECM) components, such as laminin, fibronectin, collagen, and their functional domains (Arg-Gly-Asp (RGD) motif) actively promote cellular adhesion via interactions with integrin receptors [20]. The specific conformation of the RGD amino acid sequence in the ECM proteins determines its specificity for different integrin subtypes [21]. Following the discovery of this RGD small active domain, numerous other adhesion peptide sequences have been isolated [22]. Some are specific to particular cell types or functions through binding to distinct integrin subtypes [23]. The RGD sequence is one of the most effective cell recognition motifs. The RGD sequence stimulates cell adhesion on artificial surfaces, involves a cascade of four overlapped reactions such as cell attachment, cell spreading, actin-skeleton formation, and focal-adhesion formation, and is important for transmitting cell signals related to cell behavior and cell cycle [24-25]. RGD peptides do not only trigger cell adhesion effectively, but can also be used to address selectively certain cell lines and elicit specific cell responses [26]. So, RGD peptides immobilized on a substrate enables cell adhesion and mimics the cellular signals [27]. Therefore, Choi`s group designed Cystein (Cys) terminated RGD tripeptide sequence for specially immobilizing on Au surface via thiol-gold (S-Au) coupling method (Figure 6.2). The designed peptides ($C(RGD)_4$ and RGD-MAP-C) were synthesized from Peptron (Korea).

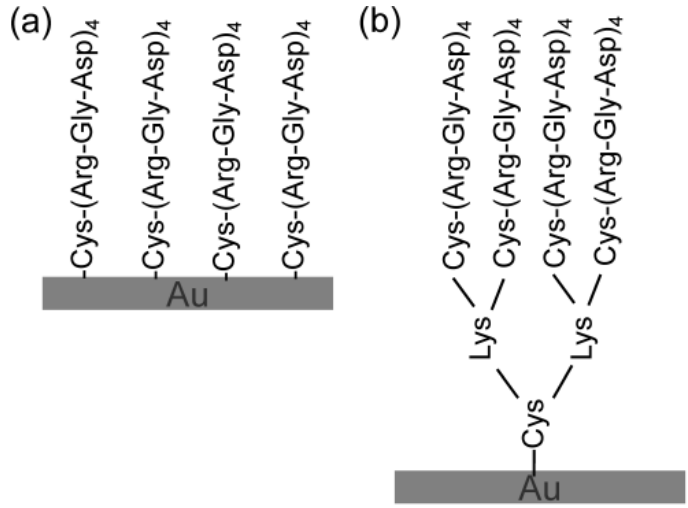

FIGURE 6.2
Schematics of the $C(RGD)_4$ (a) and RGD-MAP-C (b) peptide immobilized on Au surface. Figure reproduced with permission from: ref. 3, © 2007 Springer.

In addition to natural biomolecules and peptides, some non-native proteins/peptides have been shown to promote cell adhesion. Poly-L-lysine modulates cell adhesion via a non-receptor- mediated cell binding mechanism [11]. Positive charges on poly-L-lysine attract the negatively charged cell membrane resulting in electrostatic bond formation [11]. Prior to the PLL immobilization the Au surface is functionalized with MUA-11 self-assembled monolayer (Figure 6.3).

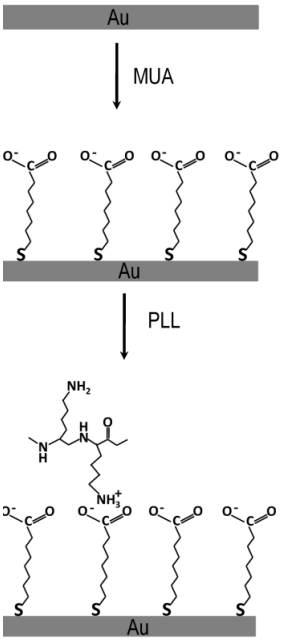

FIGURE 6.3
Immobilization of PLL on MUA functionalized Au surface. (Reproduced with permission from: ref. 11, © 2013 Elsevier).

In vitro nanoscale assembling of molecular building blocks such as nucleic acids, proteins and phospholipids, biological organisms have been used as a versatile tool in nanotechnology. In a recent study, thiol self-assembly was achieved by reacting thiol containing compounds with clean gold surfaces (Figure 6.4). Sulfhydryl groups on molecules will covalently bind to gold, thus allowing molecules to be arranged two dimensionally over a gold surface [3,19]. This is very useful since gold conducts electricity and makes for excellent electrical contacts, thus electrochemical measurements can be made on such samples. Therefore, cell adhesion molecules were mutagenically modified with cysteine residues (an amino acid containing a thiol group) [1,3,19]. Exposing a gold surface to such engineered molecules results in self-assembled monolayer's of cell adhesion molecules.

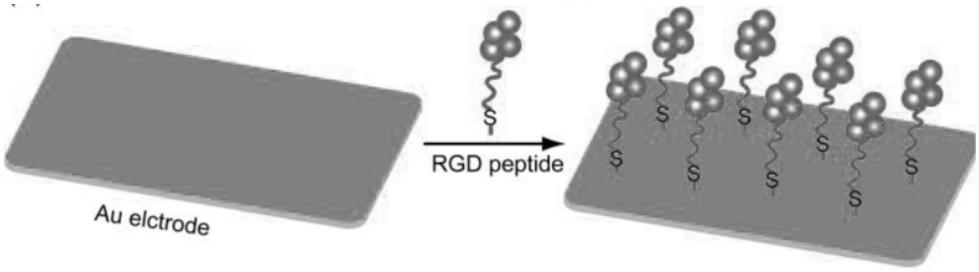

FIGURE 6.4
Self assembly of Cysteine terminated RGD peptide on Au surface. (Reproduced with permission from: ref. 43, © 2011ASP).

To develop an oligopeptide layer on the Au surface, the Au substrate was incubated in the $C(RGD)_4$ solution diluted in distilled water at 37°C for 10 hours [1,3,9,19]. Different concentrations of the $C(RGD)_4$ peptide varying from 0.05 mg/ml to 0.1 mg/ml can be applied for the formation of cell immobilization platform [6]. The optimum cell adhesion efficiency is achieved using a peptide concentration of 0.1 mg/ml [1].

In surface engineering, nano-patterned biomaterials are of great significance to enhance receptor specific coupling or trapping target molecules. In the patterned surface biomolecules are aliened according to the receptors specificity with a desired spacing. On a cell chip the adhesion molecules are nano structured according to the receptor availability on the cell surface. Therefore, cells are firmly attached to the chip surface that can withstand several washing steps during the electro analysis process. In the past decade several top down (Externally directed nanopatterning: Nanoimprint lithography, Scanning probe lithography, Atomic manipulation) and bottom up (Self-assembly) processes are employed for patterning biomolecules in a desired pattern. Self-assembly of biomaterials has become a popular method due to its simple and ease of fabrication process. Recently, Choi`s group introduced a new modification of self-assembly method, termed as Mask Guided Self Assembly Method (MGSAM) [1] (Figure 6.5). For this, a porous alumina (AAO) membrane was fabricated by a two-step anodization method, as previously described [1,9-10]. In brief, nanoporous AAO was obtained from aluminum anodized at a constant voltage of 40 V in oxalic acid solution. The alumina layer formed during the first long period of the anodization process was removed by wet etching [28]. This treatment revealed the periodic nano-concave patterned surface of the aluminum. The nano porous alumina membrane was placed on the freshly cleaned, smooth Au surface and fixed by adding a drop of acetone. Subsequently, a treatment consisting of 0.01 mg/ml of various peptides diluted with DI water was added separately on the porous AAO membrane and was maintained at 12 hours at 4^0C. The electrode was placed in a 2 M NaOH solution for 3 min to remove the AAO membrane form the Au

surface, followed by washing with DI water and drying under nitrogen steam (the peptide-modified electrodes are denoted as Au/C(RGD)$_4$, Au/RGD-MAP-C, and Au/PLL, respectively) [1,9].

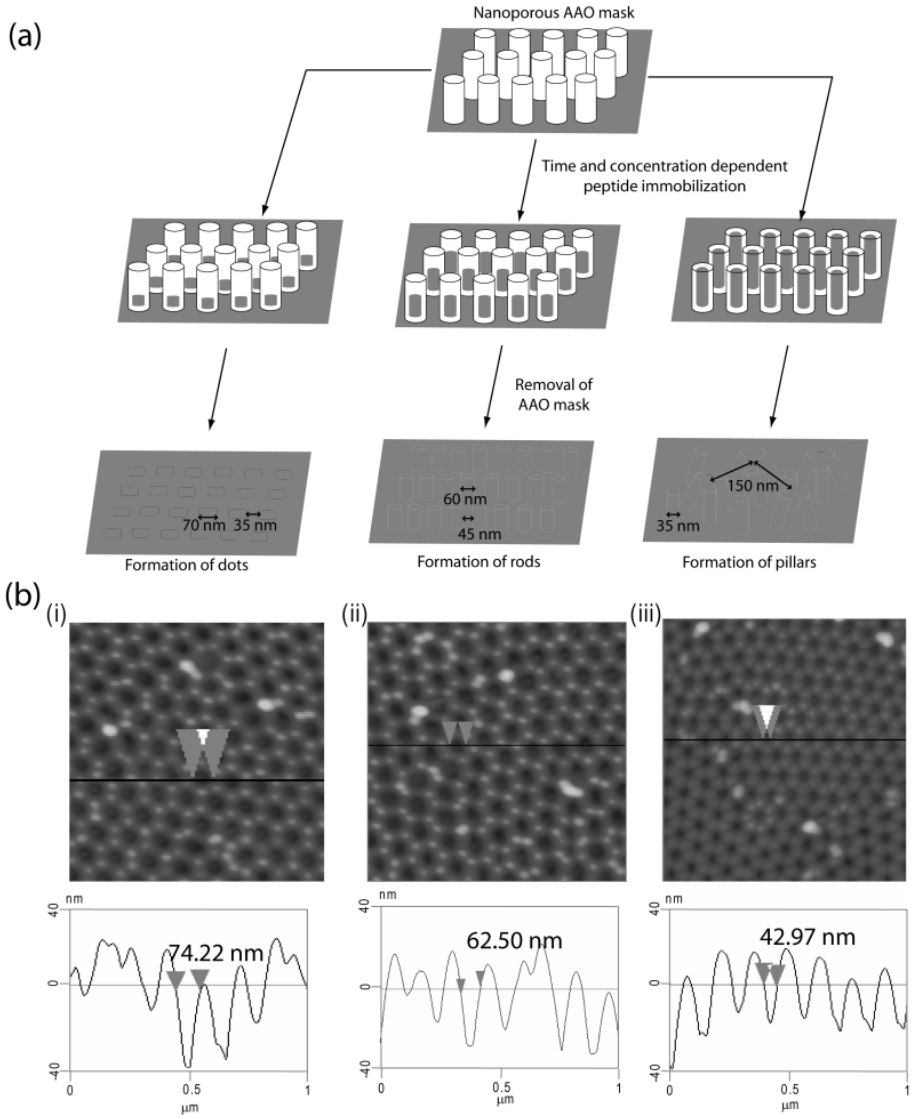

FIGURE 6.5
(a) Schematic of fabrication of various topographic RGD nanopattern, (b) AFM images of mask with varying pore size with their respective cross section analysis. (Reproduced with permission from: ref. 9, © 2012Elsevier).

Cell immobilizations

In vitro immobilization of living cells is an important process in the fabrication of a cell-based chip [29]. The interaction between cell-cell and the adhesion of cells onto the chip surface can be a reliable candidate for cellular attachment without loss of viability. The cell adhesion process involves complex mechanisms; however, most are related to integrin-mediated cell adhesion because integrin connects cell cytoskeleton to the ECM components that provides strong attachment [30-31]. At the development of a neuronal cell chip with PC12 cells established from a rat pheochromocytoma cells, a major drawback is that the cells anchor to the chip surface weakly because of insufficient amounts of positively charged ECM proteins [32]. Modification of the chip surface with ECM proteins such as collagen was reported to enhance the attachment of the cell types [33]. However, the surface modification with the ECM proteins is not effective for measuring electrochemical signals due to the protein natures [34-35]. Choi`s group immobilized newly designed different architectures of cysteine modified RGD tri-peptide sequence (C(RGD)4, RGD-MAP-C) on gold (Au) surface using the self-assembled monolayer (SAM) technique to promote the binding of PC12 cells because the strong integrin affinity of RGD influences the binding capacity of cell immobilization.

The RGD motifs successfully linked the αvβ3 domain of integrin to the Au surface [2], but the large portion of the motif that was not used for cell attachment blocked electron transfer around the cell surface and decreased the sensitivity of electrochemical signals [3]. It is well known that cellular behaviors (e.g., adhesion, migration, proliferation, and differentiation) are quite sensitive to the bioactivity, interspacing, and density of surface RGD ligands on artificial ECM materials [36-37]. Therefore, the spatial organization of the integrin-specific domain of ECM components on artificial substrates has been investigated to improve cell adhesion and maintain high electrical sensitivity; the nano-scale RGD ligand patterns increased cell adhesion more effectively than monolayer peptides [1,3,9].

Besides studies related to investigation of ECM materials to facilitate cell adhesion on the artificial surface, research regarding the fabrication of nanopatterned surfaces has been conducted to determine the influence of surface topology on cell adhesion. The spacing and height of Au nanoparticles deposited on a surface, which is subsequently coated with ECM protein, influences cell adhesion, motility, and spreading [38-39]. We recently observed that RGD peptides containing cysteine residue can be fabricated easily on Au surface (e.g., as a homogeneous nanodot array using the self-assembly technique), and promote cell adhesion without decreasing the sensitivity of electrochemical detection. Our group fabricated two types of cysteine-modified peptides, $C(RGD)_4$ and RGD-MAP-C, and PLL peptide nanodots, rods and pillars on Au surfaces via the self-assembly technique through an AAO mask [1,9]. The performance of the fabricated nanostructure was intensively evaluated with respect to the cell adhesion speed, attachment strength, spreading, cofilin phosphorylation, and mitochondrial activity. Cell functions significantly increased on the 3D-RGD-MAP-C nanopatterned surface compared to the RGD-MAP-C monolayer and nanodot surface, regardless of the cell line. Among the peptide nanostructures, nanopillar array was more suitable for cell adhesion and spreading than nanorod array due to the increased binding sites for integrin receptor on the cell surface that contribute to the formation of a strong link between the cells and Au.

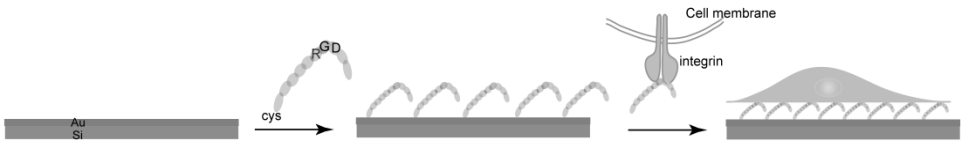

FIGURE 6.5
(a) Schematic of fabrication of various topographic RGD nanopattern, (b) AFM images of mask with varying pore

Electrochemical measurements

Cell-based chips are special devices that employ living cells immobilized on a metal surface as sensing elements, combined with sensors to perform real-time bioassays dynamically and rapidly, and have numerous applications ranging from biomedicine to environmental detection [40]. It is used for detecting the cellular responses to intracellular and extracellular stimuli. Interaction between stimulus and cell recorded as electro-physiological parameters produce responses using simple electrochemical detection system [2].
The electrochemical measurements carried out with a CHI660C Potentiostat (CH Instruments). The commonly used three-electrode configuration is employed for the electrochemical measurements, while standard silver (Ag/AgCl) served as the reference and a platinum wire as the counter electrode (Figure 6.1). Prior to the electrochemical measurement cell chip electrode with living cells needs to be washed twice with a 10 mM PBS buffer (pH 7.4) containing NaCl- 0.138M and KCl -0.0027M. Finally, electrochemical measurements performed using 2 ml of same PBS as the electrolyte. Before the measurement, the buffer solution should be first bubbled thoroughly with high-purity nitrogen for 30 min. A stream of nitrogen is then blown gently across the surface of the solution in order to prevent aerobic oxygen throughout the measurement.

Voltammetric Behavior of Different Cell Lines

Cell is the basic structural and functional unit of a tissue and obviously possesses a unique functional response, which varies with the tissues from which cell line was derived. Therefore, electrochemical response from cells immobilized on chip obviously should show cell line specificity.
The rat pheochromocytoma (PC12) cells on a collagen immobilized chip showed quasi-reversible redox behavior when subjected to cyclic voltammetry analysis using a potential window -0.2V to 0.8 V at a 100 mVs^{-1} scan rate, with a anodic current peak at +75 mV and cathodic peak at + 350 mV, whereas HeLa cells originated from human endothelium gave anodic peak at -75 mV and cathodic peak at + 150 mV [Figure 6.7]. This indicates distinguishable differences in redox behavior of two kinds of cells due to the differences of their origin that agreed with our hypothesis. The difference between the potential peaks $|E_{pc}-E_{pa}|$ exceeded 100 mV and the peak current ratio $I_{pa}/I_{pc} \geq 1$, which indicated the distinct quasi-reversible characteristics of the both cell [1]. These results demonstrated the advantage of the gold electrode over low conductive metal, semiconductor or non-metal based electrodes by offering faster electron transfer kinetics.

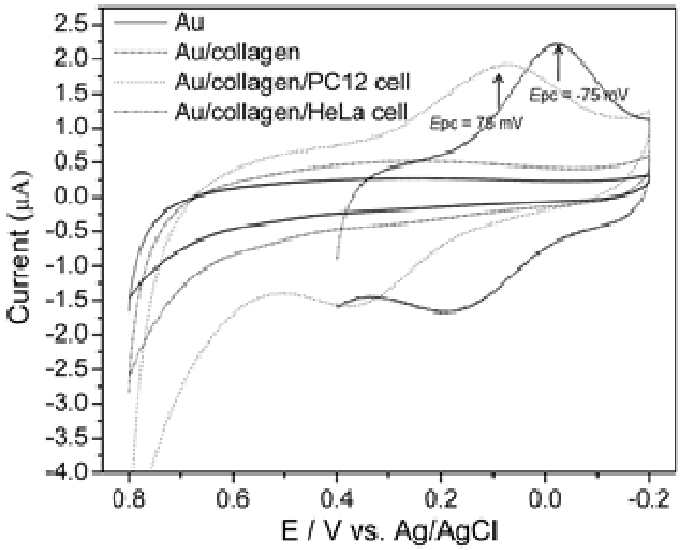

FIGURE 6.7
Redox behavior of PC12 and HeLa cell on collagen modified Au surface. CV was measured using PBS (0.01 M, pH 7.4) as electrolyte at a scan rate of 100 mVs−1 and the whole experiment was conducted at 27 ± 1°C. The experiment was repeated thrice maintaining identical condition. (Reproduced with permission from: ref. 41, © IARIA, 2011. ISBN: 978-1-61208-145-8).

The cell line specific CV signal was further confirmed by another sensitive amperometric method, differential pulse voltammetry. Considering anodic peak potential that was obtained from CV technique a potential window of -0.2 to 0.4 V was applied to measure DPV from both the cell lines at a scan rate of 100 mVs^{-1}, with 50mV pulse amplitude and 50 ms pulse width. The well distinguished DPV signal was measures from PC12 and HeLa cell. Figure 6.8 shows PC12 cell gave peaks at + 75 mV and HeLa cell at -75 mV, whereas no peaks were observed from bare Au surface indicating that peaks are certainly appears from the cells when they were immobilized on the Au electrode surfaces. Therefore, the cell line specific electrochemical signals were proved by the both of the amperometric method.

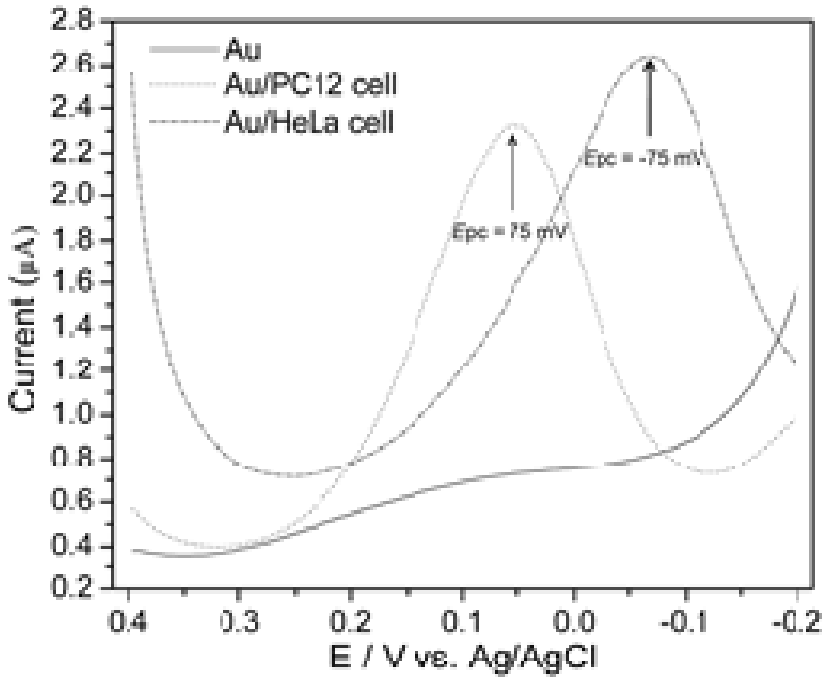

FIGURE 6.8
Differential Pulse voltammogram of PC12 and HeLa cell on collagen modified Au surface. DPV was measured using PBS (0.01 M, pH 7.4) as an electrolyte at a scan rate of 100 mVs−1. Pulse amplitude and pulse width were 50 mV and 50 ms, respectively. (Reproduced with permission from: ref. 41, © IARIA, 2011. ISBN: 978-1-61208-145-8).

Voltammetric Behavior of Different Phases of Same Cell Line

The cell immobilized electrode was treated with 2 mM thymidine in a culture medium (RPMI 1640) for 18 h, followed by a 8 h release (replaced by fresh medium) and again 2 mM thymidine for another 18 h to block cell at synthesis phase. Similarly, another cell immobilized electrode was treated initially with 2 mM thymidine as mentioned before for 18 h, followed by a 4 h release in fresh medium and then, 100 ng/ml Nocodazole was treated for another 10 h to block cell at mitosis phase. Thus, the cell chip was prepared for the measurement of electrochemical signal of the cells at the different phases of the growth cycle. A cell chip with the same number of non-treated cells served as control in parallel.

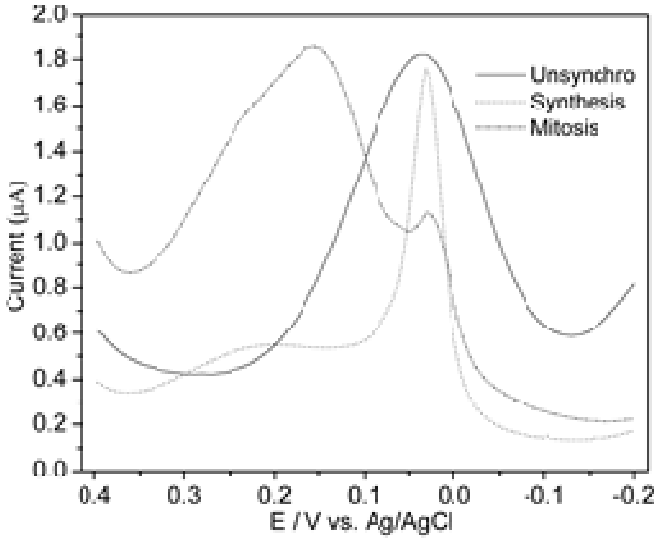

FIGURE 6.9
Differential Pulse voltammogram of PC12 cell synchronized at synthesis and mitosis phase as compared with unsynchronized (control). All the experimental condition was maintained as mentioned before. (Reproduced with permission from: ref. 41, © IARIA, 2011. ISBN: 978-1-61208-145-8).

Considering the cell line specificity of electrochemical signal we assume that same cell at different stages of its growth cycle might have different redox. During cell growth, cells pass through a number of complex processes, including prophase, prometaphase, metaphase, anaphase, and telophase, leads to several changes in the cell physiology and morphology. These cytological changes might be responsible for alterations in the electrochemical behavior of the cell. To prove this hypothesis PC12 cells synchronized at synthesis and mitotic phase of its cycle were subjected to DPV analysis. When the cells immobilized on the Au electrode were synchronized at synthesis stage, a sharp electrochemical signal appeared at +50 mV, whereas peak was observed at +150 mV when the cells were synchronized at mitotic stage during DPV measurement (Figure 6.9). Both the peaks from synchronized cells showed remarkable differences with unsynchronized cells. These differences in DPV signaling from identical cells in different phases (synthesis, mitosis) may have been due to changes in the redox properties of morphologically-altered cells [10]. Therefore, the specific DPV signals from cells in synthesis and mitosis phase which is completely different from unsynchronized cells might be useful for detection of metastatic cells of unknown origin.

Applications of cell chip for sensing environmental toxicant

Integration of living cells with metal-electrode is a novel approach that has significant advantages in tissue engineering and for studying cellular electro-physiologic states [2]. Potential uses for cell-based electrochemical systems have a wide range of applications in the field of pharmacology, medicine, cell biology, toxicology, basic neuroscience, and environmental monitoring [40]. Alteration in the cellular electro-dynamic systems gives information about the effect of a stimulus on living systems. Establishment of strong cell-substrate interaction is essential for obtaining proper functional rather than analytical information. We recently introduced cell chip technology capable of effectively

measuring changes in cell viability upon exposure to different kinds of environmental toxins [5,11] or anticancer drugs [4] based on simple and rapid electrochemical techniques. These electrical or electrochemical methods also have been incorporated into cell-based sensor arrays and electrical sensing devices for the detection of signal-frequency patterns produced by cells in growth media [40]. These whole cell-sensing systems employ sensor cells whose electro physiologic state varies upon exposure to toxic substances. The toxin treated cells produce readily measurable differences in signal intensities that used as new tools for cell viability assay [5]. These whole-cell sensing systems can be visualized as an environmental switch that is turned on in the presence of toxins or stressful conditions. In a recent study, HEK-293 cells seeded on a peptide coated Au surface and allowed culture medium containing different concentrations of bisphenol-A (BPA) and dichlorodiphenyltrichloroethane (DDT) were subjected to electrochemical measurement [6]. Figure 6.10a shows reduction peak current (I_{pc}) were decreased linearly at the concentration of BPA from 0 to 7.5 µM (Fig. 10b). The effect of DDT on CV response of HEK-293 cells also showed similar results to (Figure 6.10c). Figure 6.10d showed a significant negative linear correlation between DDT concentration and I_{pc}, indicating decreasing viability and proliferation of the HEK-293 cells.

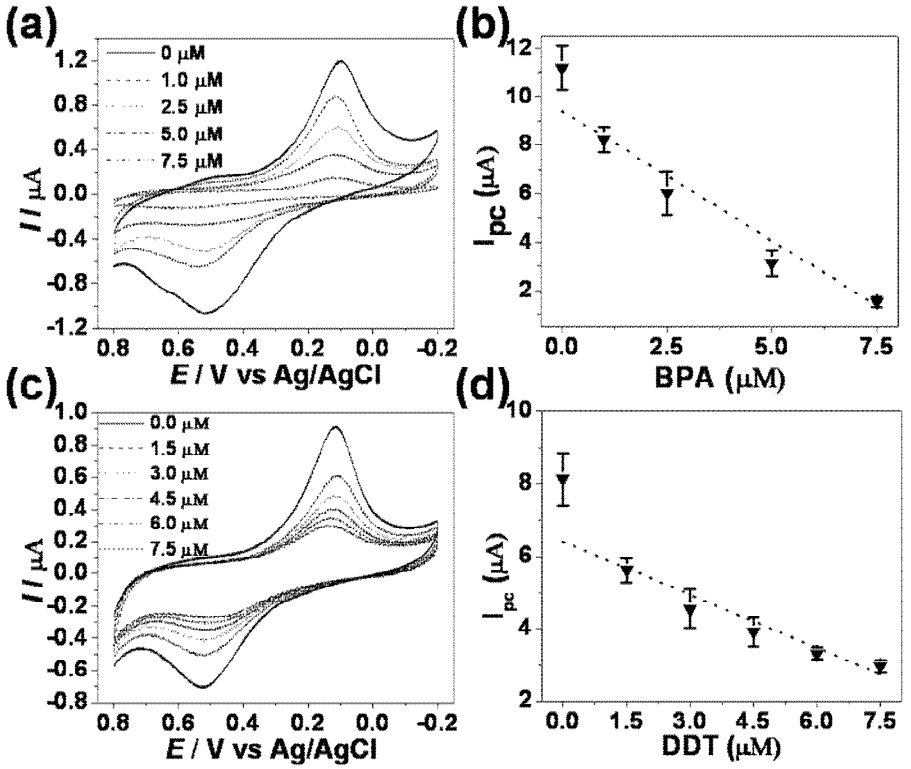

FIGURE 6.10

Cyclic voltammogram of (a) 0–7.5 µM bisphenol-A (BPA) and (c) 0–7.5 µM dichlorodiphenyltrichloroethane (DDT) treated cells; arrow indicates I_{pc} decreased 1.12–0.15 µA and 0.84–0.29 µA respectively, with the increasing concentration of toxicant. Linear plots obtained from various concentrations of (b) BPA and (d) DDT treated cells, every point corresponds to the average value of three independent measurements (error bars indicate the standard deviation). CV was measured using PBS (0.01 M, pH 7.4) at 100 mVs−1. (Reproduced with permission from: ref. 6, © 2010Springer).

In addition, we found that the redox phenomenon at the cell-electrode interface is critical for detecting the electrochemical characteristics of target cells, which vary depending on the cell line [41]. Recently, we also observed that the electrochemical properties of each cell depend on the cell cycle stage, which is used as a potential label-free technology for cell cycle monitoring [10]. It is well known that cells tend to show cycle-dependent characteristics, which are defined by a sequence of events (G1, G2, M and S-phase) in which several specific nuclear changes occur. Among these numerous cytological changes M-phase and S-phase are most vulnerable to environmental or endogenous stimulations which is used as an environmental switch for sensing systems that is turned on in the presence of toxins or stressful conditions [11]. Therefore, controlling the cell cycle on a chip at S and M phases' specific electrochemical signal has been achieved by electrochemical readout [10] (Figure 6.11). Recently, these phase specific signals were employed in sensing phase specific effect of environmental toxin.

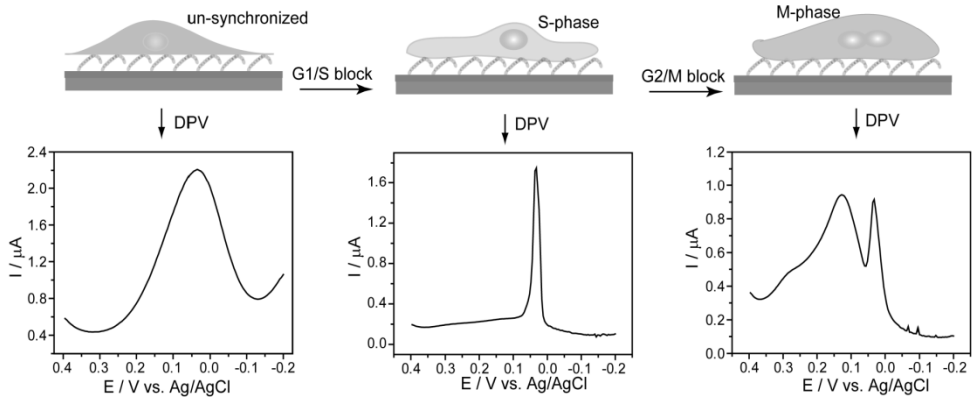

FIGURE 6.11

Figure shows synchronized S-phase (middle), M-phase (right), and unsynchronized (left) cells with their respective DPV signals (down arrows indicate respective signals). (Reproduced with permission from: ref. 10, © 2011ACS).

In a study, a chip containing both G1/S and G2/M-phase, subjected to PCB and/or BPA toxicity and electrochemical measurements were performed. The result shows that G1/S peaks sharply decreased due to 500 nM BPA treatment remaining the intact G2/M peaks (Figure 6.12a). But, peaks for G2/M decreased when 50 nM PCB was treated without affecting G1/S peak (Figure 6.12b). These results suggested that BPA toxicity affect the cells of G1/S and PCB affects G2/M phase. Whereas both the peaks decreased when a mixture of low concentrations (200 nM BPA and 20 nM PCB) of both toxin added. Moreover, no peak was detected when high concentration of mixed toxin (600 nM BPA and 60 nM PCB) added which indicates complete death and washout of cells from the electrode (Figure 6.12c). The neurotoxic doses of BPA and PCB are in agreement with a previous study [1,5] that reported 150 nM BPA and 20 nM PCB are toxic for PC12 cells. Therefore, the decreased phase specific electrochemical signal is certainly responsible for the effect of toxin used in this study. So, analysis and quantification of the current peak obtained from DPV signal intensities can be useful indirectly but accurately for determining the dose effect of the respective toxicant on completely synchronized cells.

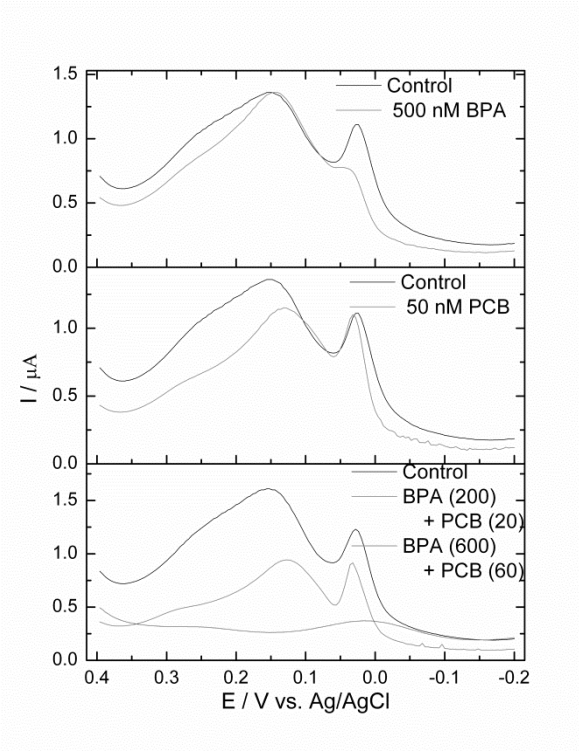

FIGURE 6.12
Phase specific toxicity of BPA and PCB are analyzed based on the two peaks obtained from cells 6 h released from G1/S,(top) image shows BPA toxicity affects mostly on G1/S peak whereas (middle) PCB affect G2/M peak, but, both the peak decreased when mixture of both the toxin treated (bottom) and no peak found when high concentration of toxin used. (Reproduced with permission from: ref. 11, © 2013Elsevier).

After confirming the complete synchronization cells at G1/S and G2/M phases, a varying concentration of PCB and BPA was exposed for sensitive electrochemical detection of cell viability. For the effective toxicity measurement 3.5×10^5 cells/ml of cells were synchronized on each chip because high density are not suitable for proper synchronization as well as for electrochemical measurements [10]. Prior to recording DPV current responses G2/M phase synchronized cells were exposed to several concentrations of PCB and G2/M phase synchronized cells to BPA. Figure 6.13a shows that the current responses from G2/M cells exposing various PCB concentrations from 20 nM to 120 nM. A dose dependent decreased in DPV current signals were recorded as functions of treated PCB concentrations (Figure 6.13c). Where, the current peaks from initial concentration (20 nM) remained unchanged comparing with non-treated control, indicating the sub-toxic dose. But, the reduction peak showed a negative linear correlation when cells were exposed to 40 nM to 120 nM concentrations of PCB (Figure 6.13b inset) which indicates cytotoxicity of PCB. Several previous study reported that electrochemical signals have positive linear correlations with the concentration of viable cells; therefore, signal decrease observed from toxin-treated cells certainly attributed to the loss of cell viability [4,5,6,11].

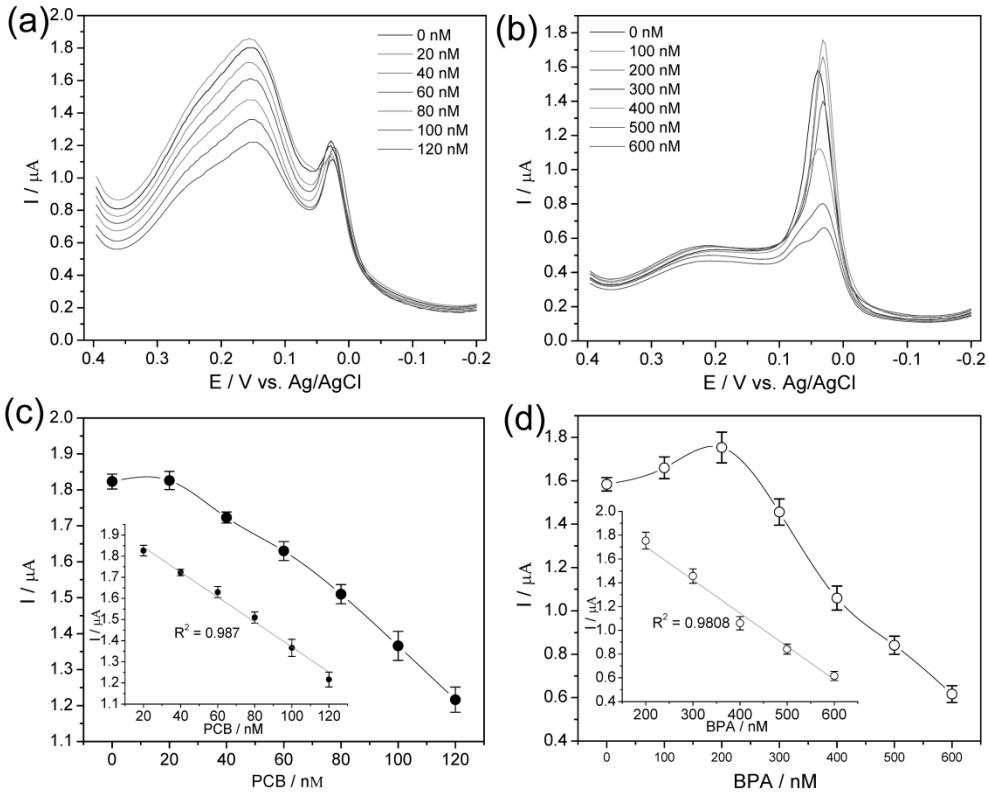

FIGURE 6.13

Concentration dependent cyto-toxicity assay; (a) effect of PCB on cell synchronized at G2/M phase and (b) effect of BPA on cell synchronized at G1/S phase. Dose response curve obtained from PCB treatment on G2/M synchronized chip (c) and BPA treatment on G1/S synchronized chip (d). (Reproduced with permission from: ref. 11, © 2013Elsevier).

Perhaps, the current responses from BPA exposed G1/S cells are plotted in Figure 6.13b, where unlikely to the other toxicants, BPA shows dose dependent dual effect [5]. The current peaks increase for 100 nM to 200 nM BPA treatments; whereas, decreased with further increasing concentrations (Figure 6.13d). The reduction peak showed a negative linear correlation when cells were exposed to 400 nM to 600 nM concentrations of BPA (Figure 6.13d inset). This finding is completely coincided with our previous report where BPA toxicity was analyzed on unsynchronized cells [5]. Therefore, it is depicted that cells at different phases of it's cycle can be susceptible for different environmental toxin which can be useful for monitoring the effect of mixed toxicity from environmental sources accurately using the developed synchronized cell based chip.

Based on the above discussions, it is suggested that whole cell either in unsynchronized or synchronized state are able to monitor single or multiple toxins from bulk environmental sample. However, technical challenges still remain before the devices will become widely used for toxicity testing. Particularly, the lack of compatibility of the miniaturized cell chip platform with bulk environmental sample is the main drawback of this rapid detection method. However, incorporation a micro method that can filter the sample before exposed to the chip chamber can overcome this limitation.

Applications of cell chip for diagnosis

Cell-chip based sensor devices are now becoming practical tools for the rapid screening of chemicals and drugs, and several have been developed specifically as toxicity screening assays. Besides these numerous environmental monitoring, the distinct cell line specific redox behavior of the cell based chip has explored the opportunity of its diagnostic application. In the recent past, activity of several extracellular biochemical parameters such as effect of glucose and potassium on neurotransmitter release [42] were monitored efficiently using the cell based chip. We observed dopaminergic behavior of PC12 cells using cell immobilized chip [42]. Glucose and potassium activated dopamine release from neuronal cell were also confirmed voltammerically using the cell based chip. In Figure 6.14, the increased current peaks due to the glucose and potassium treatment for PC12 cells were contributed by increased exocytosis of intracellular dopamine [43].

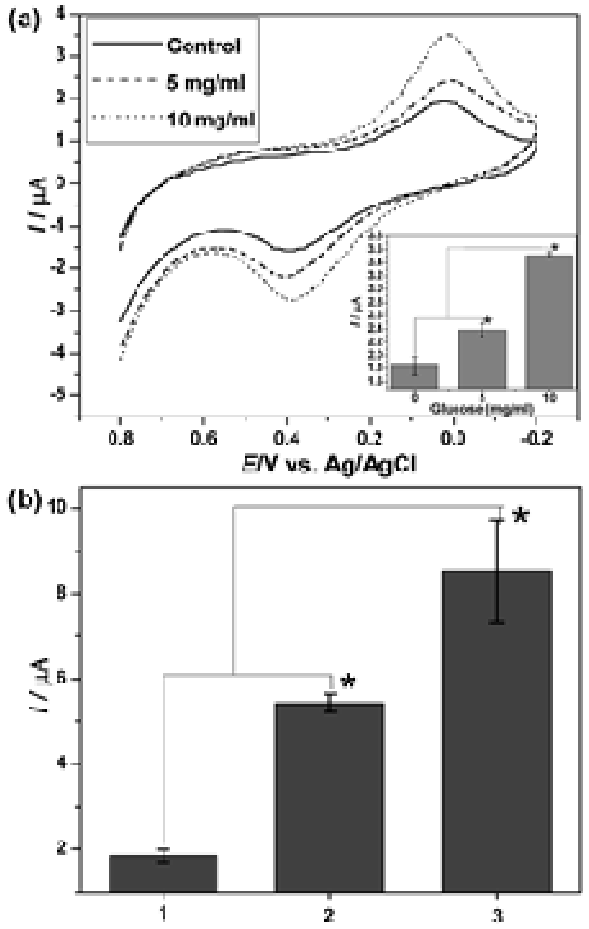

FIGURE 6.14
a) CV of glucose treated PC12 cell, inset shows current increase significantly with doses. b) Current peak from cells treated with 30 µM KCl combined with (1) 0 mg/ml, (2) 5 mg/ml and (3) 10 mg/ml glucose. (Reproduced with permission from: ref. 41, © 2011ASP).

Therefore cell chip based voltammetric monitoring of clinical specimens derived from different system of a patient can provide clear clinical information. For this cell from each system of the body should be used to achieve respective information of the system. This system specific clinical information can be achieved by analyzing and quantifying the voltammetric information of the cell chip. Finally, doctor can easily depict the clinical state of a patient from the system specifics clinical information of the chip. This quick response and analysis makes it possible for one to use as portable and disposable cell chip based assay and as early warning systems of the clinical state of a patient. However, technical challenges still remain before the use of the clinical samples such as urine, sputum, stool and other discharged specimens because of the biocompatibility concern. The external samples need to be processed before exposed to the chip chamber. Therefore, researchers are looking for the new cell based chip by combining micro fabrication and microfluidic technologies that can processed the clinical specimens to ensure biocompatibility to miniaturized cell chip platform in real-time. This chip can also be integrated with other medical equipment for automation and real-time monitoring.

Conclusions

This chapter focused on establishing strategies to develop a living cell chip based on electrochemical detection. As a model system; neural cell such as rat pheochromocytoma cells and human fibro-blastoma cells were chosen as the main analytic candidates, whereas human fibro-blastoma cells, human embryonic kidney cells and human epithelial carcinoma cells were also subject to electrochemical investigation by several researchers. The electrochemical measurements such as cyclic voltammetry and differential pulse voltammetry were conducted to examine the redox behavior of the model cell immobilized on electrode. Based on this redox behavior cell viability was determined electrochemically. However, in order to improve cell adhesion and enhance electrochemical signal cell adhesion molecules were organized on the electrode in a nanoscale array. The performance of the nanoscale peptide modified electrodes were checked and found to have positive effect on cell adhesion, spreading, proliferation and electrochemical signal transmission. Nanopatterend peptide modified cell chip proved to be potent for determination of environmental toxicity sensitively. Furthermore, fabricated nano-bio-platform was applied for artificial regulation of cell cycle on chip based electrochemical detection method. Finally, the synchronized cell chip at a definite phase of a cycle was applied for sensitive phase specific electrochemical determination of cyto-toxicity of environmental toxicant. This system works well in terms of synchronization of cells into the specific phase of its growth cycle and its electrochemical readout. It can be used as a future nano-biochip in developing sensitive cell based diagnostic devices.

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