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Integrated microfluidic system with electrochemically actuated on-chip pumps and valves

Hiroaki Suzuki*, Rei Yoneyama

Institute of Materials Science, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8573, Japan

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Abstract

A microfluidic system was constructed by integrating on-chip micropumps and check valves that function by means of a hydrogen bubble that is generated or extinguished electrochemically. Essential elements consisted of thin-film three-electrode systems, including a platinum black working electrode. Micro flow channels and containers for electrolyte solutions were formed on a polydimethylsilixane (PDMS) substrate. The growth and shrinkage of the bubble were controlled reproducibly by setting the working electrode potential at a constant value. The elastic nature of the bubble clogged in a valve compartment in the middle of the flow channel hindered the effective passage of the solution. The bubble was also effective in separating two different solutions. By making the valves open and close cooperatively, a solution could be introduced into the system and transported in the flow channel. When two dye solutions were transported and merged in a flow channel, sheath flows were observed, reflecting a low Reynolds number. As a model system, two solutions containing luciferin and luciferase were introduced separately, transported, and mixed. Chemiluminescence originating from the enzymatic reaction was observed.

Keywords: Pump; Valve; Hydrogen; Bubble; PDMS; Sheath flow

1. Introduction

With the progress of the μTAS or Lab-on-a-Chip technology, microfluidic transport is becoming more important. Over the last decade, electroosmosis has been used extensively in relation to the separation of biochemical molecules, such as DNA. This very simple method is very advantageous in moving a fluid freely even in complicated microflow channels connected with each other. However, the high driving voltage, which is usually on the order of hundreds or thousands of volts and the accompanying power consumption present problems. The setup also requires a voltage source which is much larger than the chip itself. This is an unacceptable drawback when using the system on or near a human body. Another possibility to achieve controlled fluid handling is to use pumps and valves. This conventional approach is considered to be a better choice in constructing microhealth-care system whose realization will be desired in the coming decades. However, the current researches are still focusing on the development and improvement of the respective pumps and valves [1]. Their integration into a microsystem and the control of fluid in complicated flow channels do not seem to be totally successful at present. We believe a strategy that leads to successful integration will be to simplify the structure and function of the pumps and valves. Some of the previous successful approaches to achieve a higher level of integration seem to follow the line [2].

In constructing desired components, the application of a bubble is promising [3,4]. We have already developed a micromachined syringe pump that sampled an external solution with a hydrogen bubble produced or extinguished electrochemically [5]. By precisely controlling the potential of the working electrode using an on-chip three-electrode system, a low operating voltage (∼1 V) and power consumption on the order of μW have been realized. Like conventional microsyringe pumps, the simple on-chip syringe pump will generate an effective driving force in microfluidic systems. The bubble used in the pump can also be used to construct a check valve. Although clogging by bubbles often results in malfunctioning of some liquid transport systems, such as the electroosmotic systems, the phenomenon can advantageously be used to realize a simple check valve. Because bubbles are elastic, they can adjust themselves to the structure in the area and hinder the passage of fluid. By using the simple components, a microsystem on which different solutions can be withdrawn and transported in microflow

* Corresponding author. Tel.: +81-29-853-5598; fax: +81-29-855-7440.
E-mail address: hsuzuki@ims.tsukuba.ac.jp (H. Suzuki).

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channels can easily be achieved. In this report, the fabrication and performance of such an on-chip microfluidic system will be reported.

2. Experimental

2.1. Materials and reagents

Glass wafers (#7740, 3 in., 500 μm thick) were purchased from Corning Japan, Tokyo. A polyimide precursor solution, Semicofine SP-341, was purchased from Toray Industries, Tokyo. A precursor solution of polydimethylsiloxane (PDMS), KE-1300T, was purchased from Shin-Etsu Chemical, Tokyo. A thick-film photoresist, SU-8, was purchased from MicroChem, MA, USA. A photosentitive hydrophilic polymer, ENT-2000, was purchased from Kansai Paint, Osaka, Japan. Methyl Orange was purchased from Wako Pure Chemicals Industries, Osaka. Methylene Blue and Orange G were purchased from Merck KgaA, Darmstadt, Germany. Precursor solutions containing luciferin and luciferase, Phota lite, were purchased from Kikkoman, Chiba, Japan. The other reagents used to fabricate and evaluate the device were purchased from Wako Pure Chemicals Industries and Kanto Chemicals, Tokyo. Distilled deionized water was used throughout to prepare buffer solutions. The concentrations of the pH-indicator and the dyes dissolved in the solutions were 10 mM for Methyl Orange and Orange G and 0.28 mM for Methylene Blue.

2.2. Instruments

The thickness of the membranes was measured using a Veeco Dektak 3ST profiler. To grow platinum black and AgCl on the working and reference electrodes and to confirm the potential of the latter, a three-electrode system consisting of a commercial reference electrode (Horiba 2080A-06T) and a platinum plate counter electrode was used. The corresponding pattern on the chip to be processed was used as the working electrode. In controlling potential or current in fabricating or evaluating the device, 1–3 sets of Hokuto-Denko HA-151 potentiostat/galvanostat were used. The current or potential output was recorded with a TOA Electronics PRR-5011 strip chart recorder. The movement of a bubble or color change in the flow channels was observed under a Nikon SMZ 1000 optical microscope.

2.3. Structure and fabrication of the pumping system

The system was constructed with a glass substrate with electrodes (20 mm × 20 mm) and a PDMS sheet (Fig. 1). Two sets of fluidic transport mechanisms consisting of a syringe pump and two check valves were integrated along with flow channels and containers, and placed symmetrically

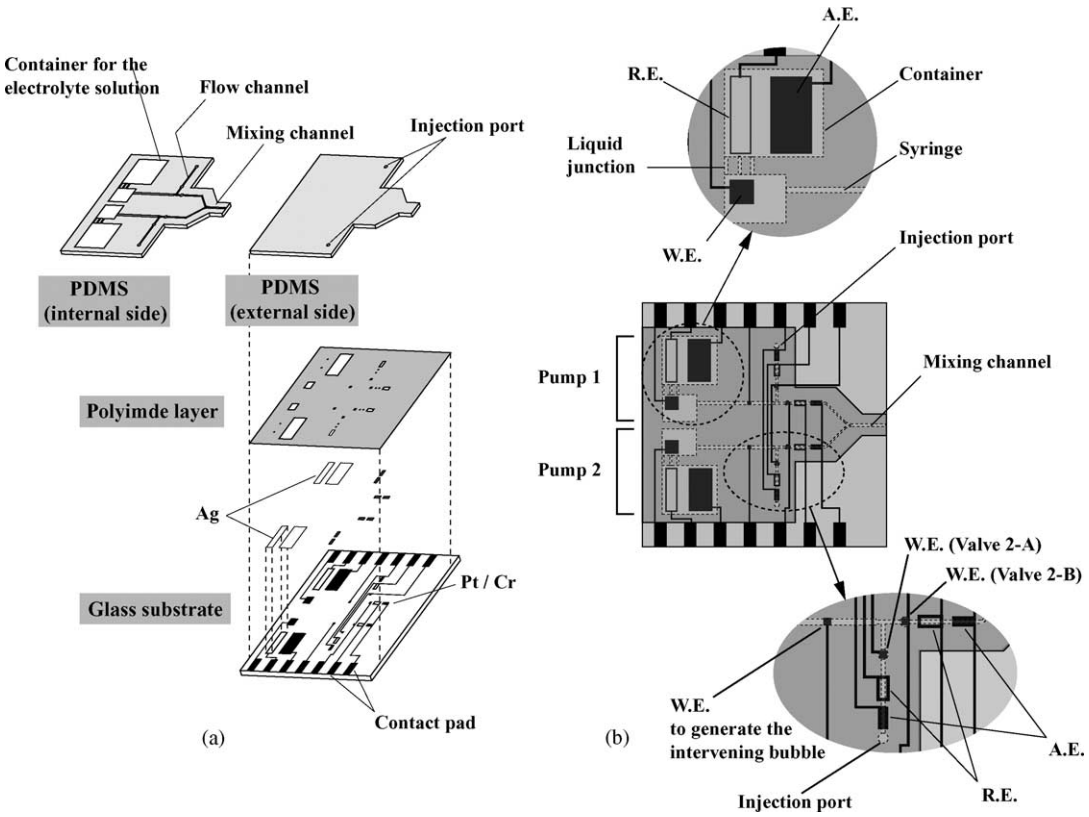

Fig. 1. Structure of the electrochemical liquid transport system with integrated on-chip micropumps and valves: (a) construction of the system; (b) completed system showing the mutual relation between the respective components.

as shown in Fig. 1. Two different solutions could be introduced and transported in the chip. Metals used for the electrodes were sputter-deposited and patterned using photolithography. Backbone patterns were formed with a 300 nm-thick platinum layer with a 40 nm-thick chromium underlayer. Same materials were used for the respective working, reference, and auxiliary electrodes for the pumps and valves. The active areas of the electrodes were delineated with a 1.7 μm-thick polyimide layer. In order to generate and shrink the bubble effectively, the working electrodes were platinized. A constant current (0.3 mA for the pumps and 0.1 mA for the valves) was impressed to the working electrode for the pump or the valve for 120 s in a 0.1 M H_2SO_4 solution containing 81 mM H_2PtCl_6 and 1.0 mM $Pb(CH_3COO)_2$. After the electrode was rinsed with distilled deionized water, the electrode was polarized by applying −0.1 mA for 60 s and +0.1 mA for 60 s alternately several times. For the reference electrodes, Ag/AgCl electrodes were used. Their surface was covered with a polyimide layer [6]. The AgCl layers were grown from two pinholes formed there by applying 100 nA for 10 min for the pumps and 5 min for the valves in a 1.0 M KCl–HCl buffer solution (pH 2.2, 25 °C). Because gas-evolving reactions from the auxiliary electrodes are detrimental for the operation of the system, the Ag/AgCl electrode was also used there [5]. The compartment for the working electrode was separated from that for the reference and auxiliary electrodes to suppress diffusion of hydrogen into the latter compartment. The two compartments were connected with three 150 μm-wide liquid junctions filled with the hydrophilic polymer (ENT-2000). Corresponding electrodes for the two valves were connected with common lines, whereas contacts to the electrodes in the pumps were taken independently.

Microflow channels and containers for the electrolyte solution were formed by casting the precursor solutions of PDMS on template structures formed with SU-8 to obtain a 2 mm-thick sheet. The depth of the structures replicated in the PDMS sheet was 70 μm. The mutual relation among the electrodes, the flow channels, and the containers is described in Fig. 1b. Through-holes were formed at two injection ports for the flow channels. The flow channels extended from the injection ports to a mixing channel by way of the vicinity of the micropump. A circular compartment of 400 μm in diameter was formed for the valves. A Y-shaped portion was formed at the mixing channel, where two different solutions flowed in. Unnecessary areas of the sheet were removed. When the glass substrate and the PDMS sheet were aligned and sandwiched between two PMMA substrates and fixed with bolts and nuts, pressure was exerted effectively only on the periphery of the flow channels and the containers, establishing a tight sealing. Reservoirs for sample solutions were formed in the vicinity of the injection ports in one of the PMMA substrates.

For the micropump, a substantial gas bubble must be produced both rapidly and effectively. Therefore, an acidic electrolyte solution, a 1.0 M KCl–HCl buffer solution (pH 2.2), was used to fill the containers for the pump. On the other hand, a sample solution used to fill the flow channel was used as the electrolyte solution for the valves. Here, a challenging problem was to separate the acidic electrolyte solution and the sample solution, which had different pHs. In order to do that, an intervening bubble was also generated between the two solutions. When filling the containers and the flow channels that way, the KCl–HCl buffer solution was first introduced into the entire flow channels and containers in a vacuum. Then, after the separating bubble was grown, introduction and flushing of the sample solution were repeated several times to exchange the KCl–HCl buffer solution in the flow channel. Critical parameters concerning the pumps, the valves, and the flow channels are summarized in Table 1.

Table 1
Critical parameters of the system

Component	Materials and dimensions
Pump	
Working electrode	Platinum black/Pt/Cr, active area: 1000 μm × 1000 μm
Reference electrode	Ag/AgCl (0.9 mm × 3.5 mm), active area: two pinholes of 50 μm
Auxiliary electrode	Ag/AgCl, active area: 1.9 mm × 3.5 mm
Electrolyte solution	1.0 M KCl–HCl (pH 2.2)
Syringe	70 μm × 450 μm × 6.0 mm (190 nl)
Valve	
Working electrode	Platinum black/Pt/Cr, active area: 200 μm × 200 μm
Reference electrode	Ag/AgCl (0.4 mm × 0.9 mm), active area: two pinholes of 50 μm
Auxiliary electrode	Ag/AgCl, active area: 0.4 mm × 0.9 mm
Electrolyte solution	Sample solutions
Compartment	400 μm diameter (9.0 nl)
Flow channel	
Injection port—pump	70 μm × 150 μm × 4.6 mm
Pump—*Y*-intersection	70 μm × 150 μm × 6.9 mm
Mixing channel	70 μm × 200 μm × 3.0 mm

2.4. Procedures for checking the function of the pumps, valves, and system

The rates of growth and shrinkage of bubbles were determined by measuring the time required for the edge of the bubble to move from one predetermined position to another or for the bubble to fill a circular compartment or be extinguished. Based on the data presented later, the potential of the working electrode for the micropump was set at −1.4 V when growing the bubble and at 0.0 V in shrinking it. Similarly, the potential for the valves was set at −1.2 and 0.0 V in growing and shrinking it, respectively. Because the generated bubble shrank slowly and spontaneously under the open-circuit condition, its size was adjusted intermittently by applying the reductive potential.

In measuring the maximum critical pressure that the pumps and valves can withstand, test devices were

additionally prepared. On a test chip, a single pump was formed and connected to a flow channel whose cross-section was the same as the syringe for the pump. A silicone tube was connected to the end of the flow channel, the other end of which was connected to a commercial syringe filled with the KCl–HCl buffer solution. The height of the edge of the water column in the latter syringe was changed and measured with respect to the position of the flow channel. On another chip, a valve was formed in a single flow channel whose cross-section was the same as the one in in the system. The other procedures were the same as those for the pump. The movement of the bubble in the pump and valve was checked under the microscope. The effectiveness of sealing was also checked from the diffusion of Orange G.

The effectiveness of the intervening bubble in the pump was checked by observing a pH change caused by mixing. After a bubble that intervenes between the solutions was generated, the solution on the side of the flow channel was replaced with water containing Methyl Orange. All these experiments were conducted at room temperature.

3. Results and discussion

3.1. Function of the micropump

In order to withdraw solutions from the injection ports and transport them through the flow channels, the capacity of the pumps was made as large as possible. Fig. 2a shows the dependence of the rates of growth and shrinkage of the hydrogen bubble on the applied potential. The rates were reproducible among chips. When the volume change was $8.0\,\mathrm{nl\,s^{-1}}$, the velocity of the solution in the flow channel was $0.74\,\mathrm{mm\,s^{-1}}$.

Fig. 3 shows the variation of the current during the growth and shrinkage of the bubble in the syringe of the pump. The generated currents were reproducible. The voltage between the working electrode and the auxiliary electrode, which was closely related with power consumption, was largest when the bubble was grown and was about 1.5 V. Because the electrolyte solution for the micropump was separated from sample solutions with the intervening bubble and the reservoirs for the reference and auxiliary electrodes were relatively large, the change in pH and the activity of Cl^- ions were considered to be small. Therefore, the ionic environment in the vicinity of the working electrode and the reference electrode was preserved well resulting in reproducible pumping.

The pump could generate considerable pressure as long as the electrolyte solution was in contact with the working electrode. In measuring the critical maximum pressure the pump can exert, however, it was found that the limiting factor was not the pump itself but the sealing between the PDMS sheet and the glass substrate. With all the factors taken into consideration, the pump could move a solution under a background pressure of 240 mm H_2O and the normal operating potential.

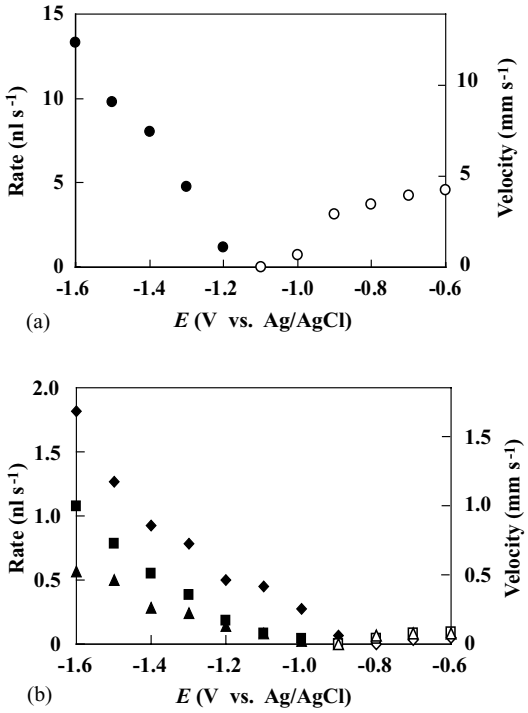

Fig. 2. Dependence of the rate of volume change on the potential of the working electrode for the pump (a) and the valve (b). The rates of growth and shrinkage are shown in the same figure. The closed symbols are for growth, and the open symbols are for shrinkage. The pHs of the solutions used for the valves was 4.0 (◇,◆), 7.0 (□,■), and 10.0 (△,▲).

3.2. Function of the valves

Unlike the pumps, the valves used sample solutions for the electrolysis. The behavior of the valve was examined in a 0.1 M citrate buffer (pH 4.0), a 0.1 M phosphate buffer (pH 7.0), and a 0.1 M borate buffer (pH 10.0) filling

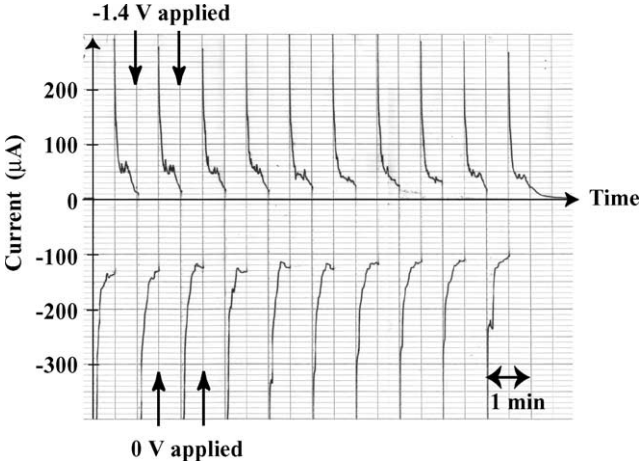

Fig. 3. Variation of the current generated from the working electrode for the pump during withdrawing and flushing. The cathodic and anodic currents correspond to the currents during flushing and withdrawal, respectively.

the flow channel. Needless to say, the reactions became non-reversible or sluggish as the pH of the solution increased. As a result, a larger overpotential was required to generate the bubble rapidly as the pH became higher (Fig. 2b). Compared with the growing change, the rate of shrinkage was much smaller. However, even at neutral pHs, the time needed for the bubble to open the channel was on the order of several seconds because the volume of the bubble filling the compartment was approximately 9 nl. The rates (especially for the shrinking change) depended on the structure around the working electrode [5]. With the relative size of the electrode and the compartment in the present system, the electrolyte solution did not effectively make contact with the working electrode even if the surface of the electrode was platinized. The small rate of shrinkage reflects the difficulty in contacting the electrolyte solution. The dependence of the rates on the solution pH will not pose any problems if the opening and closure of the valves are controlled not by the time but by the charge generated during the electrolysis. Otherwise, the elements for the valve, including the electrolyte solution, could be collected in a limited space and separated from the sample solutions with a diaphragm [7,8]. Although the structure becomes a little complicated, the approach might be advantageous to maintain the reference electrode potential constant and minimize the Ohmic drop.

A peculiar feature of the valve is that there are no ups and downs in its structure, as seen in many previous microvalves [1]. As a result, the transport of fluid was smooth minimizing the possibility of clogging. The growth often started from different portions of the working electrode. As bubbles grew, they merged into a single bubble and fitted in the cavity. The maximum pressure which the bubble could withstand was examined by means of the leakage of Orange G and the detachment of the bubble from the compartment. When pressure was exerted, the bubble deformed. However, as the bubble adjusted itself to the geometry of the flow channel, a satisfactory sealing was achieved. The limiting pressure distributed between 100 and 200 mm H_2O for both tests. Although the results of the two tests were about the same and the values of the latter were slightly higher, the bubble could withstand a pressure reaching 300 mm H_2O, depending on the case. Compared with the other types of valves, the critical pressure was not high. However, with the sequential opening and closure in the fabricated flow channels, no problem was experienced.

In producing hydrogen for valves by the electrolysis of a sample solution, changes in the solution component caused by redox reactions including the pH change caused by the electrolysis of water might be a concern. However, the amount of bubble produced in the valve was approximately 0.4 nmol and was considered to be negligible even in the microflow channel. Also, because the growth or shrinkage proceeds only around the same portion of the solution, no significant alteration of the components will occur in the rest of the electrolyte solution.

3.3. Isolation of the electrolyte solution with a bubble

In separating the sample solution from the electrolyte solution for the micropump, the use of an immiscible liquid such as silicone oil could be one possibility. However, this approach will become more difficult as the flow channels are miniaturized due to the dominating effect of surface tension. A hydrogen bubble could solve this problem again. As long as there is a working electrode in the flow channel, the bubble can be grown irrespective of the size of the flow channel.

In order to check if the bubble functioned as expected, the acidic electrolyte solution (1.0 M KCl–HCl buffer solution (pH 2.2)) was introduced into the container for the micropump, and the bubble was grown to an appropriate size. After the solution in the side of the flow channels was replaced with pure water by repeating withdrawal and flushing, the solution of Methyl Orange was used filled on the other side of the micropump. The pH change of water caused by the diffusion through the periphery of the bubble was examined. No significant color change was observed within 10 min. When the bubble was moved repeatedly within the syringe, no significant enhancement of mixing was observed for at least 20 cycles.

3.4. Introduction of external solutions and mixing

One of the two sets of mechanism was used to transport a solution following the procedure summarized in Table 2. In the following discussion, the valve between the injection port and the pump is referred to as A, whereas that between the pump and the mixing channel is referred to as B. After valve A was closed and valve B was opened, the hydrogen bubble of the pump was grown to the vicinity of the end of the syringe. Then, valve A was opened. Valve B was closed and the hydrogen bubble in the pump was shrunk, which introduced the external solution from the injection port (Fig. 4a). After valve A was closed and valve B

Table 2
Procedure to handle the pumps and valves to transport solutions

Number	Status of the valves and pumps			Event
	Valve A	Valve B	Pumps	
1	Close	Open	Off	
2			On (bubble grown)	Internal solutions flushed
3	Open	Close	Off	
4			On (bubble shrunk)	External solutions introduced
5	Close	Open	Off	
6			On (bubble grown)	Introduced solutions tranported to the mixing channel

The blanks in the columns for valves mean that the previous status is continued.

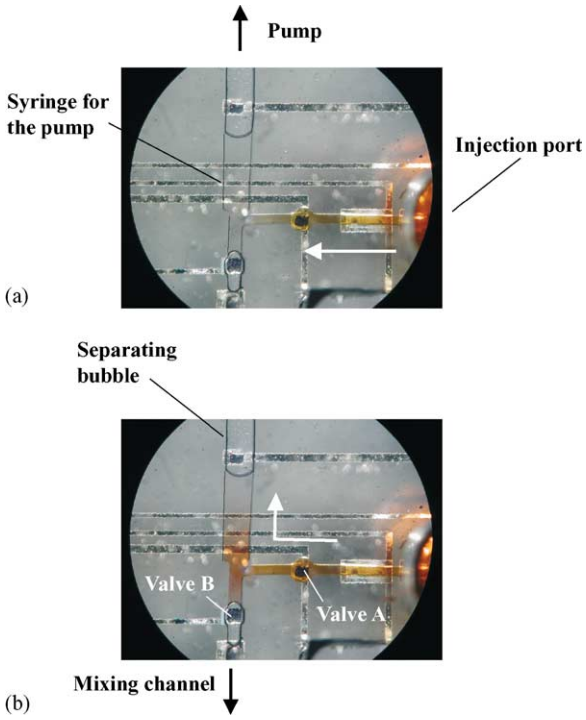

Fig. 4. Introduction of an Orange G solution from the injection port on the right. The solution entered from the injection port in (a) and moved to the syringe of the pump in (b).

was opened, the hydrogen bubble was grown again, which transported the introduced solution to the flow channel leading to the mixing channel (Fig. 4b).

Following the same procedure, two solutions could also be withdrawn from two ports and transported in the system (Fig. 5). The Reynolds number calculated based on the parameters mentioned earlier was 0.81 when the volume flow was $8.0\,\mathrm{nl\,s^{-1}}$. When the solutions flowed into the mixing flow channel at the end, sheath flows of the two solutions could be observed (Fig. 6).

3.5. Enzymatic reaction in the system

To demonstrate the practical applicability of the system, the following enzymatic reaction was used as a model.

$$\mathrm{Luciferin(Red) + ATP + O_2} \xrightarrow{\text{Luciferase}} \mathrm{luciferin(Ox) + AMP + PPi + CO_2 + H_2O} + h\nu \quad (1)$$

Here, PPi indicates pyrophosphate. A solution containing luciferin and ATP was withdrawn from one of the injection ports, and another solution containing luciferase was withdrawn from another injection port. The two solutions were transported through the flow channels to the mixing channel following the procedure described earlier. As is well known, components in two sheath flows do not mix easily unless specially designed structures are used. The time required for diffusion is proportional to the square of the distance and is expressed as follows [9]:

$$\tau_\mathrm{d} = \frac{L^2}{2dD} \quad (2)$$

where L is the distance, D the diffusion coefficient of the molecules in the solution, and d the dimension considered in the problem. If D is $\sim 10^{-5}\,\mathrm{cm^2/s}$, as often found, and L the $\sim 0.1\,\mathrm{mm}$, τ_d will be on the order of several seconds. This indicates that even the mixing by diffusion has an effect depending upon the size of the flow channel.

With our system and the normal operating condition, the length of the mixing channel should be more than several centimeters if the reaction is allowed to proceed while the solutions are flowing. In other words, additional space will be required to use the system by this mode of operation. In the present experiment, the two solutions introduced into the mixing channel were stopped considering the above situation and the capacity of the pump. This mode of operation is considered to be a natural choice as the volume of the handled solutions decreases. As expected, the

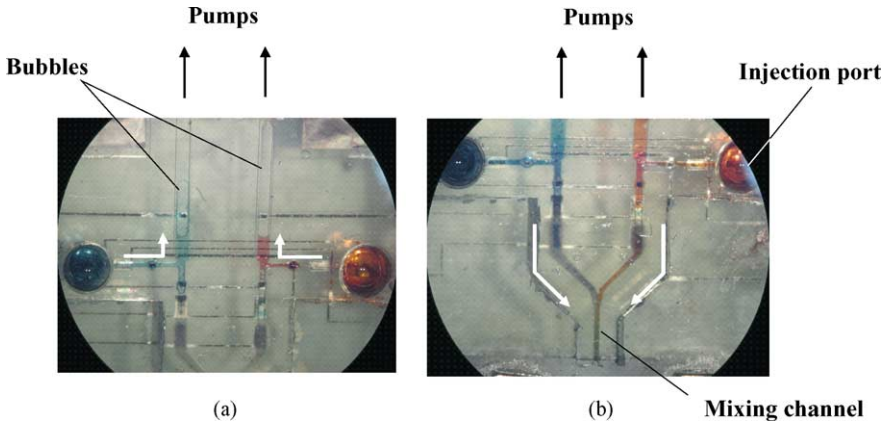

Fig. 5. Introduction and transport of two dye solutions (left: Methylene Blue; right: Orange G) through the microflow channels. The circles on the left and right are the injection ports. The two solutions were introduced by the pump in (a) and transported downward to the mixing flow channel in (b).

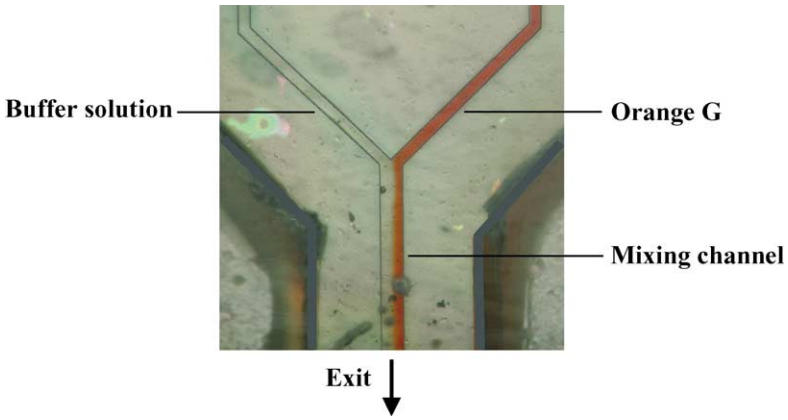

Fig. 6. Sheath flows of the two solutions observed in the mixing channel.

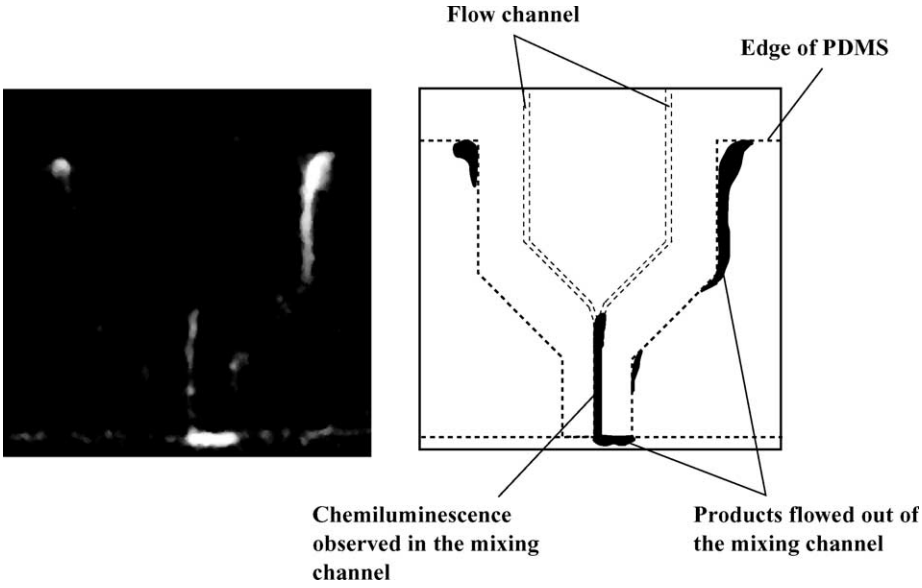

Fig. 7. Chemicalluminescence caused by the mixing of the two precursor solutions. The figure on the right indicates the positions of the chemiluminescence.

two solutions mixed rapidly. Fig. 7 shows the area around the flow channel, in which two solutions flowed in and then mixed by diffusion after the flow was stopped. Chemiluminescence originating from the enzymatic reaction was observed.

As already demonstrated in a previous study, electrochemical detectors can easily be integrated in the flow channel [5]. Based on this strategy, more pumps, valves, and sensors could be integrated. A problem encountered here is the increasing number of elements, leads, and contact pads. As has already been used in the semiconductor technology, the problem will be circumvented by incorporating a multiplexing device to select the right pumps, valves, and sensors [2].

4. Conclusions

Simplification of the structure and function of components will be the first requirement to construct a sophisticated μTAS or Lab-on-a-Chip. An electrochemically generated hydrogen bubble is effective to realize such simple pumps and valves. As demonstrated in this study, two different solutions can be introduced into the system and merged in a flow channel by operating the respective pumps and valves cooperatively. The electrochemical principle gives us a freedom to change the flow rate precisely and reproducibly. Also, the operating voltage and power consumption are much smaller than the other counterparts based on different operational principles. The advantages will make the construction of a more sophisticated Lab-on-a-Chip more realistic.

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Biographies

Hiroaki Suzuki received his BE and ME degrees in applied physics and his PhD degree in bioelectronics and biotechnology from The University of Tokyo in 1981, 1983, and 1993, respectively. In 1983, he joined Fujitsu Laboratories Ltd., Japan. In 1996, he moved to the Institute of Materials Science, University of Tsukuba, Japan, where he became associate professor of materials science. His current research interests include microbio/chemical sensors and μTAS.

Rei Yoneyama received his BE and ME degrees in materials science from University of Tsukuba in 2001 and 2003, respectively. In 2003, he joined Mitsubishi Electronic Corporation, Japan. At present, he is working on a master's degree at the Institute of Materials Science, University of Tsukuba, Japan.