Article

Journal of Biomedical Nanotechnology
Vol. 9, 880–885, 2013
www.aspbs.com/jbn

Microfluidic Chip for the Detection of Biological Toxic Effects of Polychlorinated Biphenyls on Neuronal Cells

Soeun Park[1], Jeong-Woo Choi[2], and Young-Kee Kim[1,*]

[1] *Department of Chemical Engineering and Research Center of Chemical Technology, Hankyong National University, Anseong, Gyeonggi-Do 456-749, Republic of Korea*
[2] *Interdisciplinary Program of Integrated Biotechnology and Department of Chemical and Biomolecular Engineering, Sogang University, Seoul 121-742, Republic of Korea*

The aim of this study was to develop a microfluidic neuronal cell chip device to monitor the toxic effects of polychlorinated biphenyls (PCBs) on PC-12 neuronal cells. PCBs adversely influence the activities of neuronal cells in the nervous system. In PC-12 cells, the production and secretion of dopamine decreases in response to PCB exposure. The microfluidic device that we developed to measure the amount of dopamine by cyclic voltammetry is composed of a control layer, a fluidic layer, and a gold electrode-patterned glass wafer. The control channel in the control layer functions as a microvalve to control the flow of the fluidic channel in the fluidic layer. The fluidic layer consists of 3 reaction chambers as well as fluidic channels. Three electrodes, including the working electrode, counter electrodes, and a reference electrode, are placed in a fluidic chamber. The electrochemical signals of dopamine, either from a standard dopamine solution or from the culture supernatant from cultured PC-12 cells, were obtained using a fabricated microfluidic neuronal cell chip by cyclic voltammetry. When PCBs were added to cultures of PC-12 cells, the amount of dopamine secreted from the PC-12 cells decreased due to the reduced activity of PC-12 cells. The fabricated neuronal cell chip was capable of detecting the toxic effect of dopamine on neuronal cells at concentrations of 10 μg/L and over. The practicality of the developed microfluidic neuronal cell chip was validated using river water spiked with PCBs.

KEYWORDS: *Microfluidic Device, Neural Cell Chip, PC12, Polychlorinated Biphenyls (PCBs), Persistent Organic Pollutants (POPs).*

INTRODUCTION

The term persistent organic pollutants (POPs) encompasses various organic substances that are harmful to the ecosystem.[1] Dichlorodiphenyltrichloroethane (DDT), mirex, aldrin, lindane, polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs) and poly chlorinated dibenxofurans (PCDFs) are common POPs.[2] They enter living systems through dermal absorption, inhalation, and intake of contaminated soil and food.[3] POPs are toxic substances causing cancer, endocrine disruption, immune disorders, and neuronal impairment.[4]

PCBs have a particularly dangerous effect on aquatic ecosystems.[5] In many commercial and industrial areas, PCBs were utilized to make lubricating oil, heat-exchange fluids, diluents, hydraulic fluids, and dielectric fluids for capacitors and transformers.[6–7] Although PCBs have been banned in most countries of the world since the 1970s, the residue of PCBs produced and used in some third countries continues to threaten ecosystems and human health.[5, 8] The main route of exposure to PCBs is by ingestion of contaminated sea food.[3] PCBs cause irregular menstruation, sensory deprivation, and various cancers. Therefore, many researchers have investigated the toxicity of PCBs.[9–10]

Pereira and Rao monitored the toxicity of PCBs by using rats. One group of male and female rats was fed normal rodent chow and water, while the other group was fed a diet mixed with PCBs dissolved in corn oil. The PCB-consuming rats had larger livers, and they showed hepatotoxicity and hepatomegaly.[11] In another study, Costa et al. assessed the viability of neuronal cells cultured in a medium with PCBs by using the MTT (3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyl-tetrazolium bromide)

*Author to whom correspondence should be addressed.
Email: kim@hknu.ac.kr
Received: 4 January 2012
Accepted: 16 February 2012

1550-7033/2013/9/880/006 doi:10.1166/jbn.2013.1497

assay and measured the proportion of living and dead cells by the Trypan blue exclusion assay.[12] Gichner et al. found that tobacco plants in grown in PCB-contaminated soil were less developed than those grown in non-contaminated soil because of serious DNA damage.[13] However, these methods required complicated processes to estimate the toxicity, and the estimates obtained were indirect. Thus, this study focused on the development of a direct and simple method for assessing toxicity with a neuronal cell chip on a microfluidic device.

Microfluidic devices are powerful tools for analytical chemistry, biology, diagnostics, and biomedical research.[14–15] Several microfluidic devices have been developed to detect glucose,[16] dopamine, catechol,[15] and bacteria.[17] Cell-to-cell signal transfer was investigated using a microfluidic device, whose dimensions approximated those of the cell.[18] Microfluidic devices can be miniaturized and integrated, thereby affording advantages such as easy handling, portability, short operating time, low cost, reduced consumption of sample and reagents, and high sensitivity.[16, 19–21] Among electrochemical analysis systems, cyclic voltammetry (CV) is widely used to measure electric signals.[22–23] An electrochemical analysis tool using CV has been applied to measure the levels of harmful chemicals such as polyphenol,[24] phenothiazine,[23] and bisphenol-A.[25]

PC-12 cells, derived from rat pheochromocytoma, can synthesize acetylcholine, catecholamines, and norepinephrine and have been used to explore exocytosis in electrochemical studies.[26] Dopamine, one of the catecholamines produced by PC-12 cells, is an important neurotransmitter in the central nervous system,[27–29] and is regarded as a good indicator of the physiological status of neuronal cells. Therefore, the neurotoxicity of specific chemicals can be monitored by measuring the amount of dopamine secreted from PC-12 cells.

In this study, we fabricated a neuronal cell chip based on a microfluidic device and used it to monitor the toxic effects of PCB-contaminated samples on the physiological status of neuronal cells (PC-12). Since active PC-12 cells produce dopamine, the biological activity of PC-12 cells in a cultivating chamber was evaluated by measuring the dopamine concentration using CV on a microfluidic device. This microfluidic device, which is prepared using a nano-fabrication technique, can detect extremely low concentrations of dopamine. Since the intracellular messenger is delivered to the neural signal system within several seconds of exposure to a toxicant, the microfluidic device is suitable for exploring the nervous reactions of animals to environmental toxicants. A microvalve system of fluidic channels and control channels allows sequential operation of the analysis procedure on a microfluidic chip. Multiple reaction chambers in a single microfluidic chip can monitor numerous samples simultaneously.

EXPERIMENTAL DETAILS

Materials

Polydimethylsiloxane (PDMS; Sylgard 184, Dow Corning, Midland, MI, USA), custom-made photoresist (PR) patterned wafers (SeouLin Bioscience, Seoul, Korea), gold microelectrode patterned glass wafer (Amed Inc., Seoul, Korea), and fluid controller (Fluidigm Corporation, San Francisco, CA, USA) were obtained for the construction of the microfluidic device. Reagent-grade dopamine (H8502), phosphate-buffered saline (PBS, P3813), and chlorotrimethylsilane (CTMS, 386529) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). 2,2′,3,3′,5,5′-Hexachlorobiphenyl (PCBs, C-133N) was obtained from AccuStandard, Inc. (New Haven, CT, USA). PC-12 neuronal cells (CRL-1721) were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). Dulbecco's modified eagle medium (DMEM, LM 001-07) and trypsin-EDTA solution (LS 015-01) were obtained from WelGENE (Daegu, Korea). Penicillin-streptomycin (P/S, 15140163) was supplied by Gibco (Grand Island, NY, USA). The river water used to prepare spiked samples was collected from the Anseong River, Gyeonggi-Do, Korea.

Fabrication of the Microfluidic Device

PDMS, which is the common substrate for microfluidic devices due to its high chemical and mechanical stability, was used to make neuronal cell chip devices.[30] This polymer also shows good biochemical compatibility and a low cost of disposal.[31] The PDMS and hardener were mixed in ratios of 10:1 for control layers and 20:1 for fluidic layers. The control layer and the fluidic layer were fabricated using PR-patterned control wafer and fluidic wafer, respectively. The dimension of the control channel was 103 (width) $\times$ 15 (height) μm, and that of the fluidic chamber was 300 (width) $\times$ 300 (length) $\times$ 21 (height) μm. The control layer was aligned onto the fluidic layer, and they were placed on the gold microelectrode patterned glass wafer as shown in Figure 1. The fabricated microfluidic device and the experimental arrangement, composed of control channels, fluidic channels, 3 reaction chambers, and microelectrodes, are shown in Figure 2. The fluidic reaction chamber was placed on the gold microelectrode patterned wafer, and the control channel was positioned on the fluidic channel. By increasing pneumatic pressure in the control channel, the diaphragm between the control and fluidic channels expanded into the fluidic layer to close the fluidic channel.

Cultivation of PC-12 Cells in the Microfluidic Cell Chip

PC-12 cells were incubated in a tissue dish for 36 h prior to inoculation into the microfluidic device. PC-12 cells in fresh medium were introduced into the y-directional fluidic

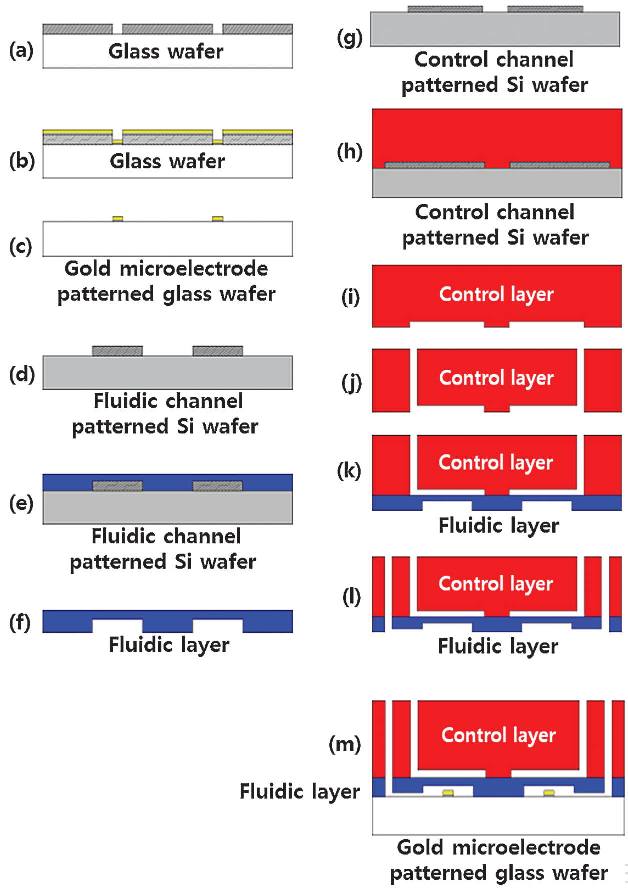

Figure 1. Schematic illustration of fabrication process for microfluidic devices. The fabrication of gold microelectrode patterned glass wafer: (a) SU-8 photoresist (PR) is patterned and developed on the glass wafer; (b) Au/Cr mixture is deposited onto the electrode glass wafer; and (c) PR is removed from the glass wafer. The fabrication of the fluidic layer: (d) SU-8 PR is patterned on the fluidic silicon wafer, (e) PDMS is molded on the wafer, and (f) PDMS is peeled from the fluidic channel patterned silicon wafer. The fabrication of control layer: (g) SU-8 PR is patterned on the control silicon wafer, (h) PDMS is molded on the control silicon wafer, (i) PDMS is peeled from the control silicon wafer, and (j) the control layer is punched. Bonding the control and fluidic layer on the gold microelectrode patterned glass wafer: (k) the control layer is aligned on the fluidic layer, (l) combined layer is punched, and (m) combined layer is aligned on the gold microelectrode-patterned glass wafer.

channel to fill the reaction chamber with a mixture of PC-12 cells and medium while the *x*-directional fluidic channels were closed by a microvalve. Then, all microvalves were closed and the PC-12 cells were cultured in a 5% CO_2 incubator at 37 °C for 36 h.

Experimental Procedure for Detection

The standard dopamine solutions (dopamine dissolved in DMEM) were assessed using CV for dopamine concentrations of 4, 6, and 8 μM. The dopamine secreted from active PC-12 cells in the neuronal cell chip was measured

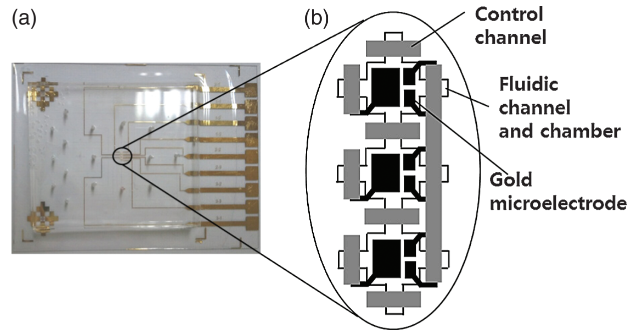

Figure 2. Image of the fabricated microfluidic device containing 3 reaction chambers. (a) Whole microfluidic device and (b) main structure of the microfluidic device composed of control channels, fluidic channels, reaction chambers, and gold microelectrodes.

using CV at 12-h intervals for 36 h after culture was begun. When the cultivation of PC-12 cells in reaction chambers was completed, the consumed medium in each reaction chamber was changed with sample solutions containing 0.1, 1, 10, and 100 μg/L of PCBs by injecting a mixture of sample and fresh medium (1:1 volume ratio) through the *x*-directional channel while the *y*-directional channel is closed. After the solutions of all reaction chambers were completely changed, PC-12 cells were further cultivated in a 5% CO_2 incubator. The activity of PC-12 cells was monitored at 12-h intervals for 36 h. Lastly, a spiked sample of Anseong River water was tested using the fabricated microfluidic device to validate its feasibility in field applications.

RESULTS AND DISCUSSION

Measurement of the Standard Dopamine Solution

This study was aimed to measure the toxicity of PCBs on neuronal cells by detecting dopamine secreted from PC-12 cells. Dopamine is a redox chemical; hence, it can be measured using CV. Prior to PC-12 cell cultivation experiments, standard dopamine solutions of 4, 6, and 8 μM were examined to confirm the performance of the fabricated microfluidic device in dopamine detection. Figure 3 shows the electric current changes of samples according to various dopamine concentrations. As the dopamine concentration of the solution increased, the electric current changes in high and low peaks increased in proportion, confirming the dopamine-detecting capability of the microfluidic device.

Measurement of PC-12 Cell Activity in Culture Experiments

PC-12 cells were cultivated in the fluidic reaction chambers of the microfluidic device for 36 h, and the secreted dopamine was measured every 12 h. The CVs of the experiment are shown in Figure 4(a) in the potential range from 0.6 to −0.3 (versus Ag/AgCl). Figure 4(b) shows the

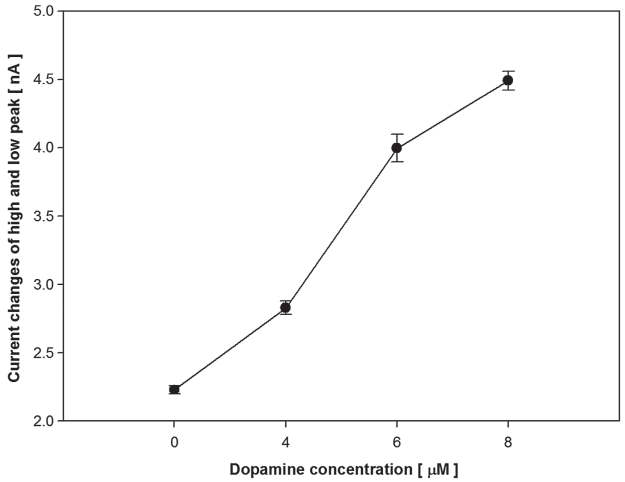

Figure 3. Electric current changes in high and low peaks according to the concentration of the standard dopamine solution.

electric current changes in high and low peaks in the CVs in Figure 4(a). As time passed, the current change between the peaks increased, and the electric current change from 12 to 24 h increased rapidly; however, the electric current change from 24 to 36 h was relatively low due to lower growth rate. From this observation, it is apparent that PC-12 cells were activated, that dopamine secretion increased over time, and that an analysis time of 36 h is sufficient to obtain meaningful experimental data.

Effect of PCBs on the Activity of PC-12 Cells

The effect of PCBs on PC-12 cells was measured by introducing sample solutions of PCBs into the microfluidic device after stabilized cultivation of PC-12 cells for 36 h. Control solutions without PCBs and samples containing PCBs were injected into different reaction chambers of the microfluidic device for comparison. When the samples were injected into the reaction chambers, the fluidic channels between chambers had to be closed to prevent cross flow between chambers. The control solution was prepared by mixing fresh medium and distilled water in the same volumetric ratio, and sample solutions were prepared by mixing fresh medium and PCB solutions instead of distilled water.

The electric current changes were monitored every 12 h for 36 h with sample solutions of 0, 0.1, 1, 10, and 100 μg/L concentrations of PCBs to demonstrate the effect of PCBs on PC-12 cell activity. The data from this experiment are shown in Figure 5. The electric current changes in control solution increased steadily, since the PC-12 cells were growing continuously. In the 0.1 and 1 μg/L PCB samples, the electric current changes were similar to that of the control solution, suggesting that PC-12 cells were not affected by low concentrations of PCBs. In contrast, the electric current changes in 10 and 100 μg/L PCB samples decreased after 12 h, demonstrating that the activity

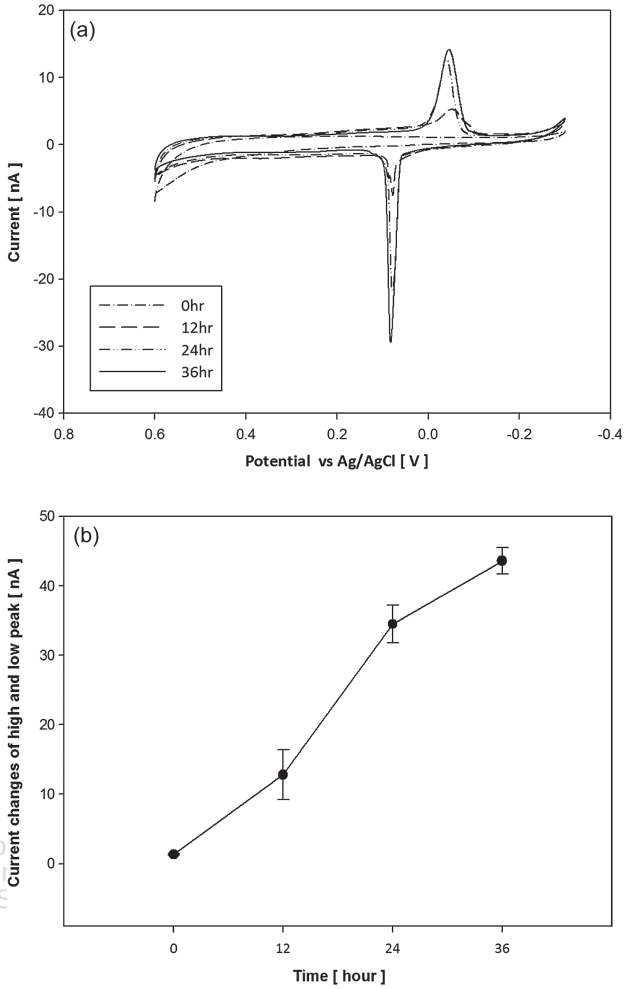

Figure 4. Electrochemical signals of PC-12 cells during 36-h cultivation. (a) cyclic voltammograms and (b) electric current changes in high and low peaks.

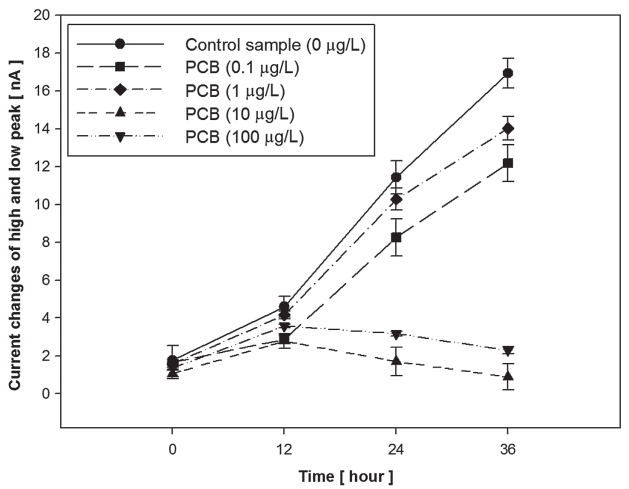

Figure 5. Electric current changes in high and low peaks using samples at various PCB concentrations.

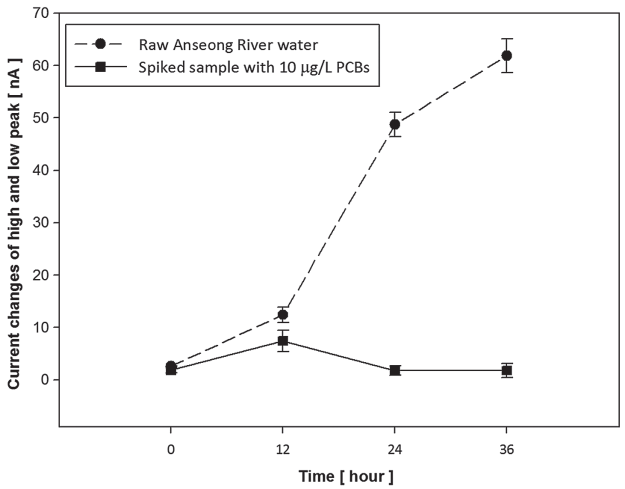

Figure 6. Result of feasibility experiment performed using the spiked sample.

of PC-12 cells was negatively influenced by relatively high concentrations of PCBs due to the toxic effect of PCBs on PC-12 cells.

It is important to detect low environmental concentrations of PCB residues, since the Korean Ministry of Environment stipulates an effluent quality standard of 3 μg/L. The neuronal cell chip is based on a microfluidic device that can successfully detect the toxic effect of 10 μg/L PCB samples. However, it is difficult to measure the exact concentration of PCBs. In comparison with the experimental results for 10 and 100 μg/L PCB samples, the electric current changes between 10 and 100 μg/L PCB samples were meaningless. Therefore the proposed neuronal cell chip system has potential as a toxicity assessment tool in conjunction with a threshold detection level, but not as a quantitative analysis tool.

Toxicity Assessment of the Spiked Sample

To validate the feasibility of field application, the fabricated neuronal cell chip system was used to measure the toxicity of PCBs by using a spiked sample (10 μg/L PCBs) of raw Anseong River water to culture PC-12 cells. The electric signals from the PC-12 cells were monitored (Fig. 6). The electric signal of the microfluidic device increased continuously over time. However, the signal from the samples spiked with 10 μg/L PCBs did not increase significantly. Thus, the feasibility of the proposed toxicity assessment system using a neuronal cell chip on a microfluidic device was validated.

CONCLUSION

The microfluidic device on a gold microelectrode patterned glass wafer was developed to electrochemically monitor the toxic effect of PCBs on PC-12 neuronal cells. The electrochemical signals from the microfluidic device were measured by CV. To confirm its ability to detect dopamine, the microfluidic device was tested with standard dopamine solutions of various concentrations. The microfluidic device monitored the activity of PC-12 cells by detecting the dopamine secreted by PC-12 cells. The fabricated microfluidic neuronal cell chip was then used to detect toxic effect of PCBs, in concentrations ranging from 0–100 μg/L, on PC-12 neuronal cells. The microfluidic neuronal cell chip was capable of detecting the toxic effect of PCBs on PC-12 cells at concentrations of $\geq$10 μg/L. The fabricated chip system was successfully applied to detect the toxicity of PCBs in the spiked sample of river water containing 10 μg/L PCBs, thereby validating its feasibility in field application. Hence, these results show that the microfluidic neuronal cell chip is an excellent tool to detect the biological toxic effect of POPs with high sensitivity.

Acknowledgment: This research was a part of the project titled "Development of Sustainable Remediation Technology for Marine Contaminated Sediments" funded by the Ministry of Land, Transport and Maritime Affairs, Korea.

REFERENCES

1. R. Lohmann, K. Breivik, J. Dachs, and D. Muir, Global fate of POPs: Current and future research directions. *Environ. Pollut.* 150, 150 **(2007)**.
2. G. H. Eduljee, Persistent Organic Pollutants: Environmental Behaviour and Pathways of Humman Exposure, edited by S. Harrad, Kluwer Academic Publishers, Norwell **(2001)**, pp. 1–28.
3. A. Binelli and A. Provini, POPs in edible clams from different Italian and European markets and possible human health risk. *Mar. Pollut. Bull.* 46, 879 **(2003)**.
4. K. E. Zimmer, M. Montaño, I. Olsaker, E. Dahl, V. Berg, C. Karlsson, A. J. Murk, J. U. Skaare, E. Ropstad, and S. Verhaegen, *In vitro* steroidogenic effects of mixtures of persistent organic pollutants (POPs) extracted from burbot (Lota lota) caught in two Norwegian lakes. *Sci. Total Environ.* 409, 2040 **(2011)**.
5. 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 **(2003)**.
6. E. C. Bonefeld-Jørgensen, H. R. Andersen, T. H. Rasmussen, and A. M. Vinggaard, Effect of highly bioaccumulated polychlorinated biphenyl congeners on estrogen and androgen receptor activity. *Toxicology* 158, 141 **(2001)**.
7. M. V. Gallo, L. M. Schell, A. P. DeCaprio, and A. Jacobs, Levels of persistent organic pollutant and their predictors among young adults. *Chemosphere* 83, 1374 **(2011)**.
8. M. Noël, L. Barrett-Lennard, C. Guinet, N. Dangerfield, and P. S. Ross, Persistent organic pollutants (POPs) in killer whales (Orcinus orca) from the Crozet Archipelago, southern Indian Ocean. *Mar. Environ. Res.* 68, 196 **(2009)**.
9. G. Ross, The public health implications of polychlorinated biphenyls (PCBs) in the environment. *Ecotoxicol. Environ. Saf.* 59, 275 **(2004)**.
10. C.-Y. Yang, T.-S. Huang, K.-C. Lin, P. Kuo, P.-C. Tsai, and Y. L. Guo, Menstrual effects among women exposed to polychlorinated biphenyls and dibenzofurans. *Environ. Res.* 111, 288 **(2011)**.
11. C. Pereira and C. V. Rao, Toxicity study of maternal transfer of polychlorinated biphenyls and diethyl phthalate to 21-day-old male and female weanling pups of Wistar rats. *Ecotoxicol. Environ. Saf.* 68, 118 **(2007)**.

12. L. G. Costa, V. Fattori, G. Giordano, and A. Vitalone, An *in vitro* approach to assess the toxicity of certain food contaminants: Methylmercury and polychlorinated biphenyls. *Toxicology* 237, 65 (**2007**).

13. T. Gichner, P. Lovecká, L. Kochánková, M. Macková, and K. Demnerová, Monitoring toxicity, DNA damage, and somatic mutations in tobacco plants growing in soil heavily polluted with polychlorinated biphenyls, *Mutat. Res., Genet. Toxicol. Environ. Mutagen.* 629, 1 (**2007**).

14. Z. L. Zhang, C. Crozatier, M. Le Berre, and Y. Chen, In situ biofunctionalization and cell adhesion in microfluidic devices. *Microelectron. Eng.* 78, 556 (**2005**).

15. 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. *Sens. Actuators, B* 108, 688 (**2005**).

16. 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. *Sens. Actuators B* 145, 553 (**2010**).

17. D. A. Boehm, P. A. Gottlieb, and S. Z. Hua, On-chip microfluidic biosensor for bacterial detection and identification, *Sens. Actuators B* 126, 508 (**2007**).

18. Y. Yamaguchi, T. Arakawa, N. Takeda, Y. Edagawa, and S. Shoji, Development of a poly-dimethylsiloxane microfluidic device for single cell isolation and incubation. *Sens. Actuators, B* 136, 555 (**2009**).

19. W.-G. Koh and M. Pishko, Immobilization of multi-enzyme microreactors inside microfluidic devices. *Sens. Actuators, B* 106, 335 (**2005**).

20. W.-H. Huang, F. Ai, Z.-L. Wang, and J.-K. Cheng, Recent advances in single-cell analysis using capillary electrophoresis and microfluidic devices. *J. Chromatogr. B* 866, 104 (**2008**).

21. L. Zhu, L.-Y. Hou, and W.-Y. Zhang, A new fabrication method for glass microfluidic devices used in micro chemical system. *Sens. Actuators, B* 148, 135 (**2010**).

22. F. Scholz and M. Hermes, The determination of the redox state of a dissolved depolariser by cyclic voltammetry in the case of electrochemically reversible systems. *Electrochem. Commun.* 1, 345 (**1999**).

23. E. Madej and P. Wardman, Pulse radiolysis and cyclic voltammetry studies of redox properties of phenothiazine radicals. *Radiat. Phys. Chem.* 75, 990 (**2006**).

24. P. A. Kilmartin and C. F. Hsu, Characterisation of polyphenols in green, oolong, and black teas, and in coffee, using cyclic voltammetry. *Food Chem.* 82, 501 (**2003**).

25. M. A. Kafi, T.-H. Kim, J. H. An, and J.-W. Choi, Electrochemical cell-based chip for the detection of toxic effects of bisphenol-A on neuroblastoma cells. *Biosens. Bioelectron.* 26, 3371 (**2011**).

26. L. A. Sombers and A. G. Ewing, Electroanyalytical Methods for Biological Materials, edited by A. Brajter-Toth and J. Q. Chambers, Marcel Dekker, Inc., New York (**2002**), pp. 279–327.

27. H.-F. Cui, J.-S. Ye, Y. Chen, S.-C. Chong, and F.-S. Sheu, Microelectrode array biochip: Tool for *in vitro* drug screening based on the detection of a drug effect on dopamine release from PC12 cells. *Anal. Chem.* 78, 6347 (**2006**).

28. H.-F. Cui, J.-S. Ye, Y. Chen, S.-C. Chong, X. Liu, T.-M. Lim, and F.-S. Sheu, *In situ* temporal detection of dopamine exocytosis from l-dopa-incubated MN9D cells using microelectrode array-integrated biochip. *Sens. Actuators, B* 115, 634 (**2006**).

29. J. D. Joseph and R. M. Wightman, Electroanalytical Methods for Biological Materials, edited by A. Brajter-Toth and J. Q. Chambers, Marcel Dekker, Inc., New York (**2002**), pp. 255–278.

30. 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 (**2009**).

31. J. S. Go and S. Shoji, A disposable, dead volume-free and leak-free in-plane PDMS microvalve. *Sens. Actuators, A* 114, 438 (**2004**).