

Application of on-chip cell cultures for the detection of allergic response

Yasutaka Matsubara*, Yuji Murakami¹, Masaaki Kobayashi, Yasutaka Morita, Eiichi Tamiya

School of Materials Science, Japan Advanced Institute of Science and Technology, 1-1 Asahidai, Tatsunokuchi, Ishikawa 923-1292, Japan

Received 28 May 2003; received in revised form 24 July 2003; accepted 13 August 2003

Abstract

In this report, the development of a microfluidic cell chip for monitoring allergic response is described. A rat basophilic leukemia cell line (RBL-2H3), a tumor analog of rat mucosal mast cells, has been used as a model to observe its allergic response upon antigen stimulus. The cells were cultivated on a poly(dimethylsiloxane) (PDMS) chip, the surface of which was modified by several methods. The PDMS chip, which comprised a cell cultivation chamber and microfluidic channels, was fabricated by conventional molding methods. In order to detect the allergic response, a fluorescent dye, quinacrine, was introduced inside the cell compartment that included histamine. The cells were stimulated with dinitrophenylated bovine serum albumin (DNP-BSA) after incubation with anti-DNP IgE. When exocytosis events occurred, the microfluidic system detected the fluorescent signal of quinacrine, which was released from RBL-2H3 cells by using a photomultiplier tube (PMT) fitted onto a microscope.

© 2003 Elsevier B.V. All rights reserved.

Keywords: Cell chip; Microfluidics; PDMS; RBL-2H3; Allergic response; Quinacrine

1. Introduction

Recently, the development of biochips based on MEMS technologies has been the main target of scientific and industrial attention. Various biological materials can be applied to a biochip. Typically, biomaterials, such as DNA, enzymes, proteins and microorganisms (McCormick et al., 1997; Linder et al., 2001; Chou et al., 1999; Karube et al., 1977) are arranged and immobilized on a chip with microscale structures fabricated by photolithography-based techniques. These chips have been used for genetic analyses (Kricka and Wilding, 1996), clinical applications, drug screening (Effenhauser et al., 1997) and environmental monitoring (van der Berg et al., 1993). They are referred to as micro-total analysis systems (μ TAS) (Manz et al., 1990). μ TAS devices have potential advantages such as reduction of reagents and samples consumption, short-time analysis, high sensitivity, portability that enables on-site monitoring, and disposability. These chips must include various elements

for the introduction of samples, separation, pre-treatment, reaction, post-treatment and detection. It is also necessary to handle liquids or gas in microfabricated channels in the chip. Therefore, microfluidics has become an important component for μ TAS devices.

The first generation of microfluidics was fabricated on glass or silicon (Terry et al., 1979; Wooly and Mathies, 1995). The channels were fabricated in a planar substrate by photolithography or micromachining techniques, and covered with a plate of a similar material. The channels fabricated on glass and quartz was sealed by high-temperature treatment. Anodic bonding was also used to seal hybrid quartz–silicon devices. These sealing processes are complicated, time-consuming with low device yield, and require high-temperature conditions, which are not desired for bio-materials. Furthermore, these materials are fragile and too expensive to implement as a disposable system. Accordingly, these limitations hinder the rapid development and testing of new concepts in microfluidic systems.

Polymers have become attractive materials in comparison to glass and silicon for the fabrication of microfluidic systems (Becker and Locascio, 2002). In contrast to glass and silicon, polymers are inexpensive and less fragile. The processes to fabricate channels in polymer devices are based on replication techniques such as casting, molding,

* Corresponding author. Tel.: +81-761-51-1663;
fax: +81-761-51-1665.

E-mail address: yasutaka@jaist.ac.jp (Y. Matsubara).

¹ Present address: Toray Industries, Inc., 2-1, Sonoyama 3, Otsu, Shiga 520-0842, Japan.

and embossing. The devices can be sealed thermally or by using adhesives.

There are many reports about microfluidic systems that utilize poly(dimethylsiloxane) (PDMS) (McDonald et al., 2000; Beebe et al., 2000; Jo et al., 2000). Numerous reasons for why PDMS is the preferred material for the lab-scale production of microfluidic systems, can be listed. For example, PDMS is optically transparent down to 280 nm. It can be produced with high fidelity by casting polymer against a mold, which has sub-micron-scale structures. It can seal reversibly to itself, or irreversibly after exposure to oxygen plasma. Besides, PDMS is nontoxic for use with biological materials in aqueous solution.

Recently, Duffy et al. have developed a system that allows a rapid and low cost fabrication of mold masters having microstructures that are larger than 20 μm . They refer to this technique as rapid prototyping (Duffy et al., 1998). In this technique, a mold master is fabricated by photolithography using SU-8 photoresist, a photocurable epoxy. Photomask patterns are drawn using computer programs such as Illustrator© (Adobe), and printed onto transparency films. These materials and techniques are suitable for fabricating microfluidic systems rapidly and inexpensively.

Biosensors are analytical devices, which include immobilized biomaterials such as enzymes, antibodies, proteins, microorganisms and mammalian cells. In these systems, a control of the interaction of biomaterials with the surfaces of manufactured materials is important. Our final goal is to develop the next generation of highly integrated chips using mammalian cells as the testing elements. As the first step for this purpose, on-chip cultivation of mammalian cells and expression of their functions are important.

In this paper, we report the fabrication of a microfluidic cell chip using RBL-2H3 mast cells. The cells are cultivated on the PDMS chip, which is fabricated by the rapid prototyping technique. Mast cells show an allergic response to an antigen stimulus. Upon allergen stimulus, the cells release histamine as well as a fluorescent dye artificially introduced into histamine granules. The microfluidic system can detect the onset of fluorescence using a photomultiplier tube (PMT) fitted onto a microscope.

2. Experimental

2.1. Fabrication of PDMS chip

Mask patterns for channel systems were designed with Illustrator 9.01 (Adobe) and printed onto a transparent film via image setter (Dolev 800 V, 2540 dpi equivalent, Scitex) by an external service (Yamada Photo Process Inc.). A negative photoresist (NANO XP SU-8 50, MicroChem. Corp.) was spun (500 rpm; 5 s, slope; 5 s, 2000 rpm; 20 s, slope; 5 s and end) on the optically polished side of a silicon wafer and pre-baked to drive off the solvent ($T = 90^\circ\text{C}$, $t = 1\text{ h}$). The transparent film was used as a photomask and the pho-

toresist was exposed (60 s) to make features $\sim 70\text{ }\mu\text{m}$ high using a mask aligner (MJB 3, Karl Suss). After post-baking ($T = 90^\circ\text{C}$, $t = 1\text{ h}$), the photoresist was developed in SU-8 developer (MicroChem. Corp.) to reveal a mold composed of a pattern of negative photoresist. To make PDMS chips, a curing agent and PDMS pre-polymer (SILPOT 184 silicon elastomer kit, Dow Corning Asia) were thoroughly mixed in a 1:10 weight ratio. The pre-polymer mixture was degassed at a pressure of 100 mTorr in a desiccator with a mechanical vacuum pump for 1 h to remove any air bubbles in the mixture and to ensure complete mixing between the two parts. The pre-polymer mixture was poured onto the master mold, and cured for 1 h at 80°C in an oven. After curing, the PDMS replicas were peeled off from the master mold. We refer to it as PDMS chip.

The cover lid was also made in PDMS. Three connectors were placed on a flat glass plate to be aligned to the injection point of the PDMS chip. The pre-polymer mixture was poured onto it, and cured for 1 h at 80°C in an oven. After curing, the lid was peeled off from the glass plate. The PDMS chip was covered with its lid and connected with silicon tube in order to introduce reagent and samples in the microfluidic channels.

2.2. Surface treatment of PDMS

Unmodified PDMS presents a hydrophobic surface and is uncharged. Therefore, we treated the PDMS surface to render it hydrophilic. The first method of surface treatment is exposure to oxygen plasma using a Reactive Ion Etching system (SAMCO, RIE-10NR). The PDMS chip was rinsed in ethanol, dried under a stream of nitrogen, then placed in a Reactive Ion Etching system and treated for 10 s at 6.7 Pa, 200 W in oxygen plasma. This treatment makes the surface of the PDMS hydrophilic in the presence of silanol groups. The second method is soaking the PDMS in 1 M HCl. This treatment makes the surface hydrophilic because of the hydrolysis of Si–O–Si bonds. The third method is to immobilize γ -aminopropyltriethoxysilane (γ -APTES) on the oxidized surface by silane coupling reaction. A plasma-oxidized PDMS chip was dipped for 45 min into acetone including 1% (v/v) γ -APTES. Then, the PDMS was rinsed twice in acetone and dried under a stream of nitrogen. Hydrophilicity of modified surfaces was measured with a contact angle meter (Kyowa Interface Science Co., Ltd., CA-A) by the droplet method.

2.3. Detection of the allergic response

Rat basophilic leukemia (RBL-2H3 line) cells, mast cells, were purchased from Japan Health Sciences Foundation, and also were kindly donated by Dr. Ozawa and Dr. T. Masujima (Hiroshima University). They were maintained in culture using RPMI1640 medium (Gibco) with 15% fetal bovine serum (COSMO BIO) along with 100 $\mu\text{g}/\text{ml}$ penicillin and 100 $\mu\text{g}/\text{ml}$ streptomycin (Gibco). This cell line has been

widely used as a model to study the mechanisms underlying the exocytosis process.

Histamine, a small molecule secreted by mast cells, is difficult to detect both optically and electrochemically. Thus, we use the fluorescent dye quinacrine, an aminoacridine. It is a weak base that has been used to identify acidic compartments of various cells. This dye is effectively introduced into low pH compartments of the cell granules by mass action (Kolber and Henkart, 1988). When allergen stimuli evoke exocytosis, the cells release the dye along with histamine. The typical method for the measurement of quinacrine release from RBL-2H3 cells was carried out using a spectrofluorometer. Cultured RBL-2H3 cells were sensitized with mouse monoclonal clone IgE (0.25 $\mu\text{g}/\text{ml}$) against dinitrophenylated bovine serum albumin (DNP-BSA) for 24 h (Fig. 1A). After sensitization, the cells were rinsed with 1,4-piperazine bis(2-ethanesulfonic acid) (PIPES) buffer (25 mM PIPES, 119 mM NaCl, 5 mM KCl, 0.4 mM MgCl_2 , 5.6 mM glucose, 1 mM CaCl_2 and 0.1% BSA) twice, and PIPES buffer was added, which included 5 μM quinacrine (Fig. 1B). After 15 min of incubation, the cells were rinsed with PIPES buffer three times, and incubated for 10 min (Fig. 1C). The extracellular solution was sampled before adding DNP-BSA as an antigen. After 10 min of incubation with 20 ng/ml DNP-BSA, we sampled the extracellular solution again. The fluorescent intensity before and after the exocytosis (Fig. 1D) was measured with spectrofluorometer (excitation wavelength 420 nm, emission wavelength 507 nm).

2.4. Cell incubation on the chip

The cells were harvested at the point of confluence from a flask by incubating for 3 min at 37 °C with a trypsin–EDTA (Gibco) mixture. They were subsequently washed once in PIPES buffer, and 1 ml of medium was added. One hundred forty microliters of cell suspension (2×10^6 cells/ml) were introduced into the chamber of the chip, which was made hydrophilic by one of the methods previously described and sterilized with 70% (w/w) ethanol. After 1 h of incubation, the chip surface was gently rinsed with medium in order to get rid of non-attached cells from the chip surface and then, the chip was dipped into the medium. The cells were sensitized with 0.25 $\mu\text{g}/\text{ml}$ of IgE antibody (Sigma, mouse monoclonal) and incubated at 37 °C and 5% CO_2 for a day.

2.5. Detection with microfluidic system

The fluorescent dye quinacrine (5 μM) was passed through the flow system of the chip from the point a to b (Fig. 1). After 5 min, it was washed with PIPES buffer in order to remove the extra dye from the reaction mixture. In this time, the flow stream was controlled from a to b. DNP-BSA (20 ng/ml) solution was injected from a to c. The dye was released from the mast cells along with histamine and could be detected in the down-stream flow cell of the chip (d) by a fluorescence microscope (Nikon, TMD300, fluorescence filter; BV-2A, DM: 455 nm, BA: 470 nm, EX: 400–440 nm) with a PMT and a photon counting system

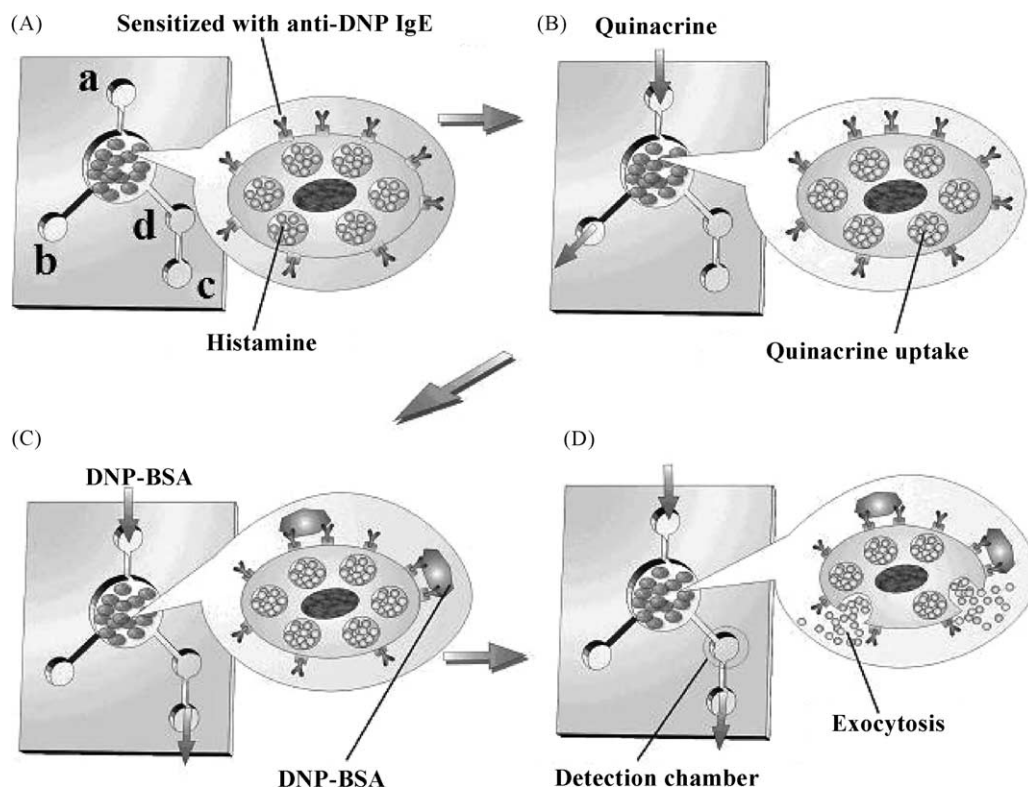


Fig. 1. Schematic illustration of microfluidic cell chip.

(Hamamatsu Photonics Inc.). The principle of the detection protocol is shown in Fig. 1. This flow system was controlled by a peristaltic pump. The flow rate of whole experiments was controlled at 20 $\mu\text{l}/\text{min}$.

3. Results and discussion

3.1. Fabrication of microfluidic system

To fabricate a microfluidic system in PDMS, we used a procedure for rapid prototyping that was described in detail elsewhere (Duffy et al., 1998). PDMS was cast against the mold master, the design of which is shown in Fig. 2, to yield elastomeric replicas (Fig. 2A). The channels were about 70 μm deep, 50 μm wide, and the diameter of the cell chamber was 10 mm. The chip design was based on those commonly used in other microfluidic devices (McDonald et al., 2000; Beebe et al., 2000; Jo et al., 2000). The cell chamber dimension was decided by the cell number, which could be arranged on the chip. A cover lid for the chip was also fabricated in PDMS (Fig. 2B).

To form enclosed channels, we aligned and sealed the PDMS chip containing microchannels reversibly to the cover lid in PDMS. Silicone tubes were connected to PDMS chip in order to introduce samples and reagents (Fig. 2C). This system was sealed reversibly. Thus, liquid flow inside the channels had to be achieved by suction using the peristaltic pump.

3.2. Cell culture on a chemically modified PDMS surface

The mast cells were incubated on PDMS chips, that were previously treated by one of the three methods

described above. PDMS comprises repeating units of $-\text{Si}(\text{CH}_3)_2\text{O}-$. In the first method, the oxidation of PDMS with oxygen plasma converted some functional groups to $-\text{Si}(\text{OH})_{4-n}\text{O}_n-$. The presence of silanol groups on the PDMS surface provided hydrophilicity. In the second method, HCl treatment also rendered PDMS surface hydrophilic. The third method, immobilization of γ -APTES, was accomplished by a silane coupling reaction. When γ -APTES was immobilized onto the PDMS surface, its propyl amine group was exposed to the surface, and was positively charged in a buffer solution at pH 7.4. Each method rendered the surfaces hydrophilic. The contact angle of a non-modified PDMS surface was about 110° , while for a surface modified by plasma, HCl or APTES treatments, it was about 5° , 45° , and 75° , respectively.

Fig. 3 shows cells incubated on a PDMS surface for 1 day. The left side of a chip was not treated (–), and the right side was treated with one of the methods previously described (+). The cells grew well on the right sides of chip because of the surface treatment. The cell population was different for each method. These surface treatments brought polarity on the PDMS surface, so the serum in the medium was more attracted to the charged surface than the non-treated one. From these results, it could be assumed that surface modification promoted the adsorption of cells. The surface, which was treated by γ -APTES, obviously provided the most suitable conditions for attaching the mast cells in comparison with the other treatment techniques.

In normal petri dish experiments, the shapes of cultivated cells indicate their vitality, and an active shape is the cylindrical one. The shapes of the cells sampled from the treated surface and the non-treated one, were also found to be different from each other. The cells that were incubated on a treated surface had a cylindrical shape. In contrast, the cells

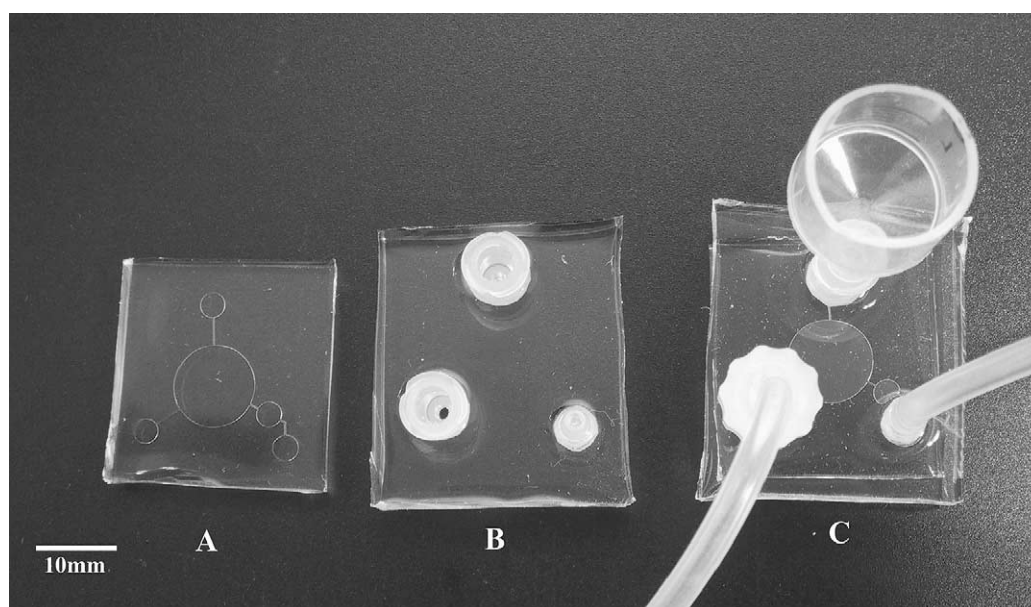


Fig. 2. PDMS cell chip. (A) PDMS chip, (B) cover lid, (C) microfluidic system.

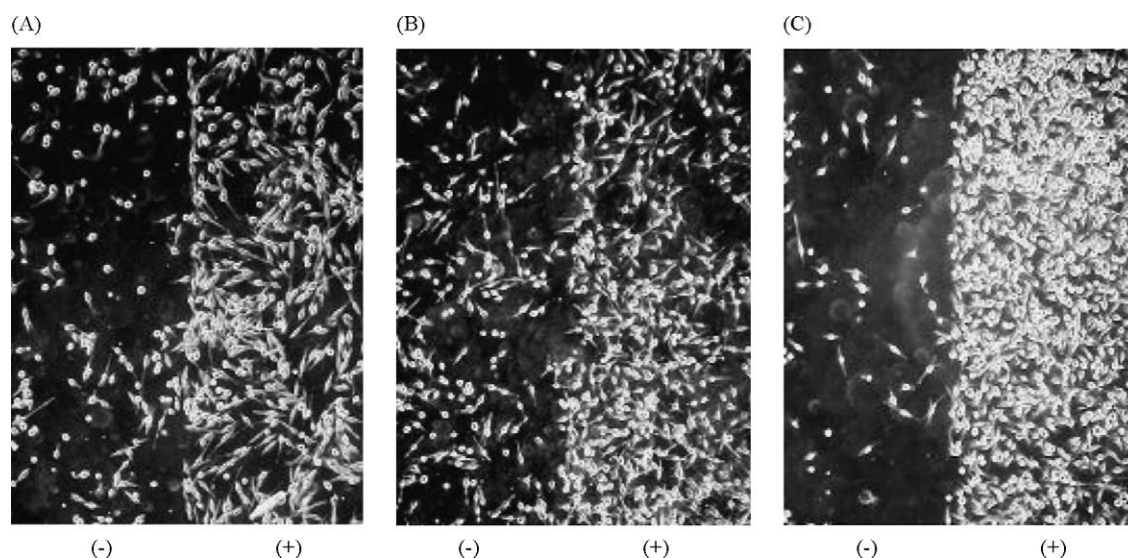


Fig. 3. Mast cells incubated on the PDMS. The left side of the PDMS shows the non-treated surface (–), and the right side shows the treated surface (+). The applied modification procedures were (A) oxygen plasma treatment; (B) HCl treatment; (C) γ -APTES treatment.

on a non-treated surface had a spherical shape. Although γ -APTES treatment attached the biggest amount of cells on the chip, the majority of these cells were found to be spherical in shape, indicating that they were inactive. On the other hand, the shape of the cells on the chip treated by oxygen plasma cylindrical which indicated that they were in active condition. Therefore, the oxygen plasma treatment was employed as the final modification step for the mast cells in further experiments.

3.3. Fluorescence detection of allergic response of cells using microfluidics

The functionality of the developed chip was confirmed with fluorescence microscopy. Our goal was to detect the fluorescent signal from a solution with a low quinacrine concentration using a microscope and a PMT. The quinacrine solution was introduced in the microfluidic system from a to c (Fig. 1B). Fig. 4 shows the variation of photon counts on the microfluidic chip. After introduction of quinacrine

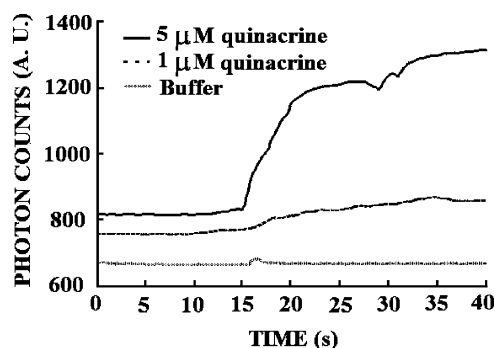


Fig. 4. Variation of fluorescence intensity on the microfluidic chip.

ranging from 1 to 5 μ M, the signal from PMT increased, as the solution reached the detection chamber (Fig. 1D). Our system could detect at least 1 μ M quinacrine dye.

In order to determine the minimum number of cells necessary to detect an allergic response using quinacrine, the quantity of dye released upon allergic stimulus from RBL-2H3 cells cultured on petri dish was measured. By comparison to a calibration curve of quinacrine fluorescence (data not shown), it was estimated that the cells in the petri dish (1.6×10^6 cells) released quinacrine to a final concentration of 0.23 μ M in the 10 ml of buffer solution upon stimulation, thus each cell released 7.19×10^{-10} mol quinacrine. Fig. 5 displays the changes in the PMT signals obtained from the petri dish before and after exposure to DNP-BSA. The number of cells arranged on the chip was about 0.8×10^5 , and the volume of cell chamber was

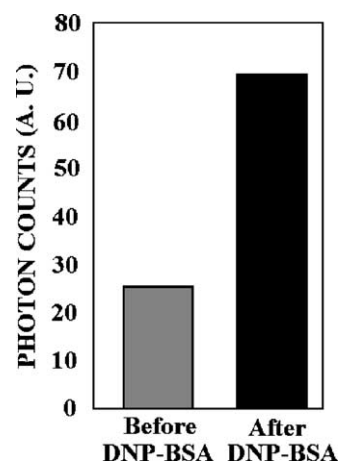


Fig. 5. Detection of the allergic response of mast cells cultured in the petri dish by monitoring the PMT signals.

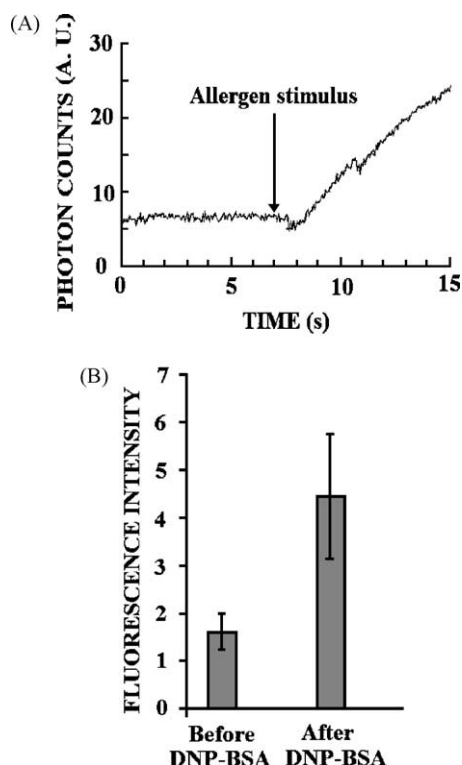


Fig. 6. Detection of the allergic response of mast cells by monitoring (A) the PMT signals and (B) fluorescence intensity.

about 5.5 μ l. The condition of microfluidic system gives cells physical stress. Supposing that the 5% of cells, which could be arranged on the chip, released the quinacrine; the total concentration of the dye solution in the cell chamber was found to be about 5.75 μ M. These results indicated that this system could detect low amounts of quinacrine, thus detecting the allergic response of the mast cells.

Fig. 6A shows the PMT signal, and Fig. 6B shows the fluorescence intensity signals from the microfluidic system, in which cells were arranged. Repetitive measurements were performed ($n = 3$) to sustain the validation of the system. Standard deviation of the fluorescence signals before and after the introduction of DNP-BSA was found as 0.3716 and 1.3085 with the mean values of 1.6081 and 4.4501, respectively. After introducing the quinacrine solution, the cell chamber was rinsed and filled with PIPES buffer. No PMT signals were collected (Fig. 6A), and low fluorescent signals were observed (Fig. 6B) before addition of the stimuli. When DNP-BSA solution was introduced into the chip, the initial step of exocytosis event took place in less than 33 ms. As the solution reached the cell chamber, the exocytosis event started and continued for at least 3 min (Ozawa et al., 1996). Consequently, quinacrine release was monitored both as a PMT signal, and as the variation of the fluorescence intensity. However, the signals from the cell chip was found to be lower than the ones obtained from the petri dish as shown in Fig. 4. There might be two reasons be-

hind such a difference between two protocols. Quinacrine, which was released from mast cells, was flown from the cell chamber to the detection one in the chip. The presence of the nonspecifically attached mast cells in the detection chamber must have caused the diffusion of the dye solution. Quinacrine also underwent further dilution with the addition of the PIPES buffer solution containing the antigen. Another reason, why the signals from the cell chip were low, was attributed to the condition of the cells under physical stress. In our supposition, 5% of the cells arranged on the chip may be eligible to release quinacrine by the exocytosis event. Nevertheless, physical stress conditions; such as environmental temperature, surface condition, and liquid flow, affected the cells and their activity. Therefore, the PMT and fluorescence signals were detected with a much lower amplitude at the detection chamber in comparison with the ones obtained from the petri dish. Even at such low signal amplitudes as shown in Fig. 6, the exocytosis event of the mast cells could successfully be detected by using our microfluidic system.

4. Conclusions

We have demonstrated the detection of the allergic response of mast cells using a microfluidic PDMS chip. The mast cells were cultivated on the chemically modified PDMS surface. The point that should be considered while adapting the mammalian cells to such an on-chip cell cultivation, is how to keep the cells in an active condition. The growth behavior of various cells is greatly affected by the type of the surface structure in vitro. This result concludes that the modification of the interface between the chip surface and the cells is crucial. Especially for microfluidic devices, patterning the reaction of cells in specific areas of a device is necessary for stimulation of the cells and detection of the response. The novel methods of self-assembled monolayer (SAM) formation on gold or micro-contact printing (μ CP) are powerful tools to pattern the biomaterials, proteins and cells on a biochip. However, these methods still have some complicated processes and not useful for modification of the specific areas on the chamber surface of PDMS-based chips. In this research, we have determined the influence of the surface modification by using the area-specific oxygen plasma treatment. The repetitive measurements for the fluorescent response of the chip showed good selectivity against the exocytosis event. Our method provides the basis for rapid mass-fabrication of numerous cell chips, which can be adopted for cell cultivation. These experimental results clearly show that the PDMS surface, which was modified hydrophilic by oxygen plasma treatment, was suitable for growing the mast cells in an active condition. These efforts to keep the cells active provided the detection of the allergic response of mast cells. This research presents a new analytical tool for both basic and applied microfluidic cell-based biosensor fabrication.

Acknowledgements

Y.M. expresses his gratitude to Dr. Tsutomu Masujima and Dr. Koichiro Ozawa of Hiroshima University for the donation of RBL-2H3 cell lines. This research was partially supported by the future program of Japan Society for the Promotion of Science (JSPS).

References

- Becker, H., Locascio, L.E., 2002. Polymer microfluidic devices. *Talanta* 56, 267–287.
- Beebe, D.J., Moore, J.S., Yu, Q., Liu, R.H., Kraft, M.L., Jo, B.H., Devadoss, C., 2000. Microfluidic technologies: a comprehensive construction platform for microfluidic systems. *Proc. Natl. Acad. Sci. U.S.A.* 97, 13488–13493.
- Chou, H.P., Spence, C., Schere, A., Quake, S., 1999. A microfabricated device for sizing and sorting DNA molecules. *Proc. Natl. Acad. Sci. U.S.A.* 96, 11–13.
- Duffy, D.C., McDonald, J.C., Schueller, O.J.A., Whitesides, G.M., 1998. Rapid prototyping of microfluidic systems in poly(dimethylsiloxane). *Anal. Chem.* 70, 4974–4984.
- Effenhauser, C.S., Bruin, G.J.M., Paulus, A., 1997. Integrated chip-based capillary electrophoresis. *Electrophoresis* 18, 2203–2213.
- Jo, B.H., Van Lerberghe, L.M., Motsegood, K.M., Beebe, D.J., 2000. Three-dimensional micro-channel fabrication in polydimethylsiloxane (PDMS) elastomer. *J. Microelectromech. S.* 9, 76–81.
- Karube, I., Matsunaga, T., Mitsuda, S., Suzuki, S., 1977. Microbial electrode BOD sensors. *Biotechnol. Bioeng.* 19, 1535–1547.
- Kolber, M.A., Henkart, P.A., 1988. Quantitation of secretion by rat basophilic leukemia cells by measurements of quinacrine uptake. *Biochim. Biophys. Acta* 939, 459–466.
- Kricka, L.J., Wilding, P., 1996. Micromechanics and nanotechnology. In: Kost, G.J., Welsh, J. (Eds.), *Handbook of Clinical Automation, Robotics, and Optimization*. John Wiley & Sons, New York, p. 45.
- Linder, V., Verpoorte, E., Thormann, W., de Rooij, N.F., Sigrist, H., 2001. Surface biopassivation of replicated poly(dimethylsiloxane) microfluidic channels and application to heterogeneous immunoreaction with on-chip fluorescence detection. *Anal. Chem.* 73, 4181–4189.
- Manz, A., Graber, N., Widmer, H.M., 1990. Miniaturized total chemical analysis systems: a novel concept for chemical sensing. *Sens. Actuat. B* 1, 244–248.
- McCormick, R.M., Nelson, R.J., Alonso-Amigo, M.G., Benvegna, D.J., Hooper, H.H., 1997. Microchannel electrophoretic separations of DNA in injection-molded plastic substrates. *Anal. Chem.* 69, 2626–2630.
- McDonald, J.C., Duffy, D.C., Anderson, J.R., Chiu, D.T., Wu, H., Schueller, O.J.A., Whitesides, G.M., 2000. Fabrication of microfluidic systems in poly(dimethylsiloxane). *Electrophoresis* 21, 27–40.
- Ozawa, K., Kobayashi, H., Kawai, E., Suzuki, E., Nonomura, Y., Masujima, T., 1996. Quantitative analysis of exocytosis visualized by a video-enhanced light/fluorescence microscope reveals two distinct components of exocytosis in RBL-2H3 cells. *FEBS Lett.* 398, 67–73.
- Terry, S.C., Jerman, J.H., Angell, J.B., 1979. A gas chromatographic air analyzer fabricated on a silicon wafer. *IEEE Trans. Electron. Devices* ED-26, 1880–1886.
- van der Berg, A., Grisel, A., Verney-Norberg, E., van der Schoot, B.H., Hep, M., de Rooij, N.F., 1993. On-wafer fabricated free-chlorine sensor with ppb detection limit for drinking-water monitoring. *Sens. Actuat. B* 13, 396–399.
- Wooley, A.T., Mathies, R.A., 1995. Ultra-high-speed DNA sequencing using capillary electrophoresis chips. *Anal. Chem.* 67, 3676–3680.