Trends in Cell-Based Electrochemical Biosensors

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Abstract: Cell-based electrochemical biosensors have contributed tremendously to the fields of biology, medicine, chemistry, pharmacology, and environmental science. With electrochemical transducers and whole cells as the recognition elements, these biosensors provide new horizons for biosensing and life science research. This review focuses on the research accomplishments on this topic over the last three years, and is divided into three sections according to the types of cellular responses. Our aim is to highlight how simple and sensitive electrochemical methods can be coupled with cells by virtue of the integration of interface control, nanotechnology and genetic engineering to generate new enabling technologies. Some specific examples to demonstrate how these sensors are useful in medicinal chemistry and drug design have also been discussed. It is hoped that this review can provide inspiration for the development of fast, selective, sensitive, and convenient detection and diagnosis platforms.

Keywords: Electrochemical, cells, biosensors, impedance, microelectrode array, review.

1. INTRODUCTION

Electrochemical biosensing technology, with intimately coupled biological recognition elements and electrochemical transduction units, has been extensively used to obtain quantitative or semi-quantitative information of plenty of analytes [1,2]. Advantages include high sensitivity, selectivity, rapid analysis and the ability to operate in turbid solutions [3]. Furthermore, the electrochemical equipments are more amenable to miniaturization, and the continuous response of an electrode system allows for on-line control. According to the type of recognition elements, electrochemical biosensors can be classified into immunological, enzymatic, non-enzymatic receptor, DNA and whole-cell electrochemical biosensors.

Whole cells, as more complex biological recognition elements, hold the promise of presenting the intact sub-cellular machinery for the identification of physiologically relevant events, and providing functional and analytical information [4-7]. The internal amplification cascades of cells can be used to increase the sensitivity of the devices. In addition, compared with enzyme, the self-sustaining whole cells are less expensive and more stable during the production process. Over the past decades, different types of cells have been incorporated into electrochemical biosensor, including microbial cells [7,8], mammalian cells [4-6] and recombinant cells (see Glossary) [9] in the immobilization format of single cell, cell layer and cell network. These cell-based electrochemical biosensors have a wide range of applications in pharmacology, medicine, cell biology, toxicology, neurosciences, and environmental monitoring [5,6,10]. Furthermore, they are of paramount importance for medicinal chemistry, because cellular electrochemical responses to various medicinal compounds provide "true" pharmacology of the medicinal components. They also give comprehensive information of the mechanistic aspects of drug activity, such as stereochemistry, diffusion, solubility, metabolism, membrane permeability, etc. This has tremendous implications for the drug discovery process and can expedite the research in this field. Moreover, as drug costs are directly tied to candidates failures, it is beneficial to predict drug toxicity and assess drug effects in situ, in real-time and on a large population of cells but with the resolution of a single cell at very early stages of development [11]. This can be readily achieved by cell-based electrochemical biosensors. Current problems facing the applications of the devices are analytical strategies, batch-to-batch reproducibility, and lack of selective placement of biological cells on the sensing sites [5]. Advancement in technology integration, nanomaterials utilization, interface control, and genetic engineering has provided a promising opportunity for meeting these challenges.

Although cell-based biosensing technology is still at its nascent development stage, several reviews have been published [1,5,6,8,10,12-17] and mostly focused on narrow classes of cells or specific applications. This paper reviews the trends of cell-based electrochemical biosensors with a wide range of whole cells in the last three years, including sensors not only in the narrowly defined sense, in which the cells act as recognition elements [16], but also for measuring cell functions. According to the cellular responses, this review discusses electrochemical biosensors based on (1) cellular activity and function, (2) cellular barrier behavior and (3) recording/stimulation of electric potential of electrogenic cells. In contrast to the first two types, where non-neural cells are usually used, neural cells, which have unique advantage of high specificity through receptor-interaction [10], are incorporated for the third type of biosensors. In view of the fact that cell immobilization constitutes an important first step toward the fabrication of sensors, a brief discussion in this regard is also presented.

2. CELL IMMOBILIZATION

As biocompatible matrices for preparation of biosensors [18], a successful matrix for cell immobilization can stably

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**Glossary**

**Recombinant cells:** the sensing elements prepared by fusing a reporter gene to the cells for eliciting a response to an analyte.

**Potentiometry:** involving potential determination between indicator and reference electrodes at zero current under equilibrium conditions for monitoring the accumulation of charge due to selective binding at the indicator electrode surface.

**Amperometry:** measuring the current resulting from the electrochemical oxidation or reduction of an electroactive species at a constant applied potential.

**Conductometry:** measuring the change in the conductance of the biological component arising between a pair of metal electrodes.

**DOX electrochemical sensor:** Comprised of 96 sets of three-electrode configuration commensurate with the dimensions of conventional 96-well plate in which the cells are cultured. During cell respiration, oxygen is consumed, and the measured current arising from the reduction of oxygen at negative potentials reduces to a threshold value. The time to achieve this threshold current is proportional to the initial cell concentration.

**Impedance spectroscopy:** a transient electrochemical method by applying a small amplitude of perturbing sinusoidal voltage to the electrochemical cell and measuring the resulting response, including non-Faradaic and Faradaic impedances. Non-Faradaic impedance is performed in the absence of any redox probe, while Faradaic measurement requires redox-active species.

**Action potential:** the sequential, electrochemical polarization and depolarization that travels across the membrane of a nerve cell in response to stimulation.

**Microfluidics:** fluidics in structures on micron and smaller-length scales, resulting in low turbulence, with laminar flows.

integrate cells and efficiently maintain their functionality. Identification of artificial surfaces is critical for preparation of cell biosensors. Whole cell immobilization on artificial surfaces often mimics what occurs naturally. For cells growing on a surface, the surface properties, such as roughness, hydrophobicity, topography, positive/negative charges, surface chemistry and specific protein or cell-surface interactions, can affect the activity and adhesion of cells to the artificial surfaces [19,20]. Recently, biocompatible nanoparticles have been extensively used to construct non-toxic biomimetic interface for immobilization of living cells [21,22], which allows adsorption of cells by the electrostatic force or weak interaction between the nanoparticles and cell membrane. Sol-gel matrices and natural polymers can entrap cells on the electrode surface. The major problem of cell entrapment is the instability of cell immobilization during continuous use and the additional diffusion barrier resulting from the entrapped materials, which can be minimized by increasing the porosity of the matrix. Although covalent binding usually leads to the decrease of cell viability when the cells are exposed to reactive groups and harsh reaction conditions, the surface modification of supports with functional groups such as amino groups, hydroxyl group and carboxylic side chain has been developed for proper anchorage of viable cells to improve cell attachment [23,24]. The grafted monolayer film with excellent hydrophilicity and biocompatibility can provide an appropriate biomimetic interface for cell adhesion, proliferation and electrochemical study [23].

### 3. ELECTROCHEMICAL BIOSENSORS BASED ON CELLULAR ACTIVITY AND FUNCTION

#### 3.1. Voltammetric Behaviours of Immobilized Cells

A living cell can be properly described as an electrochemical dynamic system [25]. Electron generation and charge transfer exist in all living cells due to the redox reactions and the changes of ionic composition and concentration in life processes [26], which have been used to characterize the viability of cells in homogeneous solution.

The tumor cells attached to a modified electrode can exhibit an irreversible voltammetric response around +0.8 V in the first scan (solid curve in Fig. (1)) [27], which is related to the oxidation of guanine [27-30]. However, at a bare carbon paste or glassy carbon electrode, the tumor cells do not show any detectable electrochemical signal, which demonstrate that the nanomaterials such as gold nanoparticles, carbon nanotubes, or carbon nanofiber can significantly reinforce the response [27,28,30]. No corresponding reduction wave was observed in the reverse scan (from positive potential to negative potential). This oxidation peak has been employed for developing a simple and rapid method to electrochemically investigate the exogenous effect, and a promising approach for electrochemical anti-tumor drug sensitivity test [27,29]. The optimal exogenous factors that affect cell viability, such as solution pH, ionic strength, temperature and components, are just consistent with cell growth conditions in culture. Comparing with conventional methods such as the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, the developed drug sensitivity test exhibits good performance characteristics, such as high sensitivity, desirable accuracy, low cost and simplified procedures.

![Cyclic voltammograms of AsPC-1 cells immobilized on gold nanoparticles modified carbon paste electrode in 10 mM pH 7.4 PBS at scan rate of 80 mV/s using a conventional three-electrode system with gold as working, platinum wire as auxiliary, and saturated calomel electrode as reference electrodes. The solid and dotted lines represent the first and second scan, respectively, and the direction of initial scan is positive.](image-url)
3.2. Electrochemical Biosensors Based on Cellular Function

Metabolism in cells, which depends on the intracellular physiological state and extracellular environment, leads to the changes of pH, oxygen and ion concentrations. A variety of cell-based electrochemical biosensors based on measurement of these changes have been fabricated [31-49]. Microorganisms such as E. coli, yeast, fungi and so on are the most used recognition elements for this type of cell-based biosensor. However, recent research has focused on more complex systems such as using animal cells or recombinant cells [7,32-34]. The detection of cellular metabolism can give direct evidence of the activation of specific receptors, which is valuable for drug screening and contains much more information than just measurement of the recognizing/binding events [35,36]. Different electrochemical methods such as potentiometry, amperometry or conductometry (see Glossary) have been used to perform the measurements (Fig. (2)).

A. Potentiometric Cell-Based Biosensors

Conventional potentiometric cell-based biosensors consist of an ion-selective electrode (ISE) or gas-sensing electrode (GSE) coated with a cell-immobilized layer [8]. The immobilized cells consume analyte to generate a potential change due to ion accumulation or depletion on electrode surface [50]. This method requires a very stable reference electrode, which is a limitation of these transducers [8]. A whole-cell potentiometric biosensor for screening of toxins has been proposed by attaching endothelial cells to a K⁺-selective membrane, on which ion transport is almost completely inhibited by a confluent cell monolayer [32]. When the biosensor is exposed to a specific class of compounds, the permeability of cell monolayer increases, and more K⁺ cation can penetrate through the attached monolayer, which produces a potential response. Interestingly, this potentiometric biosensor can be easily adapted to small-scale applications.

B. Amperometric Cell-Based Biosensors

Amperometric cell biosensors have been widely developed for determination of oxygen, glucose, lactate, and so on (Table 1) [35-48]. Most of them use oxygen sensors to detect cell respiration [37-40,42-44,46], which offer the advantage of continuous monitoring. Besides oxygen, an electroactive product or intermediate produced by specific enzymatic reaction of cells can also be detected. Among these analytes, nitric oxide (NO), adenosine triphosphate (ATP, 1, Scheme 1), glutamate, histamine (2, Scheme 1) and catecholamine family (dopamine (3, Scheme 1), norepinephrine (4, Scheme 1), and epinephrine (5, Scheme 1)) are particularly important, because they act as signals in many biological systems. They can also probably be employed to determine the effects of clinical drugs and measure extracellular stress factors in various types of cells. For example, an endothelial cellular biosensing system has been constructed by immobilizing human umbilical vein endothelial cells (HUVEC) on an electrode to detect nitric oxide, an indicator of blood vessel relaxation, through differential pulse voltammetry (DPV) [35]. Although the promising system can be used for assessing blood pressure-controlling drugs by regulating NO release, such as acetylcholine chloride (AcChCl),

![Fig. (2)](image-url)

Fig. (2). Scheme of cell-based electrochemical biosensors based on cellular functions. A1: amperometric biosensor based on detection of oxygen; A2: amperometric biosensor based on detection of electroactive species produced by enzymatic reaction.
Table 1. Amperometric Microbial Biosensors

<table>
<thead>
<tr>
<th>Target</th>
<th>Microbial</th>
<th>Limit of detection</th>
<th>Type</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe²⁺</td>
<td>Acidithiobacillus ferrooxidans</td>
<td>900 nM</td>
<td>A1</td>
<td>[39]</td>
</tr>
<tr>
<td>p-nitrophenol</td>
<td>Moraxella sp.</td>
<td>0.1 μM</td>
<td>A1</td>
<td>[40]</td>
</tr>
<tr>
<td>p-nitrophenol</td>
<td>Moraxella sp.</td>
<td>20 nM</td>
<td>A2</td>
<td>[41]</td>
</tr>
<tr>
<td>ethanol</td>
<td>Candida tropicalis</td>
<td>0.5 mM</td>
<td>A1</td>
<td>[42]</td>
</tr>
<tr>
<td>choline</td>
<td>Arthrobacter globiformis</td>
<td>80 nM</td>
<td>A1</td>
<td>[43]</td>
</tr>
<tr>
<td>L-lysine</td>
<td>Saccharomyces cerevisiae</td>
<td>1.0 μM</td>
<td>A1</td>
<td>[44]</td>
</tr>
<tr>
<td>1,3-propanediol</td>
<td>Gluconobacter oxydans</td>
<td>0.15-0.42 mg/L</td>
<td>A2</td>
<td>[45]</td>
</tr>
<tr>
<td>p-nitrophenyl-substituted</td>
<td>Moraxella sp.</td>
<td>0.1 μM</td>
<td>A1</td>
<td>[46]</td>
</tr>
<tr>
<td>organophosphates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Scheme (1). Adenosine triphosphate (1) histamine (2), and catecholamine family, including dopamine (3), norepinephrine (4), and epinephrine (5).

NOC 7 (a NO donor) and L⁵-monomethyl-L-arginine (L-NMMA), it has not taken into account the diffusion of trace analyte from cells to sensor surface. Furthermore, the materials modified on electrodes must be configured specifically to ensure both molecular selectivity and molecular transducerability for the given mammalian cell culture media.

A common disadvantage of amperometric cell biosensors is the lack of selectivity. This problem can be solved by inhibition or suppression of undesired transport mechanisms or metabolic pathways and coupling of enzymes with immobilized microbial cells to form a hybrid biosensor. For example, alcohol oxidase can be introduced to *C. tropicalis* yeast cells by culturing in a culture medium containing high concentration of ethanol [42].

Another shortcoming of cell-based sensors is long response time. By virtue of the development of genetic engineering, the dynamics of the microbial biosensing can be significantly improved by engineering the cells to anchor the desired enzyme into the periplasmic space [51]. However, when novel receptors are introduced into cells, it is important to ensure that the receptor does not interfere with normal cellular processes. Genetic engineering also offers an effective way to adjust the interface between cells and electrodes. A strategy to control the interface between Chinese hamster ovary (CHO) cells and the electrode by genetic engineering for converting activity of the expressed enzyme on cell surface into an electrical signal has been reported [34]. Construction of such electrochemically detectable interface can be extended for responding to certain factors without the
necessity of the optical system, and can serve as the basis of a compact biosensor.

The dissolved oxygen (DOX) sensor (see Glossary) can simultaneously determine dissolved oxygen with 96 sets of electrodes for studying bacteria, cells and their interaction with analytes and continuous real-time monitoring of bacteria activity [14,37,38]. Compared with methods based on fluorescent microscopy, flow cytometry and spectrophotometry, the major advantage of DOX is that it is suitable for continuous monitoring. By integration with pattern recognition techniques, this system can be used for the monitoring of bacteria activity upon exposure to antibiotics [38]. It provides a simple and high throughput system in a non-invasive manner without any preparation step for development of the next generation of analytical devices.

Nowadays, the use of the miniaturized sensor is of great demand to minimize the size of the measurement chamber, thus one can work with small numbers of cells. An integrated distance-control NO sensor has been constructed to perform on-line Ca²⁺ channel-related drug screening with transformed human umbilical vein endothelial cells (T-HUVEC) placed at a known distance from the electrodes [48]. This automated system features a unique design of an electrode set for distance control, and increases sample throughput. However, the transfer of the electrode set among wells prolongs the assay time, in this regard, microelectrode arrays exhibit incomparable superiority. A microelectrode array biochip for drug screening purposes has been developed to study the influence of reserpine, nifedipine and L-3,4-dihydroxyphenylalanine (L-dopa) on quantitative catecholamine release from PC12 cells [36]. This biochip focuses on quantifying release from multiple cells rather than resolving individual events. It can obtain high sensitivity through minimization of the distance, and consequently the solution volume. Meanwhile, nanotechnology has also enormously contributed to the development of miniaturized amperometric cell-based biosensor, in which cells are integrated into a nanosystem to perform sensing function. An innovative nano-biochip, which contained an array of nanovolume electrochemical cells, was presented [7]. In the presence of a toxin, the E. coli integrated into the chip produced β-galactosidase, leading to an enzymatic reaction upon addition of a substrate. The product of the enzymatic reaction was then oxidized at an electrode. The chamber array with nL volume enabled simultaneous measurement of eight samples, allowed minimum interaction with the water flow and resulted in increased speed and sensitivity.

C. Conductometric Cell-Based Biosensors

Conductometric cell-based biosensors are based on detection of changes in ionic species due to cell-catalyzed reactions. Even though the detection of solution conductance is non-specific, conductance measurements are extremely sensitive [8]. Microalgae were used as bi-enzyme receptor to develop a conductometric biosensor for monitoring the activities of two kinds of enzymes [49]. This biosensor overcame the disadvantage of using two kinds of pure enzymes, which faced the stability and commercial problems, and thus provided a convenient way for multi-detection array.

4. BIOSENORS BASED ON BARRIER BEHAVIOR OF ATTACHED CELLS

The cell-to-electrode interactions have showed important perspective in the development of biosensors and prosthetic devices [4]. Cells have excellent insulating properties and can affect the local ionic environment at electrode/solution interface, which leads to change of impedance [11,52-58]. Thus electrochemical impedance spectroscopic (EIS) technique (see Glossary) can be used to monitor the biological status of cells, including cellular viability and morphology, cell number, and adhesion, proliferation and apoptosis of cells on surface, and has shown great advantages for cell-based general screening of medicinal compounds in drug development [53,56-58]. This technique is inexpensive and relatively easy to perform in comparison with surface plasmon resonance and quartz crystal microbalance techniques. Theoretically, it can dynamically measure cellular movement in nanoscale with better resolution than conventional optical methods [5]. Two categories of EIS have been used for detection of cell-to-electrode interactions (Fig. 3), leading to two kinds of cell-based EIS biosensors. A common disadvantage of cell-based impedance techniques is that the response measured is the total change produced by a number of cells.

4.1. Non-Faradaic EIS Technique

Different set-ups have been designed for impedance measurement, in all of which impedance is measured as a function of time and changed environment [10]. Electric cell-substrate impedance sensing (ECIS) is the most popular non-

Fig. (3). Schemes of cell-based non-Faradaic (a) and Faradaic (b) impedance spectroscopic (non-Faradaic EIS and Faradaic EIS) biosensors based on detection of barrier behavior of attached cells.
Faradaic EIS technique [55,56]. Their applications include study of cell-cell contact, cytotoxic effect of toxin, and drug discovery. However, ECIS suffers from issues of insufficient cell coverage, leading to low sensitivity and uneven distribution of cells between wells, which hampers their usage in high-throughput screening in drug discovery. Many groups have been striving to improve the performance of ECIS. An important achievement is the invention of real-time cell electronic sensing (RT-CES), which can improve the sensitivity and the well-to-well distribution by increasing the covering area [53,57]. By constructing a set of “circle-on-line” microelectronic sensors on the bottom of wells, 80% of the surface area can be covered, which allows more sensitive quantitative detection [53]. Furthermore, the RT-CES system has raised cell impedance measurements to the heights now serving as the basis of high throughput tools for investigations of drug development and toxicity prediction. Several researches have demonstrated the capability of this system to obtain dynamic and concentration-dependent effects of toxin, drug and other chemicals on cellular status [53,57,59]. For example, the effects of antipyrene, trichloro, dimethyl formamide, cadmium (II), p-phenylene diamine, propranolol, ibuprofen, nalidixic acid, salicylic acid and glycerol on mouse fibroblasts have been studied using this system [59].

The second effort is to develop different set-ups that allow the simultaneous visualization and measurement of barrier function of cells. For this purpose, indium tin oxide (ITO), which combines electrical conductivity and optical transparency, is an attractive alternative to gold as a micro-impedance biosensor. Using an ITO–SiN₃ microelectrodes array, the effect of cytochalasin D on porcine pulmonary artery endothelial cell (PPAEC) has been monitored, thus providing a more reliable method for measurement of cellular response to drugs simultaneously by electrochemical and optical approaches [58].

With the ability to manipulate cells and control mechanical, electrical and biochemical parameters down to the nanometer scale, micro-fabrication instrumentation has allowed electrical impedance techniques to be used for studying the anchorage-dependent cells cultured on microelectrode arrays (MEAs) [53,56]. The disadvantage is the increased electrode impedance caused by the electrode polarization as the electrode size decreases [61]. To avoid polarization, a microhole-based chip can be utilized to realize high current density nearby the hole but no electric double layer for sensitive impedance measurement, which can examine the cytotoxicity of dimethylsulfoxide on L929 cells [61]. This approach provides an effective way for on-line recording of the impedance of even a single cell under specific physical/chemical environments in real time without chemical markers.

### 4.2. Faradaic EIS Technique

Faradaic EIS is a powerful tool for analyzing interfacial properties of modified electrodes during biorecognition events. The cell membranes (thickness 5-10 nm) show the capacitance and resistance of 0.5-1.3 μF cm⁻² and 10⁻¹⁻¹⁰ Ω cm⁻², respectively [62]. As a result, when cells attach to an electrode, a barrier hindering the access of the redox probe to the electrode surface is produced, thereby, resulting in an increase in the electron transfer resistance (R_e). The increase magnitude of electron-transfer resistance is related to the number of immobilized cells. This behavior has been used to detect *Escherichia coli* O157:H7 concentration [52]. Upon cell adhesion the R_e value is proportional to the logarithmic value of the cell concentration ranging from 4.36×10⁸ to 4.36×10⁴ cfu/ml, which is comparable to other label-free immunosensors. Based on this detection principle, several Faradaic EIS sensors for cell number have been developed [3,28,30,52,63-68], and the fabrication characteristics are summarized in Table 2. Some of these cell-based sensors are prepared by immobilizing antibody on electrode surface to capture cells [52,63-65]. A major problem associated with anchoring cells by immunoreaction is the low capture efficiency (CE). As a result, optimal utilization of the functional surface area (where attached cells are detected) of an elec-

<table>
<thead>
<tr>
<th>Table 2. Characteristics of Cell-Based Faradaic EIS Biosensors</th>
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<tbody>
<tr>
<td><strong>Modified electrode</strong></td>
</tr>
<tr>
<td>Ab/Au IDAM⁺</td>
</tr>
<tr>
<td>Ab/epoxysilane/ITO⁺</td>
</tr>
<tr>
<td>SAM/Au⁺</td>
</tr>
<tr>
<td>Ab/ITO IDAM⁺</td>
</tr>
<tr>
<td>Ab-Magnetic NPs/Au IDAM⁺⁺</td>
</tr>
<tr>
<td>Ab-biotin/neuraminidase/ biotin-SAM/Au⁺⁺</td>
</tr>
<tr>
<td>Polyaniline/SPCE</td>
</tr>
<tr>
<td>GNP's embedded chitosan gel/GCE</td>
</tr>
<tr>
<td>CNI-chitosan/GCE</td>
</tr>
</tbody>
</table>

*Ab refers to antibody.  
SAM refers to self-assembly monolayer.  
NPs refers to nanoparticles.  
⁺⁺ denotes the nanoparticles immobilized with antibody in this study.
5. BIOSENSORS BASED ON THE RECORDING/STIMULATION OF CELLULAR ELECTRIC POTENTIAL

Electrogenic cells (neural cells, heart muscle cells, pancreas beta cells) or a neural cell network grown in culture on microelectrode arrays or on field effect transistors are very sensitive to neuroactive compounds added to the culture medium, which can be measured by the change of membrane potential during an action potential (see Glossary).

The uptake clamp and patch clamp techniques are dominant techniques for monitoring cellular electrophysiology [70-76], particularly the study of nicotinic cholinergic receptor, which is a focus of interest for drug discovery [71,72]. However, these techniques are basically invasive methods, and the manipulation procedure needs high skill. In contrast, the cell-based biosensors using a micro-sensor array provide a non-invasive way for recording the action potential in real-time, which can reach a single cell and cell network. In this field, microelectrode array (MEA) cell-based biosensor [77,78], and the light-addressable potentiometric sensor (LAPS) [79,80] are the most popular detection methods.

5.1. MEA-Based Electrochemical BioSensor

The integration of a fixed microelectrode array with the electrodes on a silicon or glass substrate and a cell culture chamber has been demonstrated to allow for long-term, non-invasive and multisite extracellular potential recording from electrogenic cells, and is used for biosensing [77] (Fig. 5(a)). However, this technique suffers from the difficulty to precisely control the cell density and the interaction between cells and electrodes, which reduces the reliability of measurement. Microfabricated systems create new opportunities to solve the problem by integration of extracellular matrix (ECM) surface patterning technique with microfluidic channels (see Glossary). The control of surface makes it possible to reproducibly place the cells at precise points [13], and microfluidics can not only deliver soluble factors, but also help to control neuronal connectivity. More importantly, compared with cells immobilized on electrodes as sensory elements, cells in microfluidics avoid the impact to cellular physiological activity resulted from the immobilization of cells [81]. By combination of planar microelectrode arrays with microfluidic devices, extracellular electrical signals of various type of primary neural can successfully be recorded, and this bioelectrical activity can be presented for several weeks [78]. In addition, the MEA-based cell biosensor can only detect a limited number of discrete active sites, ascribing to the fabrication of electrode arrays. This shortcoming has been solved by utilization of light-addressable potentiometric sensor [79].

5.2. Light-Addressable Potentiometric Sensor-Based Biosensor

Cells are active and dynamic entities, not confined to permanent localization on a device to allow easy interrogation. Light-addressable potentiometric sensor is a commonly used semiconductor chip for developing cell-semiconductor hybrid system, by which cells can be randomly cultured and the change of cellular electric potential can be addressably
detected [79, 80] (Fig. 5b). Liu et al. [80] reported an olfactory cell-based biosensor as a real bionic technique for odorants detection based on LAPS. Using LAPS as sensing chip, the response under stimulations of the odorants or neurotransmitters was tested. The results demonstrated that this kind of hybrid system of LAPS and olfactory neurons had great potential and was promising to be used as a novel neurochip of bioelectronic nose for detecting odors.

Fig. (5). Schemes of cell-based biosensors with (a) microelectrode array (MEA), (b) light-addressable potentiometric sensor (LAPS), and (c) carbon nanofiber (CNF) array for recording/stimulation of cellular electric potential.

5.3. Nanoelectrode Array-Based Neural Probes

Electrical stimulation of nerve cells has been widely employed in neural prostheses, clinical therapies, and basic neuroscience studies [82, 83]. A variety of metals and metal alloys have been fabricated into electrodes for neural stimulation. However, only large electrodes with a low current density can be safely used due to the low charge injection limit, which makes the electrode size much larger than the neural cell. This makes it difficult to access the 3D neuronal network. Nowadays, the application of nanotechnology and nanoelectrode arrays (NEAs) shows the ability to interface solid-state electronics with living cells at the subcellular level [84]. For example, a study of the electrochemical properties of vertically aligned carbon nanofiber (CNF) arrays coated with polypyrrole and the cell culture of neural cells on such substrates [84] (Fig. 5e) shows the great potential of such nanostructured materials to extract and modulate neural signals more precisely than MEAs, while inducing much less damage to the tissues.

6. FUTURE DESIGN STRATEGIES

Future design of cell-based electrochemical biosensors mainly involves the following aspects on the priority list: new immobilization/culture materials and technology, multifunction analysis, and application of gene technology and nanomaterials. The availability of stable immobilization/culture biomaterials is one of the cornerstones, because the effectively holding of cellular function is of paramount importance for preparation of cell-based electrochemical biosensors.

Because cells respond to external stimuli with the parallel activation of different signaling pathways, simultaneous measurement of multiple signals is very useful. Recently, a kind of multifunctional integrated electrochemical sensor array has been developed for monitoring fermentation conditions [85]. It is anticipated that integration of different transducer principles, enabling multi-functional cellular processing of input- and output-signals, will be central to the design of next-generation sensing devices.

Because cells can respond to various physical parameters, for detecting single species of molecules or ions, highly specific cells are needed, which can be obtained through gene technology. Using genetically engineered cells, enzymes of interest can be expressed on cell surfaces, which allows for the acceleration of electrochemical response and enhancement of selectivity of biosensors. Furthermore, recombinant cells will contribute to the integration of the cell functions and electroactive substrates for microfabrication.

In perspective, nanomaterials for construction of new electrical-neural interface may open up many opportunities for biomedical, medicinal applications and fundamental neuroelectronics research. Successful implementation of nanoscale interface engineering is necessary to address challenges like long-term biocompatibility and neuron-device contact.

Cell-based electrochemical biosensors have been applied in biological, medical, environmental and chemical sciences. They can diagnose physiologically relevant analytes and events superior to conventional molecular-based strategies. As extensive experience has been gained by cell-based electrochemical sensors in assessing drug effect and toxicity, they will be important tools in medicinal product development studies. It can be anticipated that these biosensors will become one of the leading biotechnologies that promise future breakthroughs in numerous fields.

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ABBREVIATIONS

AcChCl  =  Acetylcholine chloride
CE       =  Capture efficiency
CHO      =  Chinese hamster ovary
CNF      =  Carbon nanofiber
DOX      =  Dissolved oxygen
DPV      =  Differential pulse voltammetry
ECIS     =  Electric cell-substrate impedance sensing
ECM      =  Extracellular matrix
EIS      =  Electrochemical impedance spectroscopic
GSE      =  Gas-sensing electrode
HUVEC    =  Human umbilical vein endothelial cells
IDAM     =  Interdigitated array microelectrode
ISE      =  Ion-selective electrode
ITO      =  Indium tin oxide
LAPS     =  Light-addressable potentiometric sensor
L-dopa   =  L-3,4-dihydroxyphenylalanine
L-NMMA   =  Nω-monomethyl-L-arginine
MEAs     =  Microelectrode arrays
MNAC     =  Magnetic nanoparticle-antibody conjugates
MTT      =  3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NEAs     =  Nanoelectrode arrays
RT-CES   =  Real-time cell electronic sensing
PPAEC    =  Porcine pulmonary artery endothelial cell
Rt       =  Electron-transfer resistance
T-HUVEC  =  Transformed human umbilical vein endothelial cells

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