Electrochemical Biochip for Drug Screening At Cellular Level

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Abstract. Drug screening at cellular level has becomes an attractive field of research. Different researchers have tried to record cellular response to drugs by electrical or optical approach using both invasive and non-invasive methods. Silicon-based microelectrode integrated microchips are useful tools for in situ temporal recording of neurotransmitter releasing from neural cells. A microfabricated electrochemical biochip is presented in this paper. Using dopaminergic cells grown on the chip, the dopamine exocytosis can be electrochemical amperometric detected non-invasively from drug incubated dopaminergic cells by the microelectrode integrated on chip. This silicon-based electrochemical chip has been designed with an electrode array located on the cell culture chamber bottom. Each electrode is individually electrical controlled. MN9D and PC12 dopaminergic cell lines have been demonstrated on this chip for drug effects study. This silicon-based electrochemical microchip provides a non-invasive, in situ, temporal detection of dopamine exocytosis from dopaminergic cells, and holds the potential for applications in studying the mechanisms of dopamine exocytosis and drug screening. It is also extendable for other cell culture and drug effects study.

Keywords: microelectrode array, cell chip, cell monitoring, in situ, temporal detection, drug screening, non-invasive

1. Introduction

Cells are basic units for life and function. Therefore, cells give scientists a better idea of a drug behavior than a biochemical mixture alone. Cellomics can provide high content screening. This technique allows living cells studies in a controlled environment where optical or electrical reveal temporal changes in the cells and cell constituents can be monitored.

Cell culture plays a major role in the development of the cell based analysis system for drug screening. The cellular responses may be used as parameters for monitoring chemical information for both pharmaceutical and chemical safety and drug efficacy profiles in vitro as screen tool1. To create a stable and uniform microenvironment for the cell growth at the microchip level, researchers 2,3,4,5 presented the microfluidic technique, but have not integrated the sensing capability yet.
Dopamogenic cells are selected as the cell model in this research to demonstrate an electrochemical microchip based platform technology in situ, for temporal and non-invasive detection of drug effected cellular response for drug screening. Invasive and non-invasive methods are targeted by different group of researchers to perform electrical stimulating on neural cell study. The conventional non-invasive method in which electrochemical electrodes need to be directly contacted to the cell membrane surface, requires researchers to manually located electrode under microscope. This method can detect dynamic response of the neurotransmitter releasing, but it is difficult to operate. The conventional invasive method is done by lysising the cells after drug incubation and all the lysised solution sent for HPLC and electrochemical analysis. This method is not able to monitor the neurotransmitter releasing dynamic process and is also not able to quantify the amount of neurotransmitter released. Only the total neurotransmitter in the cells can be detected. Large cell concentration and sample quantity are required in this method. To speed up the drug screening process, a convening non-invasive and quantity detection tool is expected. Electrochemical technique is well established and widely used for various chemical sensing as well as dopamine sensing. It is with the advantages of stability, sensitivity and compatible with microfabrication process and easy integration with cell culture chips. Therefore electrochemical sensor and amperometry technique is applied into this research.

A silicon based-electrochemical microchip for neural cell culture and drug effected cell response detection is presented in this paper. The dopamingenic MN9D and PC12 cell lines are used as cell models to demonstrate the device function, which included cell culture on chip, drug incubation and K+ stimulation as well as dopamine releasing detection. The drug L-DOPA, reserpine are used to demonstrate the device, which is with the capability to monitor drug effect on dopamine releasing and uptaking.

2. Experimental

2.1. Design and microfabrication of electrochemical biochip

A silicon based electrochemical sensor array integrated chip has been designed with 25 disc electrodes with a 5x5 layout shown in Fig. 1. The electrode is with 30µm diameter and 250µm spacing (electrode center to center). All the electrodes are located on the bottom of the cell culture chamber and each electrode is individually controlled. Microchip structure and packaged device has been shown in Fig. 1.

![Fig. 1 Microchip Structure](image)

Two pieces of 6” silicon wafer were used as substrates to make this electrochemical biochip and Au was used as electrode material. The detail microfabrication process was described by previous publication by Y. chen et al.
2.2. Cell culture on chip, cell depolarization and amperometric dopamine detection

The MN9D cell is a mouse dopaminergic neuronal cell line derived from the fusion of rostral mesencephalic neurons from embryonic C57BL/6J mice with the N18TG2 neuroblasts cells\textsuperscript{15}. The experiment condition for the neural cell culture on microchip was studied and reported by H.F. Cui \textit{et al.}\textsuperscript{16}. A thin layer of collagen coating was processed at the bottom of the chamber at 37$^\circ$C for overnight before PC12 cell culture. The protocol for the PC12 cell culture recommended by L.A. Greene \textit{et al.}\textsuperscript{17} was used in this research. Amperometric technique was used to detect the dopamine exocytosis from dopaminergic cells. All the three electrodes (working, counter, and reference electrode) used on the biochip, and all potentials were quoted versus the biochip microelectrode. The MN9D cells cultured in the balanced salt solution w/wo L-dopa were washed with the balanced salt solution for 4 times and then kept in the balanced salt solution for amperometric detection. MN9D cells were multi-stimulated by repetitively introducing K$^+$. The detail experimental condition was also reported by H.F. Cui \textit{et al.}\textsuperscript{16}. The similar drug effects testing were also done for PC12 cells. Both L-dopa and reserpine effects were tested for the PC12 cells and the dopamine exocytosis were detected both for with/without drugs effected PC12 cells.

3. Results and Discussions

3.1 Sensor calibration to dopamine

Based on the oxidation peak potential of dopamine studied by cyclic voltammograms (not shown), the amperometric recording of dopamine exocytosis from MN9D and PC12 cells as well as amperometric calibration was done at $+200$mv vs. internal microelectrode. The different volume of stock dopamine solution was injected into PBS buffer solution. The steady state current readings were reordered before and after dopamine were injected. The current difference was as the sensor response to the corresponded dopamine concentration. Based on this method, the sensor steady-state calibration to dopamine was plotted in Fig. 2. This method was used to quantify the dopamine exocytosis from the neural cells by K$^+$ stimulation with/without drug effects. The dopamine detection limitation was found at 0.12 µM(signal/noise=3).

3.2 Neural cells cultured on chip and drug effects testing

The neural cells MN9D and PC12 were successfully cultured on the micro chip which is shown in Figure 3. Once the cell density on chip surface reached 80-90% coverage, it was suitable for drug effect testing. The successful cell growth on chip established a basis to perform dopamine exocytosis \textit{in situ}, temporally detection on chip for drug screening.
Fig. 3 Neural cells growth on chip

The elevated extracellular K⁺ level caused the depolarisation of dopaminergic cells\textsuperscript{18,19}. The K⁺ stimulated dopamine exocytosis from MN9D and PC12 cells w/o L-DOPA incubation were shown in Fig. 4. H.F.Cui \textit{et al}\textsuperscript{18} reported the quantitative study and explanation. It can be found that PC12 cells are with a higher dopamine concentration inside cells, without L-dopa incubation, the dopamine exocytosis from PC12 cell also can be detected but not for the MN9D cells. After L-dopa incubation, dopamine exocytosis was significantly increased both for MN9D and PC12 cells and all can be \textit{in situ}, temporally detected by the electrochemical microelectrode. The drug reserpine was also tested for the PC12 cells and the amperometric detection of KCl stimulated dopamine exocytosis from PC12 cells will be reported by H.F.Cui \textit{et al} in other publication. It can be found that PC12 with dopamine releasing before reserpine incubation. And after reserpine incubation, the dopamine exocytosis was reduced significantly and proportional to the reserpine incubation time and shown in Fig. 5. The percentage of the ratio of dopamine signal with and without drug incubation was plotted against the drug incubation time. If the PC12 cells were incubated with reserpine for about 30mins, residual dopamine releasing can barely be detectable. Similar phenomena and mechanism were found and explained by Y. Kasai \textit{et al}\textsuperscript{20}. 

Fig. 4 Amperometric detection of KCl stimulated dopamine exocytosis from neural cells w/o L-Dopa incubation
4. Conclusions
The neurotransmitter excytosis from on chip cultured neural cells has been successfully in situ temporal non-invasive detected by using electrochemical microchip. The capabilities of using this chip for the drug screening and drug effect mechanism study have been demonstrated. It holds a potential for drug effects study on other cells as well as drug discovery and may speed up the drug screening process.

Acknowledgments
This work was supported by research funds of the Agency for Science, Technology and Research (A*STAR) to Yu Chen through Institute of Microelectronics, and Academic Research Grants of the National University of Singapore R-398-000-006-112 and R-398-000-024-112 to Fwu-Shan Sheu. This research is also supported by Miss Jong Ming Ching (Institute of Microelectronics) for the mask layout.

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