Biotechnology and Bioprocess Engineering **2004**, 9: 86-92

Microfluidic Components and Bio-reactors for Miniaturized Bio-chip Applications

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Abstract In this paper miniaturized disposable micro/nanofluidic components applicable to bio chip, chemical analyzer and biomedical monitoring system, such as blood analysis, micro dosing system and cell experiment, etc are reported. This system includes various microfluidic components including a micropump, micromixer, DNA purification chip and single-cell assay chip. For low voltage and low power operation, a surface tension-driven micropump is presented, as well as a micromixer, which was implemented using MEMS technology, for efficient liquid mixing is also introduced. As bio-reactors, DNA purification and single-cell assay devices, for the extraction of pure DNA from liquid mixture or blood and for cellular engineering or high-throughput screening, respectively, are presented.

Keywords: DNA chip, cell chip, MEMS, bio chip, micropump, micromixer

INTRODUCTION

Micro bio-analytical systems are currently gaining significant importance due to the limitations shown by conventional systems for the identification of specific biological phenomena, and in the development of new biological, diagnostic processes and user-adapted analytical systems.

As most bio-chemical analysis include liquid handling, both of microfluidic control components as well as bio-reactors are required for integrated micro bio-analytical system. Generally, the microfluidic control components include a micropump for liquid translation, microvalve for liquid control and a micromixer for reagents mixing, *etc*. These microfluidic components, especially the micropump, should be operated in low voltage and low power consumption for the application of the micro analytical system. In this paper, a micropump driven by surface tension for low voltage and low power operation, embedded with check valves for directional liquid movement, is introduced. Also, a micromixer, fabricated using microelectromechanical systems (MEMS) technology for efficient liquid mixing, is presented.

As examples of bio-reactor applications, two biochip solutions, which can play critical and significant roles in bio-processing and bio-engineering in current and near future, are introduced. One is a deoxyribonucleic acid (DNA) purification chip for the extraction of DNA from sample liquid or blood prior to DNA analysis. The other is a single-cell assay chip for cellular engineering or a high-throughput drug screening system.

In the following sections, each component and integrated micro bio-reactor chip, with their applications and experimental results, are presented.

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MICROFLUIDIC CONTROL COMPONENTS

Micropump Driven by Surface Tension

Previously, a number of micropumps have been developed using various actuation methods, including piezoelectric [1], electrostatic [2], thermopneumatic [3], electromagnetic [4], bimetallic [5] and shape memory alloy (SMA) actuations [6]. However, most of these require high operation voltages (piezoelectric and electrostatic actuation) or high operation powers (thermopneumatic, electromagnetic and SMA actuation). These devices are difficult to apply to some application fields, such as embedded medical devices, remote environmental monitoring systems and handheld chemical analyzers, *etc*. Therefore, a micropump operated by continuous electrowetting (CEW), which is the motion of a liquid drop driven by the variation of surface tension induced by a low applied voltage, with low power consumption, is proposed [7].

Fig. 1 shows the structure of the proposed micropump, which illustrates the principle of the pump actuation. In step 1, with a positive voltage applied to the electrode, the left-side surface of the mercury drop shows a higher surface tension than that of the right-side surface, because the repelling force induced from accumulated positive charge between mercury molecules being higher on right-side surface compared to the left-side surface. Therefore, the mercury drop moves toward the outlet chamber and

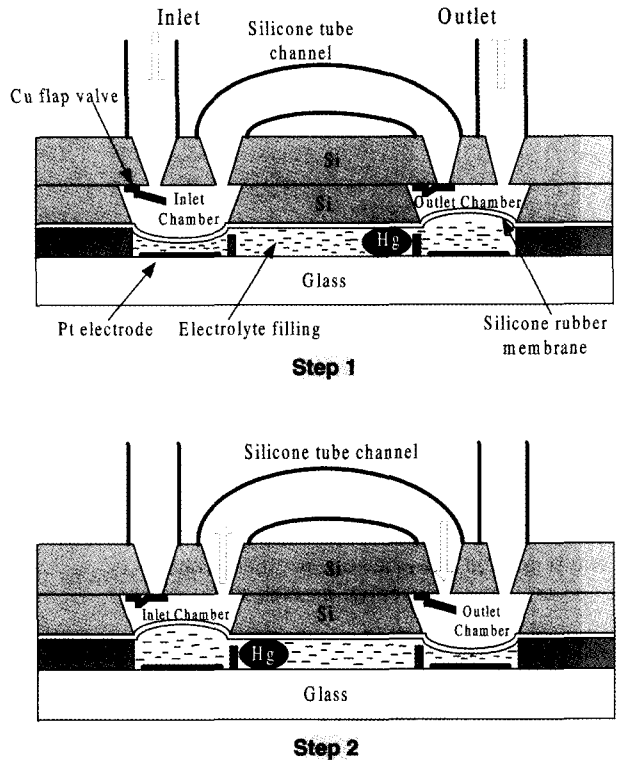


Fig. 1. Schematic view of the proposed micropump, with the principle of the pump actuation.

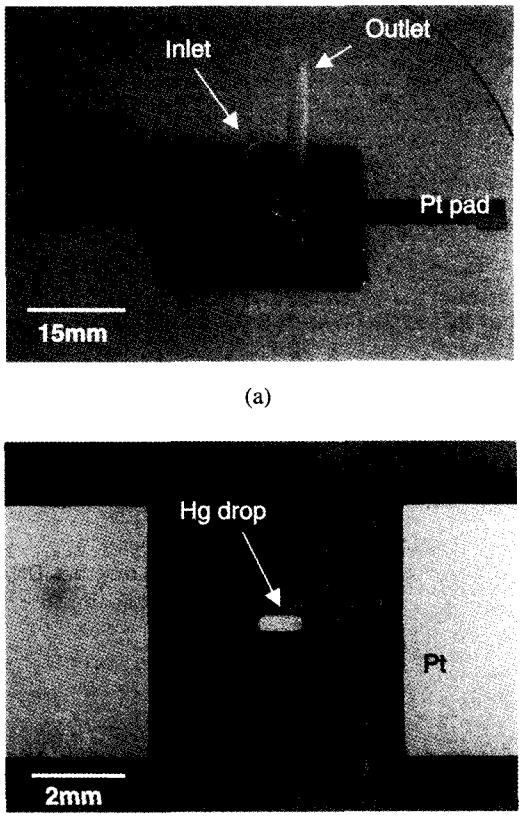


Fig. 2. Photographs of the fabricated micropump, with a magnified view of the mercury placed channel.

the membrane moves downward opening the valve in the inlet chamber, while closing the valve in the outlet chamber. In this step, the liquid flows into the inlet chamber and out of the outlet chamber. In step 2, the polarity of the applied voltage is reversed causing the mercury drop move toward the inlet chamber. The actions in the two chambers are reversed, and the liquid in the inlet chamber flows to the outlet chamber through a silicone tube channel. The oscillating mercury motion caused by alternating the applied signals generates a net flow of liquid from the inlet to the outlet.

The micropump was fabricated from silicon and glass substrates using a MEMS/micromachining technique and semiconductor processes. Fig. 2 shows photographs of the fabricated micropump, with a magnified view of the mercury placed channel.

The pumping rates of the fabricated micropump were measured using water as the pumping liquid for various applied voltages and frequencies. Fig. 3 shows the measured flow rates. The maximum flow rate was measured as 70 μL/min at a frequency of 25 Hz, with an applied voltage and power consumption of 2.3 V and below 170 μW, respectively.

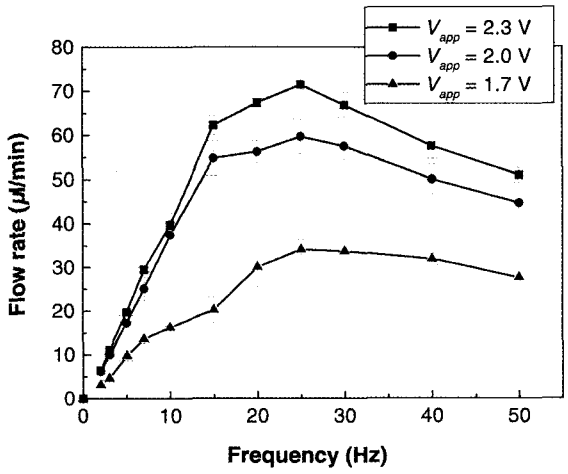


Fig. 3. Flow rates as a function of the actuation frequency.

3-Dimensional Micromixer

Rapid mixing is essential in many microfluidic systems targeted for use in biochemical analyses, drug delivery and sequencing or synthesis of nucleic acids [8,9]. Biological processes, such as cell activation, enzyme reactions and protein folding, often involve reactions that require mixing of reactants for their initiation. In this paper, a new 3-dimensional micromixer, devised to increase the mixing efficiency on a micro scale, where the Reynolds number is low, is established [10].

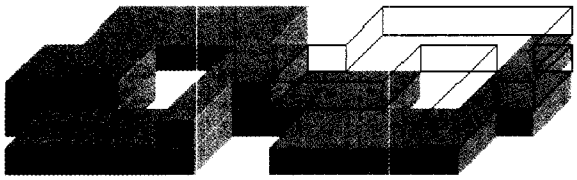


Fig. 4. Schematic diagram of the proposed micromixer.

(a)

(b)

Fig. 5. Photographs of the fabricated micromixer; (a) top view and (b) bottom view.

It has been shown that a "twisted pipe" has the potential to enhance mixing, even at low Reynolds numbers (<100). This mixing enhancement is possible because of the phenomenon known as chaotic advection [11], where simple regular velocity fields produce chaotic particle trajectories. The occurrence of chaotic advection typically indicates rapid distortion and elongation of material interfaces. This process significantly increases the area across which diffusion occurs, leading to rapid mixing. In order to obtain adequate liquid mixing at low Reynolds numbers, the geometry of a channel must be 'complicated enough' to trigger chaotic advection. However, in order for fluidic channels to be easily fabricated and integrated into microfluidic systems, the geometry should remain 'relatively simple'.

The proposed mixer consisted of two-layer channels. Mixing is a two-step process; the first step is segmentation, where a heterogeneous mixture of two fluids is formed by convection; and the second is the inter-

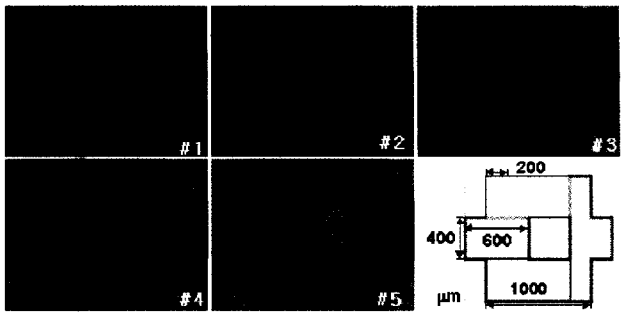


Fig. 6. Photographs at each consecutive segment stage of the fabricated mixer at a flow rate of 2 mL/min.

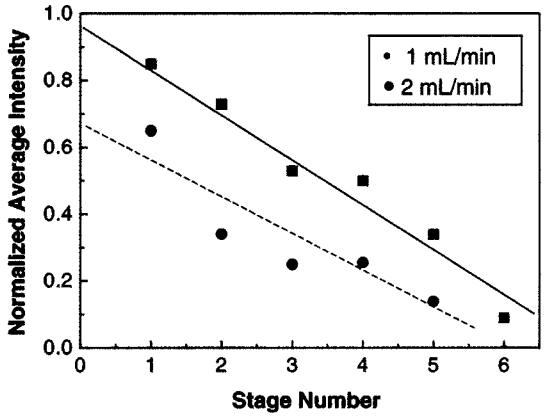


Fig. 7. Normalized average optical intensity in each stage of the mixer.

diffusion of molecules between domains. As shown in Fig. 4, the proposed mixer has a separated serpentine flow path, in order to increase the chaotic advection, as well as repeated segments to increase the interfacial area.

The micromixer was fabricated using PDMS, and is shown Fig. 5. The depth and width of the channel are about 100 μm and 400 μm, respectively. The mixer was tested using phenolphthalein, a pH indicator that changes color from colorless transparent to red at pH values higher than 8. Fig. 6 shows the mixing of 0.1 mol/L phenolphthalein and 0.3 mol/L NaOH in the micromixer with multi stages. Fig. 7 shows the normalized average optical intensity in each stage of the fabricated micromixer. After five or six mixing sections the two streams were fully mixed.

MICRO BIO-REACTOR

Disposable DNA Sample Preparation Microfluidic Chip

Nucleic acid (NA) probe assays have an enormous scope of applications in biotechnology and medicine, ranging from agriculture and farming to the detection of pathogens in foods and for genetic diagnostics on human subjects [12]. Recently, there has been much interest in

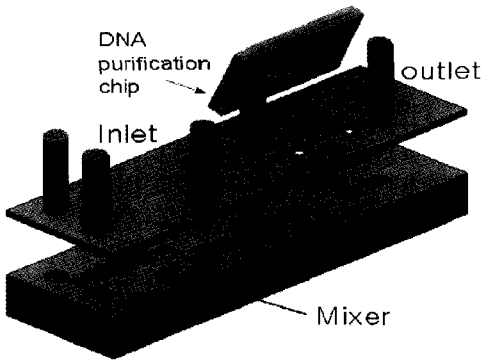


Fig. 8. Proposed microfluidic system for sample preparation of a Nucleic Acid probe assay.

the implementation of microfluidic devices for NA probe assays. Theses devices are excellent candidates for miniaturization because the performance and costs of NA probe assays can be improved on a microscale, and the same microfabricated part can be used for many different assays by just changing the nature of the reagents, with no adjustment required to the devices construction [13].

The purpose of NA probe assays is the detection and reporting of very small amounts of predetermined NA sequences in biological fluid samples. NA probe assays typically include polymerase chain reaction (PCR) to amplify the number of copies of DNA to detectable levels. The PCR technique requires a relatively pure DNA sample in aqueous solution, which is free of inhibitors during the PCR process. Therefore, the extraction and purification of nucleic acids from biological samples are critical steps that should be carefully handled in NA assays. Also, this series of complex chemical processes are the most difficult and time-consuming part.

In this paper, a new microfluidic chip has been proposed to address the sample preparation process for NA probe assays [14]. The proposed microfluidic system was composed of a DNA extraction chip integrated to the micromixer introduced in the previous section (Fig. 8). In the mixer, biological samples are mixed with lysis reagents to release their DNAs into solution, and the lysed solution is then mixed with chaotropic salt. In the DNA purification chip, the DNAs in solution will bind to the exposed SiO_2 surface when the concentration of the chaotropic salt is high [15].

Fig. 9 shows the fabricated DNA purification chip, which was designed to maximize the DNA binding surface area onto photosensitive glass. Photosensitive glass was chosen as the binding substrate because its fabrication process is simple and inexpensive compared with other alternative technologies, such as deep reactive ion etching (RIE). Fig. 10 shows the prototype microfluidic module integrated with a mixer and DNA extraction chip. The mixer is connected in series with the DNA purification chip using a silicone tube.

The fabricated device was tested by passing a chaotropic DNA starting solution through the chip. During this procedure, the DNA present in the solution binds to

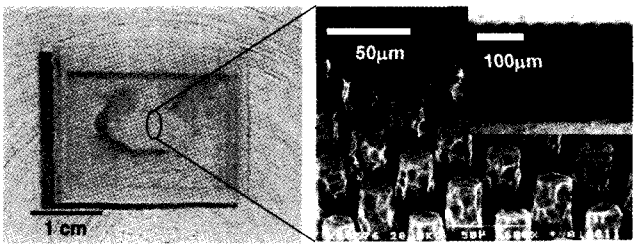


Fig. 9. Photograph of the glass microstructure for DNA binding.

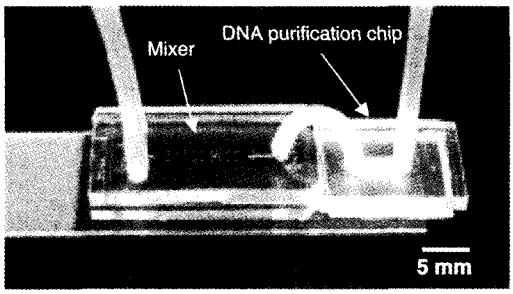
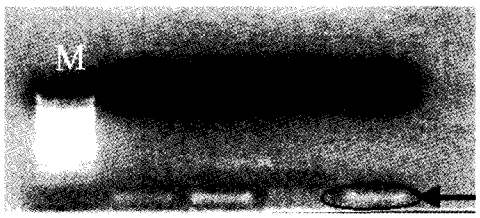


Fig. 10. Photograph of the prototype microfluidic module integrated with a mixer and DNA purification chip.

(a)



(b)

Fig. 11. Photograph of the electrophoretic gel analysis results, showing the DNA eluted from the DNA purification chip, where lane M denotes the marker: (a) high concentration sample (600 ng/200 μL) without PCR and (b) low concentration sample (5 pg/200 μL) with PCR.

the glass surface of the microchannels. Next, an ethanol based wash solution was introduced, which was intended to wash away the salts and other PCR inhibitors present in the sample. Finally, an elution reagent was introduced to the chip and held there for several minutes, releasing the DNA back into the fluid stream. This solution was processed through gel electrophoresis in 1% agarose gels, with ethidium bromide (EtBr) staining.

In this experiment, two concentrations of DNA sample - high concentration (600 ng/200 μL) and low concenp-

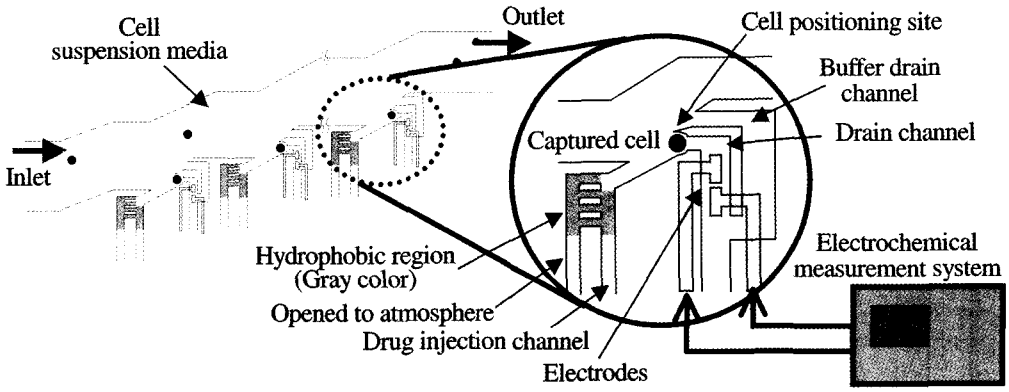


Fig. 12. Schematic illustration of the proposed single-cell assay device.

tration (100 copies/200 μL which is equivalent to 5 pg/200 μL) - were tested. Fig. 11 shows the photograph of electrophoretic gel analysis results showing the eluted DNA from the fabricated DNA purification chip. In the figure, the character 'M' denotes the marker. For high concentration sample, the presence of target DNA has been detected without PCR step, while the PCR was performed for low concentration sample before the gel electrophoresis. The amount of released DNA from high concentration sample was detected about 30 ng, which means that the binding capacity of the chip is about 15 ng/cm^2 (Fig. 11(a)). Also Fig. 11(b) shows that the fabricated purification chip can detect the gel band of the eluted DNA from a low concentration sample below 100 copies/200 μL (or 5 pg/200 μL).

Single-cell Assay Chip

Cells are usually influenced by a mixture of hormones, ions and neurotransmitters released from neighboring cells. Dynamic monitoring of a single cell is important for individual cell analysis without the influence of neighboring cells in independently controlled environments. Previously, Thielecke *et al.* reported a single-cell positioning chip with single-cells placed on ring electrodes [16]. However, it was difficult to independently control the environment of each cell located in an individual ring electrode structure. In this paper, a microfluidic chip is introduced in which a single-cell is autonomously captured in a cell-positioning site by a pre-defined fluid stream where a specific liquid flow, containing drugs or reagents, can be consecutively supplied to each single-cell through a flow injection channel [17].

The schematic view of the proposed structure is shown in Fig. 12. The cell suspension medium was introduced into the inlet due to the external pressure and single cell is positioned on each cell-positioning site. The drug or other chemicals can be injected onto each captured cell through the corresponding injection channels, and the resulting cell secretions monitored. A hydrophobic region is formed to prevent the suspension medium from flowing in or out of the isolated region, but which allows air to leak out during drug introduction.

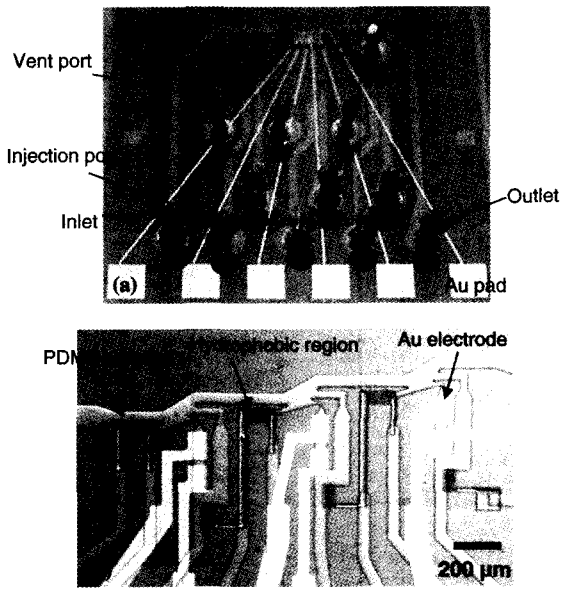


Fig. 13. (a) Photograph of the fabricated device. (b) Detailed view of the dotted region in (a).

The device consisted of a grooved PDMS and silicon substrate bonded together. The hydrophobic region was formed by a self-assembled-monolayer (SAM) coating of 1H, 1H, 2H, 2H-perfluorodecyltrichlorosilane (FDTS) onto the silicon surface, with an observed contact angle of about 105°. Fig. 13 shows a photograph of the fabricated device. The initial design of the devices had three cell-positioning sites.

In the experiment, the fluid stream, including the cell suspension medium and drug injection medium, is controlled by the external air pressure using a low-range pressure regulator. First, the feasibility of cell capturing was tested using polystyrene beads (15 μm in diameter) in distilled water. The hydrophilic region was gently filled with the flow of medium through the inlet to the outlet. A bead was then introduced through the inlet under the force of the external pressure. Fig. 14 shows a series of pictures of the bead capturing and drug injection during

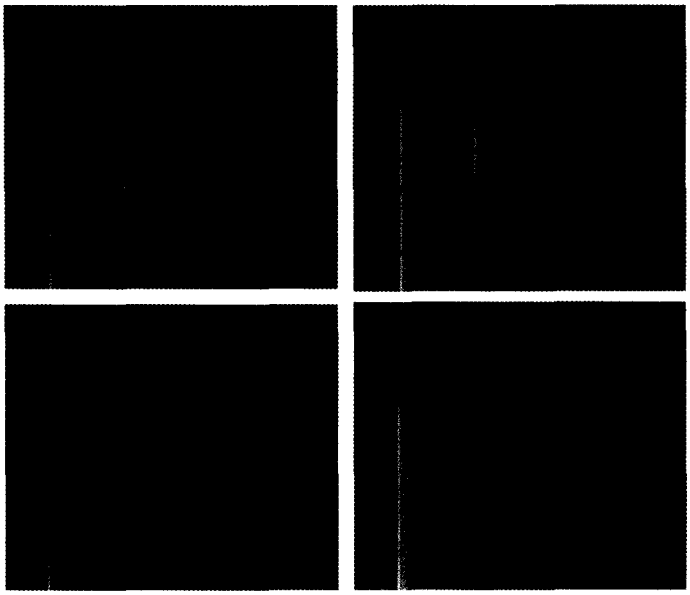


Fig. 14. Series of pictures during the cell-positioning test. (a) Capturing a bead. (b) Introducing blue ink (drug) from the injection channel, now stopped at the hydrophobic region. (c) Overcoming the capillary force by increasing the injection pressure. (d) Ink (drug) injection onto the captured bead.

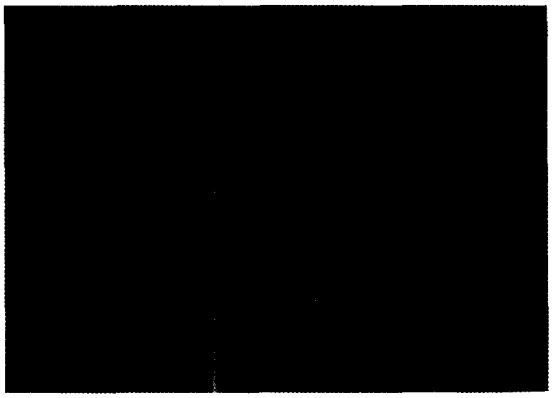


Fig. 15. Capturing of CHO DG44 cell on cell-positioning sites, with a magnified view of the captured cell.

the experiment. As shown in Fig. 14(a), a bead can be exactly placed on a cell-positioning site from the predetermined flow stream. Also, this figure shows that the medium does not entered into the hydrophobic region as it stops at the interface of the hydrophilic and hydrophobic regions. After the bead had been captured, blue ink was introduced from the injection channel. Without any injection pressure, the ink was introduced through the hydrophilic injection channel until it was blocked at the entrance of the hydrophobic region (Fig. 14(b)). The ink then goes through the hydrophobic region by applying an injection pressure (Fig. 14(c)) and finally onto the captured bead (Fig. 14(d)). During the ink injection, the air in the injection channel can leak out to the atmosphere through a hydrophobic vent channel. Fig. 14(d) shows that the excess ink stream drained out through the buffer drain channel located above the main drain channel, demonstrating that the injected drug (in this experiment the injected ink) does not flow into other captured cells in the neighborhood. The experiment using living cells is shown in Fig. 15. This photograph shows the actual live cell, CHO DG44, captured in a cell-positioning site. The cell suspension medium was phosphate buffered saline (PBS) solution (pH 7.4).

CONCLUSION

Microfluidic components, including fluidic controllers and bio reactors, for a biological assay system have been proposed and fabricated using MEMS technologies. These devices and systems are expected to enhance the performance of the biological assay system, with relatively high sensitivity and small amounts of reagents compared to conventional assay systems. The proposed microfluidic devices or systems are hoped may open new possibilities in bio-processing and bio-engineering by overcoming the limitation of conventional technologies.

Acknowledgements This work has been partially supported by the NRL program from the Ministry of Science and Technology, the KOSEF through the MICROS center at KAIST and the project of "Development of micro optical and thermofluidic devices with high functionality" from the Ministry of Commerce, Industry and Energy of Korea.

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[Received February 28, 2004; accepted April 15, 2004]