Microfluidic Neural Cell Chip for Monitoring of PCBs

Soeun Park[*] and Young-Kee Kim[*,**]

[*]Program of Integrated Biotechnology, Hankyong National Universty,
snowhee1202@hanmail.net
[**]Division of Chemical Engineering, Hankyong National University,
Anseong, Kyonggi-Do 456-749, Republic of Korea, kim@hknu.ac.kr

ABSTRACT

The microfluidic neural cell chip was fabricated to monitor neurotoxic material, polychlorinated biphenyls (PCBs), which have bad influence to ecosystem and human health through foods and contaminated water. The chip consist of gold electrode patterned layer, fluidic layer, and control layer. The gold electrode layer has three electrodes (working electrode, counter electrode, and reference electrode). The fluidic layer contains three chambers (300 μm × 300 μm × 20 μm) and PC12 cells were cultivated in each chamber. A proposed microfluidic device makes possible to accomplish experiments with three different samples. PCBs have bad influence on the activity of PC12 neural cells, which was resulted in interference of dopamine production. Electric measurement of dopamine concentration was performed by cyclic voltametry (CV). In this study, fabricated microfluidic neurla cell chip can successfully measure bad influence of PCBs on the activity of PC12 cells. This tool can be applied to monitor PCBs in aquatic environment.

Keywords: neural cell chip, PC12, microfluidic device, PDMS, PCBs

1 INTRODUCTION

Microfluidic devices can be usefully applied to make chemical or biological analysis method [1]. Especially, it is usufel for cell analysis because it is possible for miniaturizing, integrating complex multi-step procedure, reduced reagent consumption, fast and sensitive measuringy, and portable and easy handling [2-5]. In analytical chemistry, biology, diagnostics and biomedical research, microflluidics are up-to-date technology[6]. A microfluidic device can integrate sample preparation, mixing, reaction, separation, and detection [7]. The dimensions of microfluidic device are comparable to the size of cell, therefore it is good for detection of single cell signal [8].

A various polymeric materials, such as poly(dimethylsiloxane) (PDMS), poly(methylmethacrylate) (PMMA), and pollycarbonate, are used to fabricate microfluidic devices [1]. PDMS has been widely used to make microfluidic devices, because of its several advantages including high chemical, biological and mechanical stability [1, 9]. Disposable microfluidic device was designed by the micro-molding techique for biological and chemical applications by using PDMS [10].

Persistent organic pollutants (POPs) are toxic chemicals that are resistant to photolysis, chemical attack, or biological degradation. The pesticides such as DDT, mires, aldrin, lindane, polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are well-known POPs. PCBs, which is a common POPs, have a bad influence on aquatic ecosystems and it can be accumulated in aquatic animals via food chain [11]. Western countires have prohibited to use PCBs from twenty years ago, however, accumulated residues in environment are still dangerous for ecosystem and human health owing to their persistence. Also the third world countries produce and use this harmful chemicals continuously [11].

In this study, a microfluidic neural cell chip was fabricated by using PDMS. The fabricated device was used to measure extremely low concerntration of PCBs based on electric signal measurement technique. Signal transmission system in neural cells can be affected by neurotoxins, and the metabolism of neural cells can be observed quickly by electric method. This analytical tool can make possible to measure direct effect of POPs on the nervous system of animals. In the analytical system, the activity of neural cells was measured by detecting the emission of dopamine, cellular neurotransmitter, using cyclic voltammetry (CV) in presence of PCBs.

2 MATERIALS AND METHODS

2.1 Materials

Polydimethylsiloxane (Sigard 184 set) was purchased from Dow Corning Korea Ltd. (Seoul, Korea) to fabricate control and fluidic layer of microfluidic device. Photoresist (PR) patterned wafers, used as mold, and gold microelectrode patterned glasses were designed to fabricate microfluidic devices. Those are customized by SeouLin Bioscience (Seoul, Korea) and Amed Inc. (Seoul, Korea), respectively.

Reasgent grade dopamine (H8502) was purchased from SIGMA-ALDRICH Co. (St. Louis, MO, USA), and 2,2',3,',5,5'-hexachlorobiphenyls (PCBs) was obtained from AccuStandard, Inc. (C-133N, New Haven, CT, USA).

PC12 (CRL-1721), a neural cell, was purchased from American Type Culture Collection (ATCC, Washington DC, USA). Dulbecco's Modified Eagle Medium (DMEM, LM 001-07) and Trypsin-EDTA Solution, 1X (LS 015-01) were obtaind from WelGENE (Daegu, Korea). Phosphate Buffered Saline (PBS, P3813) was purchased from SIGMA-ALDRICH. Penicillin-streptomycin (P/S, 15140163) was bought from Gibco (Carlsbad, CA, USA).

2.2 Neural cell culture

PC12 cells were cultured in DMEM at 37℃, 5% CO_2 incubator (MCO-18AIC, Sanyo, Japan) for 48hours. 70-80% growing PC12 cells in culture dish area was washed 2 or 3 times by 10 mL PBS, and then was detached in 5% CO_2 incubator for 3minutes at 37℃ by adding 1mL Trypsin-EDTA.

5mL of fresh medium was added to detached PC12 cells in culture dish. Medium containing PC 12 cells was moved into 15 mL centrifuge tube and was centrifuged at 3000 rpm for 5 minutes. After removing spernatant, 1 mL of fresh medium was putted in the tube and then moved to 1.5 mL centrifuge tube. Tube was centrifuged at 3000 rpm for 5 minutes and supernatant was removed. 200 μL of fresh medium was added into the tube.

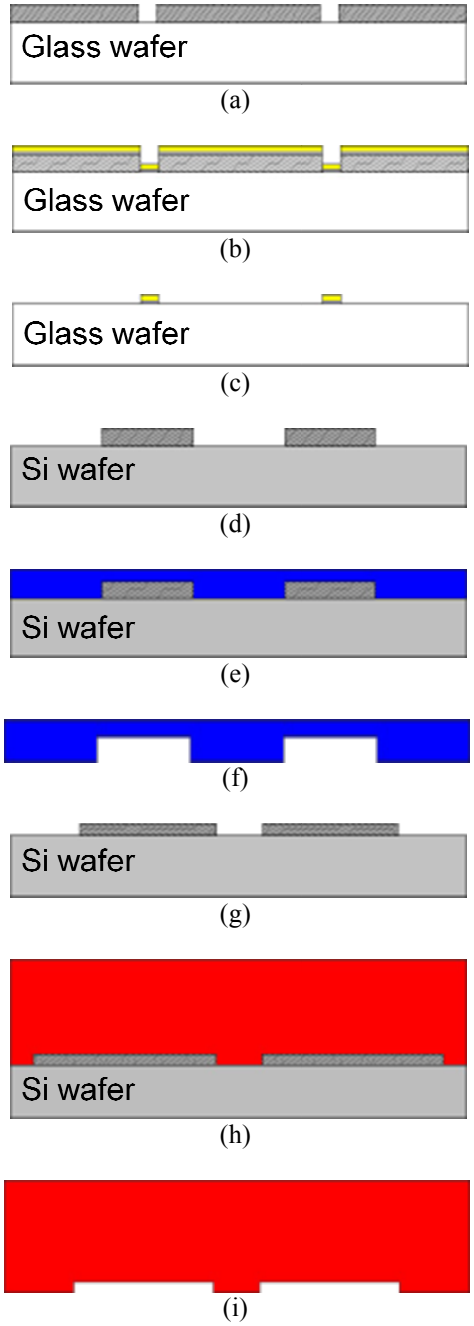
2.3 Fabrication of microfluidic chip device

PDMS and hardener was mixed in the ratio of ten to one or twenty to one to make control layer and fluidic layer, respectively. Mixed solution was left in vacuum desiccators for 2 hours to remove the bubble. PR patterned wafer was coated with CTMS vapor for 10 minutes to protect mold. Control layer was fabricated using PR patterned wafer. The dimension of control channel is 103 (width) × 15(height) μm. Square acryl mold ring (36 (width) × 43 (length) × 10 (height) μm) putted on wafer coated with CTMS and the 8 g mixture of PDMS and hardner (in the ratio of 10 to 1) poured in the acryl ring and it was baked in the 80℃ oven (KR/VS-1202V6, Vision Scientific, Korea) for 45minutes. After cooling the control layer, it was took off from the wafer and cutted verge. Punching mark of control layer was punched with micro scope and 0.75mm punch (UNI-CORE, USA), and then control layer was washed with ethyl alcohol.

Fluidic layer was fabricated using PR patterned fluidic wafer. Dimension of fluidic chamber was 300 μm (width) × 300 μm (length) × 20 μm (height). The mixture of PDMS and hardner (in the ratio of 20 to 1) was poured on the fluidic wafer and fluidic layer was made by spin coating method. The formed fluidic layer was left on the leveled off place and was baked at 80℃ oven for 35 minutes.

Control layer was aligned on the fluidic layer, and then it was baked at 80℃ oven for 90 minutes to attach the control layer on the fluidic layer. After detach the combined two layers from the fluidc wafer, punching mark of fluidic pattern was bored with micro scope and 0.75mm punch.

The bound two layers was aligned on the gold microelectrode patterned glass wafer and this microfluidic device was baked at 80℃ oven for 18 hours. Figure 1 shows the schematic fabrication procedure to make microfluidic device. The fabricated microfluidic device is shown in Fig. 2.



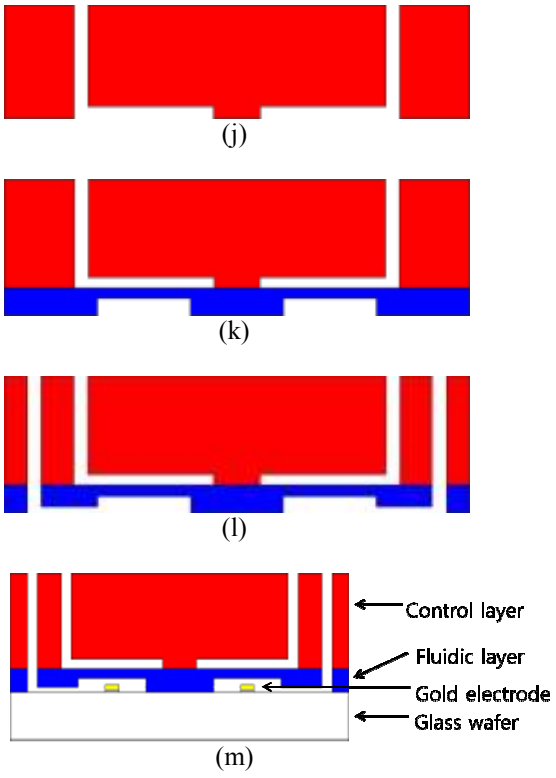


Fig. 1. Schematic illustration of microfluidic device fabrication procedure. Gold microelectrode patterned glass wafer fabrication: (a) SU-8 photoresist (PR) is patterned and developed on glass wafer, (b) Au/Cr mixture is deposited on glass wafer, and (c) PR layer is removed from gold electrode patterned glass wafer. Fluidic layer fabrication: (d) SU-8 is patterned on fluidic silicon wafer, (e) PDMS is molded on fluidic silicon wafer, and (f) PDMS is peeled from fluidic silicon wafer. Control layer fabrication: (g) SU-8 is patterned on control silicon wafer, (h) PDMS is moled on control silicon wafer, (i) PDMS is peeled from control silicon wafer, (j) control layer is punched. Binding of control and fluidic layer on gold electrode patterned glass wafer: (k) control layer is aligned on fluidic layer, (l) bound control and fluidic layer is punched, (m) alignment and binding control and fluidic layers on gold microelectrode patterned glass wafer.

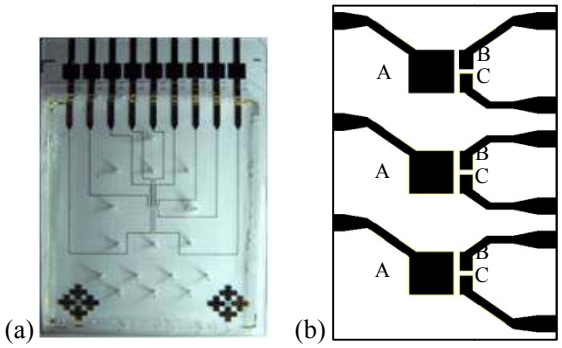


Fig. 2. Images of the fabricated microfluidic device containing three chambers (a). Gold microelectrode configuration in three chambers of the chip (b). A: working electrodes, B: counter electrodes, and C: reference electrodes.

2.4 Cultivation of PC12 cells in microfluidic device

Fabricated neural cell chip was sterilized in autoclave at 120℃ for 15minutes. PBS buffer solution containing 5% P/S was injected into control channel, and working of microvalve was confirmed using pressure controller (Fluidic controller, Fluidigm, USA). The PC12 cells in fresh medium was injected into fluidic chamber via fluidic channel and all of the microvalves were closed. PC12 cells were cultivated in 5% CO_2 incubator at 37℃ for 36 hours.

2.5 Measurement using neural cell chip

The concentration of dopamine, which is excreted from active PC12 cells, was measured using cyclic voltametry according to PCBs concentrations. Electrochemical signal of dopamine was measured in concentration of 1×10^{-4} M, 1×10^{-5} M, 1×10^{-6} M, 1×10^{-7} M, 1×10^{-8} M, 1×10^{-9} M, and 1×10^{-10} M. Dopamine was dissolved in DMEM. The presence of PCBs can influenced to activity of PC12 cells, and CV was measured every two hours. The PCB concentrations of 0.1 and 0.15 ppm was examined.

3 RESULTS AND DISCUSSION

According to concentration of dopamine, electrochemical signals were measured using cyclic voltametry. The concentrations of dopamine were 1×10^{-4} M, 1×10^{-5} M, 1×10^{-6} M, 1×10^{-7} M, 1×10^{-8} M, 1×10^{-9} M, and 1×10^{-10} M. Fig. 3 (a) shows an calibration curve between dopamine concentration and electric current change.

After PC12 cells were cultured in the microfluidic neural cell chip for 36hours, sample solution containing PCBs was injected into the chip. The electrc signals of neural cell chip were measured every two hours. The activity of cells in 0.15 ppm of PCBs decreased rapidly. On the other hand, the activity of PC12 cells in 0.1 ppm PCBs decreased after 6 hours. In reference experiment, electric current change increased steadily.

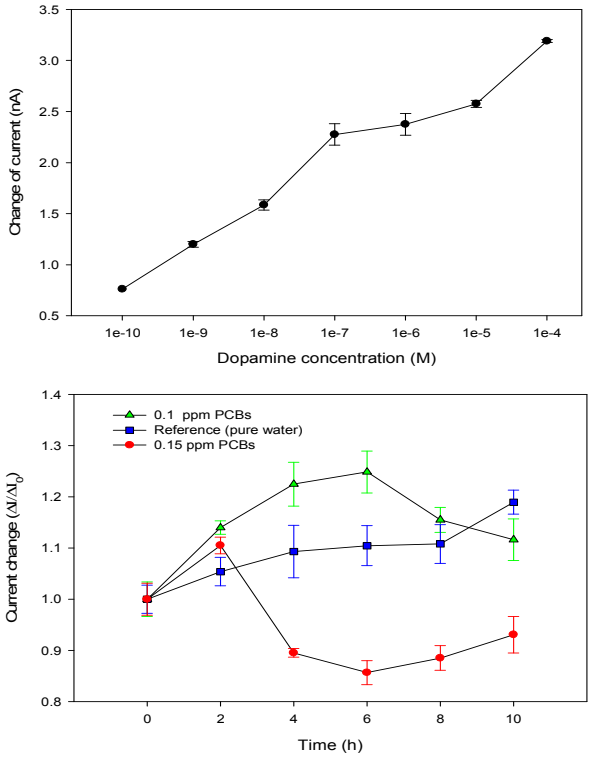


Fig. 3. Difference of low and high current obtained from cyclic voltametry (CV) measurement. (a) Electric current change of neural cell chip according to dopamine concentration, (b) Electric current change of neural cell chip in cases of samples of 1.0 ppm and 1.5 ppm PCBs comparing to samples of pure water.

4 CONCLUSION

The neural cell chip was fabricated by using microfluidic device on gold electrode patterned glass wafer. PC12 neural cells were cultivated in micro-chamber in fabricated neural cell chip. The signals of neural cell chip were measured according to dopamine concentration from 1 $\times 10^{-10}$ M to 1 $\times 10^{-4}$ M. The changes of pysiological state of PC12 neural cell were measured by using the proposed neural cell chip according to PCBs concentration.

REFERENCES

[1] M. J. Schöning, M. Jacobs, A. Muck, D. T. Knobbe, J. Wang, M. Chatrathi, and S. Spillmann, "Amperometric PDMS/glass capillary electrophoresis-based biosensor microchip for catechol and dopamine detection," Sensors and Actuators B: Chemical, 108, 688-694, 2005.

[2] R. Popovtzer, T. Neufeld, E. z. Ron, J. Rishpon, and Y. Shacham-Diamand, "Electrochemical detection of biological reactions using a novel nano-bio-chip array," Sensors and Actuators B: Chemical, 119, 664-672, 2006.

[3] F. Xia, W. Jin, X. Yin, and Z. Fang, "Single-cell analysis by electrochemical detection with a microfluidic device," Journal of Chromatography A, 1063, 227-233, 2005.

[4] Y. Wang, Q. He, Y. Dong, and H. Chen, "In-channel modification of biosensor electrodes integrated on a polycarbonate microfluidic chip for micro flow-injection amperometric determination of glucose," Sensors and Actuators B: Chemical, 145, 553-560, 2010.

[5] W.-G. Koh and M. Pishko, "Immobilization of multi-enzyme microreactors inside microfluidic devices," Sensors and Actuators B: Chemical, 106, 335-342, 2005.

[6] Z. L. Zhang, C. Crozatier, M. Le Berre, and Y. Chen, "In situ bio-functionalization and cell adhesion in microfluidic devices," Microelectronic Engineering, 78-79, 556-562, 2005.

[7] L. Zhu, L.-y. Hou, and W.-y. Zhang, "A new fabrication method for glass microfluidic devices used in micro chemical system," Sensors and Actuators B: Chemical, 148, 135-146, 2010.

[8] Y. Yamaguchi, T. Arakawa, N. Takeda, Y. Edagawa, and S. Shoji, "Development of a poly-dimethylsiloxane microfluidic device for single cell isolation and incubation," Sensors and Actuators B: Chemical, 136, 555-561, 2009.

[9] C. Karuwan, A. Wisitsoraat, T. Maturos, D. Phokharatkul, A. Sappat, K. Jaruwongrungsee, T. Lomas, and A. Tuantranont, "Flow injection based microfluidic device with carbon nanotube electrode for rapid salbutamol detection," Talanta, 79, 995-1000, 2009.

[10] J.-C. Yoo, H.-J. Her, C. J. Kang, and Y.-S. Kim, "Polydimethylsiloxane microfluidic system with in-channel structure for integrated electrochemical detector," Sensors and Actuators B: Chemical, 130, 65-69, 2008.

[11] A. Binelli and A. Provini, "The PCB pollution of Lake Iseo (N. Italy) and the role of biomagnification in the pelagic food web," Chemosphere, 53, 143-151, 2003.