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Poly(dimethylsiloxane) (PDMS) and Silicon Hybrid Biochip for Bacterial Culture

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Abstract. **In this study, a novel PDMS/silicon hybrid microfluidic biochip was fabricated and tested for the long-term batch culture of bacterial cells. The PDMS (poly(dimethylsiloxane)) cover with 3-dimensional micro-channels for flow was fabricated using Teflon tubing and hole-punch techniques, without photolithographic methods. The PDMS/silicon hybrid biochip was prepared by bonding of PDMS cover and a silicon chip that had electrodes and micro-fluidic channels defined. The absorption of liquid into PDMS cover was characterized and conditions to prevent drying of nutrient media within the micro-chamber were shown. The absorption of liquid from micro-chambers into the PDMS cover was reduced up to 2.5 times by changing the mixing ratio of PDMS and curing agent from 10 : 1 to 2.5 : 1. In addition, pre-saturation of the PDMS cover with media prior to the incubation resulted in the preservation of liquid in the micro-chambers for up to 22 hours. Optimization of the mixing ratio and pre-saturation of the PDMS cover reduced the drying time 10 times when compared to the unsaturated PDMS cover composed of 10 : 1 ratio of PDMS and curing agent. *Listeria innocua* and a strain of *Escherichia coli*, expressing green fluorescent protein (GFP), were successfully cultured in batch mode within the PDMS/silicon hybrid biochip.**

Key Words. **PDMS/silicon hybrid biochip, bacterial culture, fabrication of 3-dimensional micro-channel, absorption in PDMS**

1. Introduction

As a merger of microelectronics and biological sciences, BioMEMS is one of the most promising research fields and has the potential of solving important problems in a wide variety of areas such as microfluidics (Beebe et al., 2000), genomics (Kopp et al., 1998), proteomics (Mouradian, 2001), and metabolomics (Yasukawa et al., 2002). Microscale bioreactors and fermentors based on the BioMEMS principles constitute an important part of these applications, not only as a tool for biological techniques such as manipulation of cells (Beebe et al., 2002), but also for mimicking *in vivo* conditions to investigate intercellular interactions and behaviors (Bhatia et al., 1998).

Long-term cultivation using perfusion in a microfluidic device has been reported recently (Leclerc et al., 2003); however, batch culture is another important concern for applications such as infectious agent detection and cell-based screening of drug candidates. Specifically, the detection of small quantities of pathogenic bacteria or toxigenic substances in food, bodily fluids, tissue samples, soil, etc, are a few of the most important applications of biochips (Gómez et al., 2001). These applications necessitate not only the counting of the total number of bacteria but also detection of the number of viable cells in the samples. A silicon-based microscale biochip for electrical detection of bacterial growth was recently demonstrated for this type of application (Gómez et al., 2002). The detection of bacterial growth was accomplished by measuring the changes of impedance in the medium due to generation of ionic metabolites during bacterial growth in batch culture.

The advantages of polymer microfluidic devices when compared with the silicon, glass and quartz based devices are their low cost and rapid fabrication (Hong et al., 2001). Moreover, the mechanical flexibility of poly (dimethylsiloxane) (PDMS) allows the creation of valves and pumps within the microfluidic structures (Unger et al., 2000). PDMS is a promising material for micro-

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fluidic devices for cell cultivation due to its excellent biocompatibility, low cost, easy fabrication and more importantly its permeability which can provide oxygen to the media without additional equipment or set-up (Leclerc et al., 2003). Microfluidic devices made of PDMS have already been applied for applications related to cell culture such as endothelial cell (Borenstein et al., 2002) and liver cells (Powers et al., 2002). In spite of the merits, the lack of rapid, simple and reliable fabrication methods to make electrodes on PDMS makes PDMS/silicon (or glass) hybrid biochips a viable solution for biomedical applications. Bonding of metal and PDMS has been reported previously in a few articles using evaporated metal (Bowden et al., 1998), Si-H bond on metal (Hirayama et al., 1999). Embedding of carbon-based electrodes in micro-channel was suggested as substitute of metals (Gawron et al., 2001). Nevertheless, rapid fabrication of electrodes on PDMS in a reproducible fashion has not been reported so far.

There have been some reports on the characterization of permeation of organic and aqueous solvents in silicon-based elastic polymers (elastomers). Swelling of the PDMS network with solvent was observed with different content and molecular weight of siloxane oligomer precursors (Sivasailam and Cohen, 2000). Permeability of gas through a composite membrane made of PDMS and other polymers was measured for the separation of gases (Kimmerle et al., 1991). The absorption and diffusion properties of organic (Blume et al., 1991) and water vapors (Favre et al., 1994) in the PDMS network have also been reported. The absorption of liquid in PDMS can cause major problems in cell culture applications due to drying of a minute volume of the liquid media. For long term observation ranging from a few hours to more than 12 hours, which encountered for the cell growth and also detection of changes in environmental condition of cell culture inside the chips, this problem becomes critical. For this reason, prevention or delay of the absorption of liquid into PDMS covers is a fundamental concern for micro-scale biochips made of PDMS. There are no reports to date on bacterial cell culture inside nano-liter volume chambers within micro-fluidic devices.

In this study, a novel PDMS/silicon hybrid microfluidic biochip was fabricated and tested for the long-term batch culture of bacterial cells. The 3-dimensional microfluidic channels of the PDMS cover were made without photolithographic methods, using Teflon tubing and hole-punch. The hybrid biochip was assembled by bonding of PDMS cover and the silicon biochip, which had electrodes and micro-wells. Based on the electrical measurements of absorption of liquid into PDMS cover, the optimum ratio of PDMS and curing agent composition and operating conditions for long-term cultivation of cells were recommended. Fluorescently labeled *Listeria innocua* and green fluorescent protein (GFP) producing *Escherichia coli* were successfully cultured for more than 20 hours in these devices.

2. Device Fabrication

Microfluidic devices are made of at least two layers, one layer that contains the microfluidic channels and chambers, and the other layer being used as the cover. Due to the soft characteristics of PDMS, it is very easy to treat and alter its shape even after it is cured, for example, by cutting and boring using a blade or punch, respectively. We decided to use a silicon wafer to make the channels and wells, due to ease of integration with metallization and future electronics, and a PDMS layer to make the cover and the layer for the connecting ports. In addition, a horizontal channel was used to connect the nano-liter volume channel to fluidic ports, as discussed below. Thus, the input and output ports were placed far enough from each other to enable viewing of the wells from the top with high magnification objectives ($20\times$–$40\times$) in epi-illumination configuration with a fluorescent microscope (Eclipse E600, Nikon).

2.1. Fabrication of silicon chip

The electrodes and micro-channels/micro-chambers were fabricated in a silicon wafer. Detailed dimensions and fabrication methods for silicon biochip have been described before (Gómez et al., 2001). Briefly, micro-channels and micro-chambers were fabricated on silicon wafers with a thickness of 450 μm and (1 0 0) orientation. The depth of channels and chambers were 12 μm and it was etched by potassium hydroxide (KOH) using the thermally grown SiO_2 as a hard mask. The electrodes were defined over the oxide by lift-off, using a 5 μm thick photoresist layer (AZ4620, Clariant Co., New Jersey, USA). The metallization is formed by evaporation of a 300 Å thick layer of gold over a 300 Å thick film of titanium. These electrodes were used to measure the impedance in the chamber for electronic observation of the drying of liquid from chamber. The size of silicon biochip and micro-chamber for cell culture were 9.1 mm × 8.9 mm and 530 μm × 850 μm, respectively. The volume of the chamber in which cells were grown was 5.3 nl. The chamber has two interdigitated electrodes with five fingers each. The exposed area of each finger is 580 μm × 70 μm, and distance between the two fingers is 10 μm.

2.2. Fabrication of PDMS cover

Three-dimensional channels were needed for the connection of hybrid biochip and macro-scale fluidic

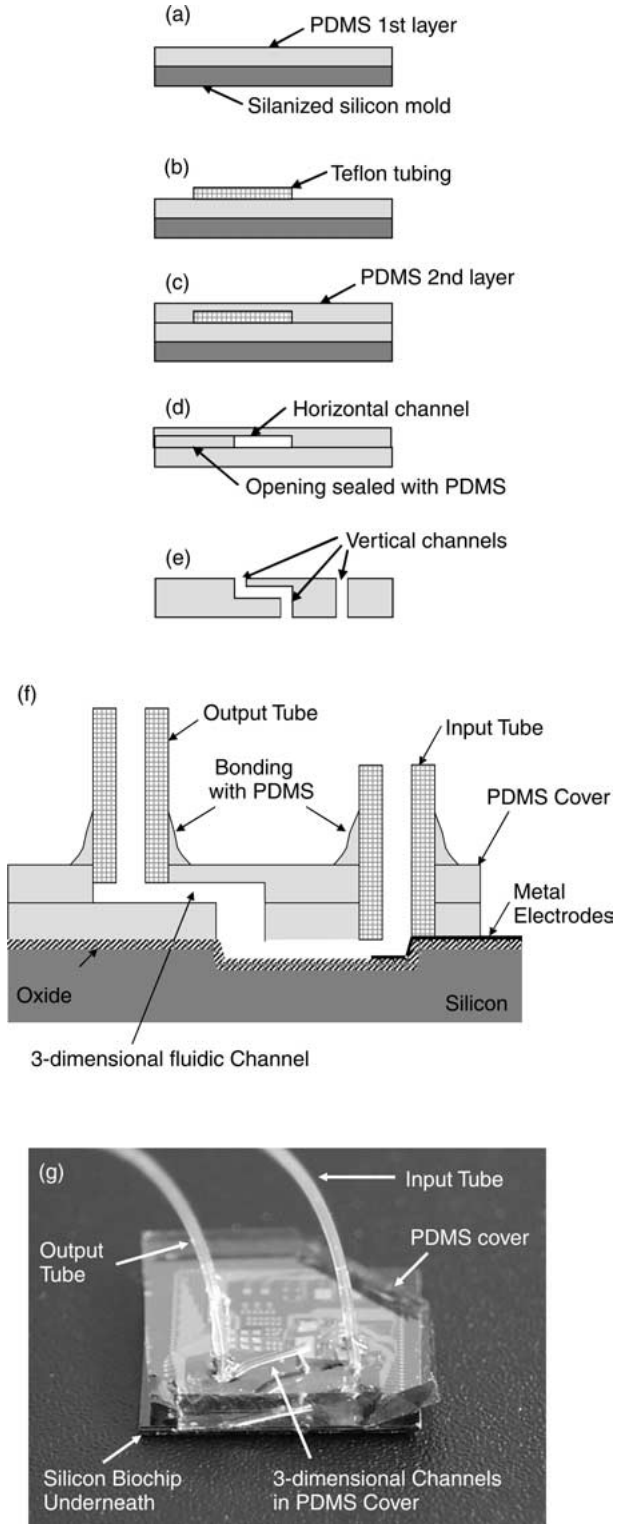

Fig. 1. *Process flow cross-sections (a–e), schematic drawing (f) an optical micrograph (h) of the hybrid PDMS/Silicon biochip.*

components and for appropriate distance between the ports. A two-layered PDMS cover was made without photolithographic methods using Teflon tubing and a hole-punch. As shown in Figure 1a, a mixture of PDMS (Sylgard 184, Dow-Corning) and curing agent was poured, approximate thickness of 500 µm, onto a silanized 3 inch silicon wafer mold and heat cured at 120 °C for 10 minutes. The surface of silicon wafer mold was silanized with vapor of trichloro(3,3,3-trifluoropropyl)silane (Sigma-Aldrich Co., Missouri, USA) for 2 hours in a desiccator to prevent the bonding of cured PDMS. The reagent was vaporized by application of vacuum using vacuum pump. For the construction of 3-dimensional micro-channels, a 7 mm long piece of Teflon tubing (Cole Parmer Co., Illinois, USA) that had outer diameter of 360 µm and inner diameter of 50 µm, was placed on the first PDMS layer. This tubing was used as a mold to create a horizontal channel to connect the channel in silicon with the output port in the second layer, as shown in Figure 1b. Another PDMS mixture was added on this first layer as shown in Figure 1c. After curing, the PDMS cover embedded with the Teflon tubing was peeled off from the mold, and cut into the adequate size for the silicon chip. The embedded Teflon tubing was pulled out of the PDMS cover from one open end of horizontal channel and then it was blocked with PDMS as shown in Figure 1d. Holes for vertical input- and output-flow of liquid were punched from both sides of the cover as shown in Figure 1e.

2.3. PDMS/silicon bonding

PDMS can be bonded on an oxidized silicon chip reversibly without any treatment, and it is reported that this bond can withstand pressures up to 20 psi. However, reversibly bonded cover could not withstand pressure even less than 10 psi for a few hours. Thus, a stronger bond was necessary for achieving higher flow rates and at least a few hours of flow. In this work, the PDMS cover layer and the oxidized silicon chip were permanently bonded to each other after oxidation of both surfaces by exposure to oxygen plasma at 200 W for 15 seconds. Teflon tubing was inserted into the inlet and outlet holes, sealed with PDMS mixture around the tubing, and cured at 90 °C oven for 10 minutes. The cross-section of final device is shown in Figure 1f. The Teflon tubing could not be permanently bonded on the PDMS, however, this bond could easily withstand 15 psi. Figure 1g shows an optical micrograph of the final device.

2.4. Hybrid biochip with input reservoir

In another version of the process, a reservoir was added at one of the ports (input port for example), where a sample can be dispensed and then pulled through the microfluidic channel by applying a vacuum at the output port. Instead of inserting an input tubing, a third thick layer of PDMS was used to form a reservoir. The

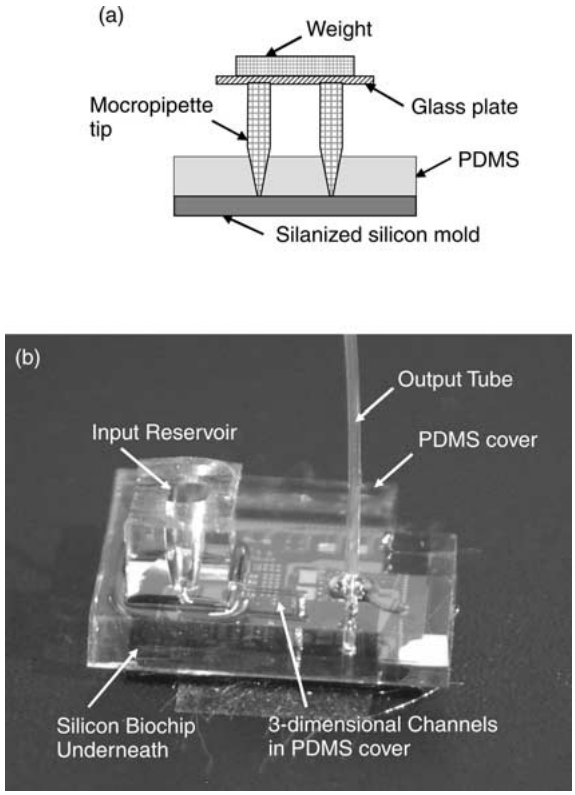

Fig. 2. Partial process flow cross-section (a) and optical micrograph (b) of the hybrid PDMS/Silicon biochip with an input reservoir.

schematic drawing of fabrication method is shown in Figure 2a. The conical shape of reservoir was made using yellow tip for micro-pipette and molding of PDMS. The 200 µl yellow tip was positioned perpendicular to the surface, and followed by pouring and heat curing processes as general PDMS molding technique. The contact of yellow tips and silicon mold was secured during curing by addition of weight on top of them. A piece of reservoir layer was cut into appropriate size after removal of yellow tip from the PDMS layer and bonded on top of PDMS cover. It was permanently bonded by application of PDMS solution. The height and diameter of reservoir was roughly 2 mm and 1 mm, respectively. The volume of the reservoir was about 0.5 µl. Figure 2b shows an optical image of this device.

3. Absorption of Liquid in PDMS

3.1. Absorption study in PDMS slabs

The PDMS covers, made of different ratio of PDMS polymer solution and curing agent solution, were used to investigate the characteristics of absorption of the liquid. The cured polymer with different ratio of PDMS and curing agent is expected to give different hardness and permeability, and pressure actuated valve and pumps made by these materials have been reported (Unger et al., 2000), using this.

The PDMS elastomer is composed of a pre-polymer (defined as the solution of PDMS before it is cured) and a cross linker. The physical and chemical characteristics of polymer changed with the ratio and properties of these components. Normally, a pre-polymer that has long chain length makes polymer more flexible, and higher concentration of cross linker makes it more rigid. PDMS is a silicon-based polymer, and its flexibility is derived from the length and distance among each PDMS chains, connected by cross linker. The mixing ratio of pre-polymer and curing agent also affects the rigidity. Higher concentration of curing agent leads to larger number of linking between pre-polymer and curing agent, resulting the formation of more rigid cured polymer.

The gaps between polymer chains allow liquid and solutes to draw closer in the polymer network. Even though PDMS is hydrophobic in nature, moisture and solutes are known to be absorbed by it. Manufacturer's suggested ratio of PDMS solution and curing agent is 10 : 1, respectively. In our experiments, PDMS covers with different hardness were made by varying the mixing ratios of PDMS solution and curing agent. The absorption rates of water into PDMS slabs that had various mixing ratios of PDMS polymer and curing agent were tested by a spectrophotometer (DU Series 600, Beckman, California, USA). The PDMS slabs were saturated with deionized water during a 12 hour long immersion step. The optical absorbance of the slab was scanned between 350 nm to 900 nm. Maximum change in the absorbance was observed at 590 nm. All of the consecutive measurements were performed at this wavelength. The optical absorbance of PDMS slab is changed due to an increase of the amount of water absorbed.

Five PDMS slabs were made with the ratio of 2 : 1, 2.5 : 1, 3 : 1, 7.5 : 1, 10 : 1 of PDMS solution and curing agent. The thicknesses of slabs were 1 mm. Each slab was cut into 1 cm × 2 cm size using a razor blade after peeling off from the mold, and then placed inside the cuvette, close to the opening of the light path. The opening of the cuvette was sealed with parafilm during the measurement. The absorption of liquid in six different samples was measured simultaneously. Control cuvettes were used that had PDMS slab without deionized water and deionized water without PDMS slab. Any variations in the data from the controls were subtracted from the actual data points.

The initial rates of increase of absorbance were similar in all cases. The amount of water absorbed into PDMS and the time when the absorbance reaches the maximum point $(t_{\max})$ decreased with increased content

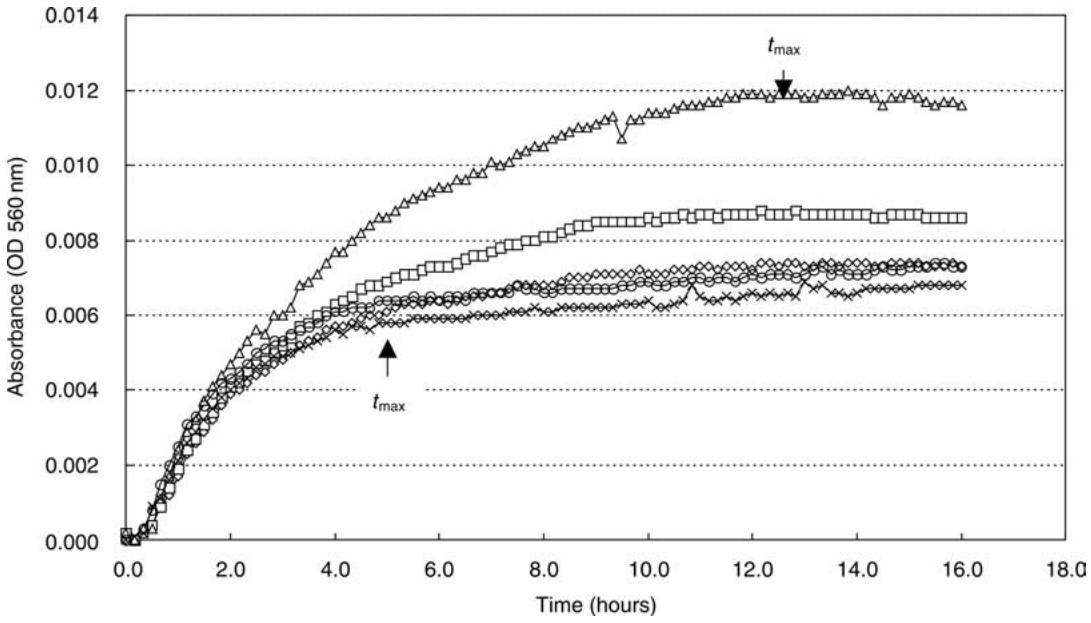

Fig. 3. *The optical absorbance of PDMS slab submerged in deionized water. The ratios of PDMS polymer and curing agent were 10:1 (△), 7.5:1 (□), 3:1 (○), 2.5:1 (◇), and 2:1 (×).*

of curing agent. The trend is clear for the 10 : 1 and 7.5 : 1 ratio while the differences among the three lower ratios were minor. As the ratio of the PDMS solution to curing agent was changed from 10 : 1 to 2 : 1, t_{max} changed from 12 hours to 5 hours. A lower t_{max} implies an earlier saturation and hence a reduction of absorption. The PDMS slab with 2 : 1 ratio of solutions showed the lowest absorption. The PDMS slab that had 2.5 : 1 ratio was chosen for further experiments, because relatively larger content of excessive curing agent in 2 : 1 mixture sometimes leads to damage on the silanized surface of mold, resulting the irreversible bonding of cured PDMS onto the mold.

3.2. Absorption of liquid in PDMS/silicon hybrid biochip

The flow path in PDMS/silicon hybrid biochip was sterilized to prevent the influence of potential bacterial contamination within the biochip on the electrical measurements of absorption. The PDMS/silicon hybrid biochip was sterilized with 70% (v/v, in deionized water) ethanol solution for more than 2 hours. The ethanol was washed out by circulation of sterilized deionized water and LB media for 1 hour and 15 minutes, respectively. Pressurized nitrogen gas was used to facilitate liquid flow. Biochips were made with different PDMS covers that have different ratios of PDMS polymer and curing agent based on the results of absorption characteristics of the PDMS slabs. The ratios of normal and harder covers were 10 : 1 and 2.5 : 1 of PDMS polymer and curing agent, respectively. Since the goal is to determine and maximize the time that the LB media would stay within the nano-liter volume chambers, electrical impedance measurements were used as a means to determine when the liquid has been absorbed from the chambers. A rapid increase of impedance reaching the air capacitance would indicate the drying of the fluid. The composition of low conductivity LB media was yeast extract (5 g/L), tryptone-peptone (10 g/L), and dextrose (3.3 g/L). The impedance at 1.1 kHz with a 50 mV A.C. signal was measured with HP4284A LCR meter (Hewlett Packard Co., California, USA).

The biochips were also tested with as-is unsaturated PDMS covers and PDMS covers saturated with the LB media. The nitrogen pressures used for unsaturated and saturated PDMS were 2 and 15 psi, respectively. Often, unwanted air-bubbles were trapped inside the channels and wells. These bubbles can be removed by blocking the output port and pressurizing the flow path, prior to the incubation. The bubbles can be removed using this technique because of the permeability of PDMS cover to air and fluid. The incubation was started immediately when the bubbles disappeared. The temperature was set at 37 °C using a micro-heater positioned under the biochip. The A.C. electrical impedance curves were measured as a function of time and results are shown in Figure 4. The impedance started to increase when the liquid in the chamber of interest (where the impedance measurement is being performed) started to be absorbed. The impedance reached the maximum threshold of $1.0 \times 10^6\,\Omega$ when all of LB media in the chamber was absorbed into PDMS cover.

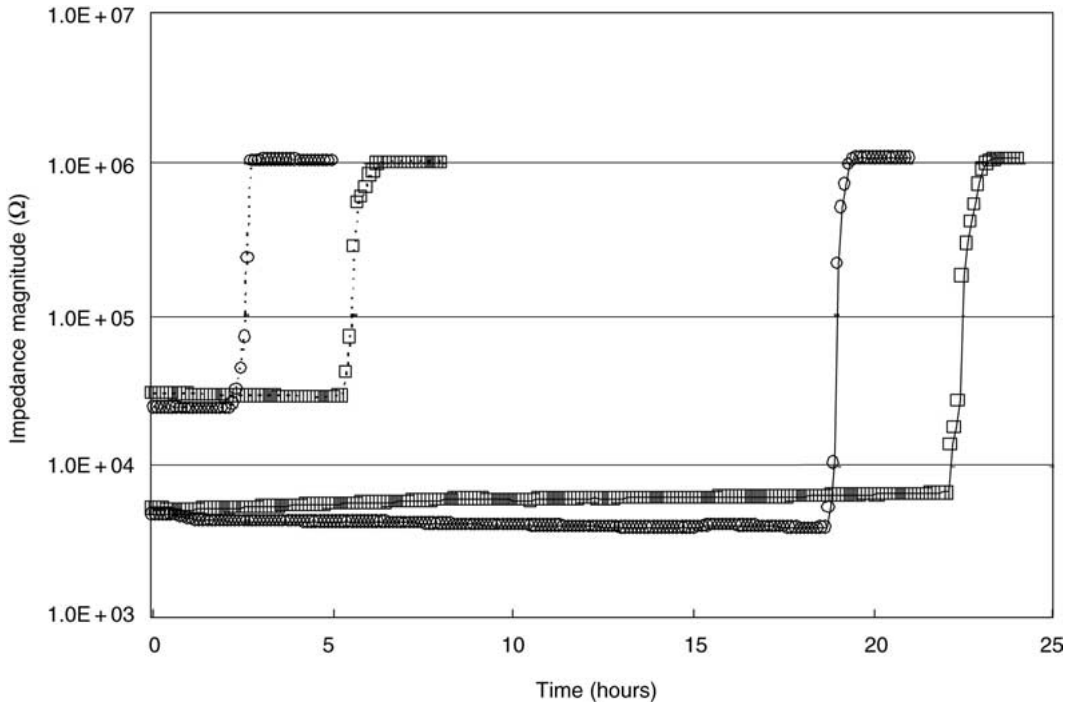

Fig. 4. *The impedance change in PDMS/silicon hybrid biochip by absorption of LB media into PDMS cover. The frequency was 1.1 KHz. PDMS covers were made of 10 : 1 (○) and 2.5:1 (□) ratio with saturated (solid line) and unsaturated (dotted line) modes before incubation started.*

As shown in Figure 4, in the case of the unsaturated PDMS cover, the complete absorption was delayed from 2.7 to 6.3 hours by the increase of the mixing ratio of curing agent from 10 : 1 to 2.5 : 1. Recalling the optical data from Figure 3, the absorption reached a maximum at around 5 hours in all PDMS slabs of ratio 2 : 1, 2.5 : 1, and 3 : 1. The reason why the absorption of unsaturated 10 : 1 completed in about 2.7 hours is actually due to the amount of liquid in 5.3 nl volume chamber. The amount of liquid was a lot less than the experiment described in Figure 3, and, hence, could be absorbed in a shorter amount of time. In the case of unsaturated 2.5 : 1 cover, the absorption was delayed up to 5 hours. This is expected to be due to the different absorption rate of moisture in the PDMS covers with different compositions. The lower concentration of PDMS polymer is expected to lead to smaller gaps among PDMS chains, larger numbers of bonds between PDMS polymers and curing agents, and a reduction of mechanical flexibility. The change of physical properties is expected in not only flexibility but also in hydrophobicity and porosity, affecting the absorption of moisture.

The largest delay in the absorption was achieved using the saturated PDMS cover. It took 19 and 23 hours for 10 : 1 and 2.5 : 1 PDMS covers, respectively, for the media to evaporate. The difference in the baseline of impedance in Figure 4 is due to chip-to-chip variation. The absorption was delayed around 20% by increasing the content of curing agent from 10 : 1 to 2.5 : 1, even the cover was saturated before incubation started. When the cover is saturated before the incubation, the limiting factor for drying is evaporation of moisture from the surface of PDMS to the outside ambient. The interaction between water molecules and PDMS chains would be changed with different content of curing agent. However, even if the evaporation rates from PDMS were same for both of the covers made of 10 : 1 and 2.5 : 1 ratios, the differences of permeability and water content of those covers are expected to be resulted in different absorption times.

3.3. Discussion on absorption in PDMS

PDMS is a well-known material for its use in separation of ethanol from aqueous solution. Diffusion of solvents through PDMS membranes has been characterized and reported, however, there are large differences among these reported data. Characteristics of solvent diffusion in PDMS were summarized and compared (Watson and Baron, 1996) and a new apparatus was suggested (Yeom et al., 1999) for a more precise measurement of permeation. The hydrophilic impurities within PDMS were claimed as the main source of large differences in permeation of liquids in PDMS. We used Sylgard 184, which is a commonly used PDMS material for microfluidic devices, however, to our knowledge there have been no reports on parameters for diffusion of liquid in this material.

The main polymer and cross linker composing the PDMS can have a large variation depending upon molecular weights and mixing ratio of polymer and cross linker, amount of additives, and etc. The absorption of fluid in PDMS is very important for cell culture applications when the solution is sustained in the micro-chambers for long time. Diffusion coefficient of water in PDMS, solubility of water in PDMS, porosity of PDMS, interaction between water and PDMS chains, effect of temperature on diffusivity, etc. are essential to obtain a fundamental understanding of absorption in PDMS based microfluidic devices, and more detailed study than what is presented here, needs to be undertaken. We present some simple analysis and explanation below.

Typically, mass transfer between different phases is described using Fick's diffusion law as $J = -D(\Delta c/\Delta z)$. In this equation, J represents the linear flux of molecule per unit area $(g/s \cdot m^2)$, which can be calculated using the diffusion coefficient $D(m^2/s)$, difference of concentration $\Delta c(g/m^3)$, and distance $\Delta z(m)$. The measured weight and calculated volume of PDMS cover used in this experiment was approximately 0.06 g and 64 mm^3, respectively. The water content of RTV 615 PDMS membrane was suggested as 0.38% (w/w) by Blume et al. (1991). PDMS cover used in this experiment would absorb around 0.228 µl of water, if similar amount of water could be absorbed as in the RTV 615 (General Electric) PDMS membrane.

Assuming the PDMS cover was completely dried before culture started, ρc between saturated and unsaturated PDMS cover used in this experiment is 3.6 $/m^3$, obtained based on the maximum content of water mentioned above. The volume of micro-chamber for cell culture was 5.3 nl, and it is only 2.3% of the amount of water that could be absorbed in the PDMS cover. The diffusion coefficient of water (25 °C) through 1.1 mm thick PS342.5 (Fluorochem Ltd., Old Glossop, UK) PDMS membrane was measured as $2.0 \times 10^{-9}\, m^2/s$ (Watson and Baron, 1996). However, the diffusion coefficient can vary significantly, an order of magnitude, with different sources of PDMS and measuring conditions. If the diffusion coefficient of Sylgard 184 is assumed to be in the range of 2.0×10^{-10} and $2.0 \times 10^{-8}\, m^2/s$, then the flux can be calculated to be from $7.1 \times 10^{-7}\, l/s \cdot m^2$ to $7.1 \times 10^{-5} \cdot m^2$. This would correspond to anywhere from 2.8 minutes to 280 minutes (4.6 hours) for the absorption of 5.3 nl of water into PDMS through 0.45 mm^2 of contact area. The contact area is simply calculated from the size of micro-chamber, 530 µm × 850 µm. Considering the drying time of LB media in unsaturated 10 : 1 PDMS cover, as shown in Figure 4 was 2.7 hours, Sylgard 184 is expected to have around five times lower diffusion coefficient than PS342.5.

The total area of our micro-channels and micro-chambers contacting the PDMS cover was 2.8 mm^2. The amount of water that can pass through 2.8 mm^2 of PDMS for 3 hours is calculated to be about 215 nl. This is close to the amount of water which saturates the PDMS cover used in this experiment, even without the consideration of the pressure that was applied for flow. Thus, PDMS cover is expected to be saturated with liquid before incubation starts, when higher pressure was used for the flow.

It should be noted that the transfer of molecules through polymer is affected by many factors, including temperature, viscosity of the liquid, characteristics of polymer, interaction between polymer and liquid, etc. The estimation performed in this experiment was extremely simplified, because of the lack of data on Sylgard 184. The LB medium also has a higher viscosity than water because of various substances dissolved in the solution. The solutes absorbed in PDMS cover can affect the permeability of liquid as well. Considering all these factors, further investigations are required to characterize the actual behavior of liquids in PDMS in order to provide a better understanding of polymer-based microfluidic devices.

4. Culture of Bacteria in the Hybrid Biochips

Listeria innocua (ATCC F4248) and GFP recombinant *Escherichia coli* (Invitrogen Life Technologies, Carlsbad, CA) were cultured in the PDMS/silicon hybrid biochips saturated with moisture. Low conductivity LB media was used for all the culture steps, from stock culture to main culture.

The *L. innocua* cells were stained with Vybrant DiI carbocyanine dye (Molecular Probes, OR) before they were introduced into the biochip. This lipophilic, carbocyanine dye diffuses laterally within the plasma membrane of the cells, resulted in staining of the entire cell. The dye is also transferred into daughter cells after proliferation, however, the concentration reduces upon each cell division. The maximum wavelength of absorption and emission of DiI dye is 549 nm and 565 nm, respectively, thus it gives orange fluorescence.

The GFP *E. coli* was transformed with pQBI T7-GFP plasmid (Quantum Biotechnologies). Ampicillin was added (50 µg/ml) in low conductivity LB media for GFP *E. coli* culture. The ampicillin resistance gene is included in GFP *E. coli* to prevent the contamination of other microorganisms and to maintain GFP gene encoded on the plasmid. The stock culture was stored in a refrigerator at 4 °C after 20 hours of culture in 37 °C

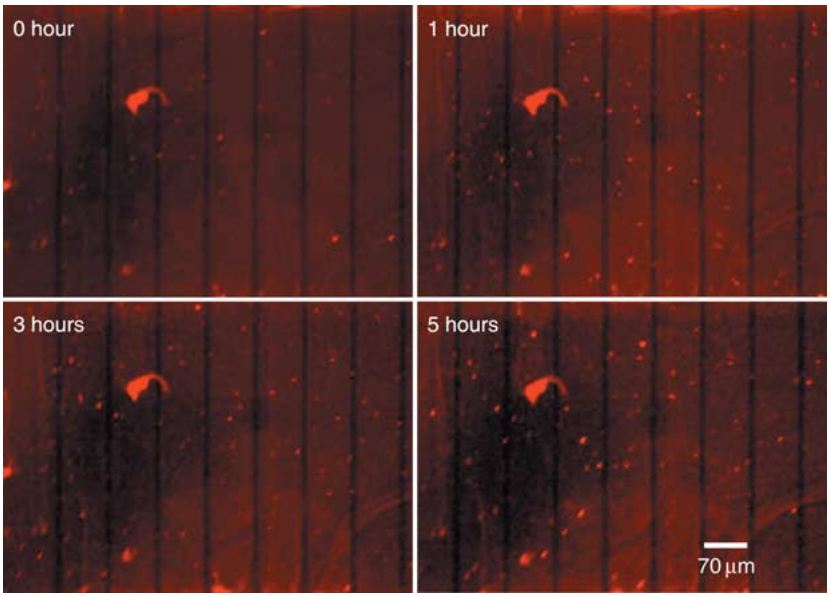

Fig. 5. *Fluorescence image of the cultivation of* Listeria innocua *in a well within the PDMS/silicon hybrid biochip.*

incubator. The cell culture solution was prepared by the following steps. The 100 µl of stock culture was inoculated into 2 ml of fresh medium as seed culture and incubated at 37 °C in static incubator without mixing. Another 100 µl of this seed culture was transferred into fresh medium again after 16 hours of growth as main culture. Actively growing cells are obtained through these two steps of cultivation by using the cells in exponential growth phases. The culture in biochip was started after injection of another diluted solution of cells prepared by addition of 100 µl of main culture solution into a new medium already set at 37 °C to avoid heat shock. Two hours of pre-culture period was given to this solution for the adaptation of cells to the new atmosphere. The images of cells grown inside the chip were taken with a Kodak digital camera (DSC240) connected to a Macintosh computer (PowerMac G4, 400 MHz) having Photoshop software.

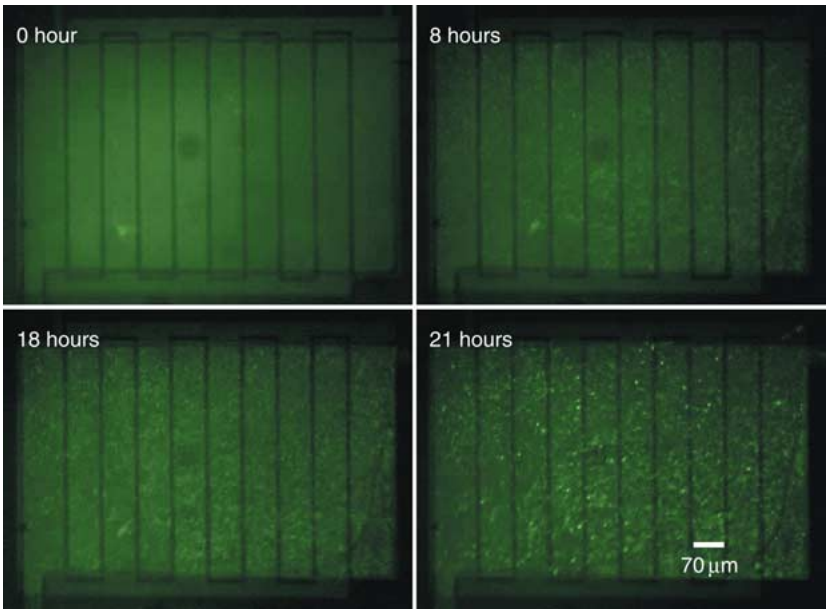

Fig. 6. *Fluorescence image of the cultivation of GFP* Escherichia coli *in a well within the PDMS/silicon hybrid biochip.*

The number of fluorescent *L. innocua* cells increased with incubation as shown in Figure 5. The brighter dots are fluorescently labeled cells in the micro-chamber. The size of cells appears different because of the differences in the intensity of fluorescence and the vertical position in the 12 μm deep micro-chambers of the biochip. However, increased number of fluorescent cells were not observed clearly after 5 hours of culture, despite the fact that the higher concentration of cells was optically confirmed by bright-field micrographs (data not shown). This was due to a loss of intensity of fluorescence during culture. The loss of fluorescence coming from not only the reduced amount of dye in each cell by proliferation, but also due to quenching of the dye. To alleviate this problem, culture of GFP *E. coli* in the chip was performed. GFP *E. coli* is expected to have a more stable fluorescence for long-term culture, comparing with fluorescence labeled microorganism. The plasmids coding green fluorescence protein are inserted in this strain. The cell gives green fluorescence when this protein is expressed and concentrated with cell growth. The pictures of the culture of GFP *E. coli* are shown in Figure 6. Since the synthesis of GFP inside the cells is a slow process, the daughter cells do not fluoresce 2–3 hours after the cell division has occurred. Thus, the number and intensity of fluorescence of cells slowly increased with cell growth. Normally, doubling time of *E. coli* is 30 minutes, and hence the concentration of cells after 20 hours of culture is thought to reach to more than 1.0×10^8 CFU/ml. The higher concentration of cells was optically confirmed. Consequently, the culture is believed to be at the stationary or death phase of growth with this concentration. There is also a possibility of the growth of non-fluorescent cells due to the loss of the plasmids because of the depletion of ampicillin in the medium. These non-fluorescent cells dominantly grow when ampicillin is depleted, because the growth rate of reversed cell is faster than the cells with the plasmids. However, considering the increase of the number of fluorescent cells and intensity of the fluorescence after 18 hours of culture, in this case, the production and accumulation of GFP is believed to be still in progress. Cell growth was observed for up to 22 hours, thus, proving the feasibility of the device for long-term batch culture of bacterial cell inside hybrid polymer-based micro-devices.

5. Conclusions

A novel PDMS/silicon hybrid microfluidic biochip was fabricated and tested for the long-term batch culture of bacterial cells. The PDMS cover that had 3-dimensional micro-channels for flow was fabricated without photo-lithographic methods. Liquid was kept in micro-chambers for up to 22 hours with the PDMS covers made of 2.5 : 1 ratio of PDMS and curing agent when they are pre-saturated with liquid before the start of the incubation. In this case, the drying was delayed by more than ten times longer than biochip made of unsaturated PDMS cover composed of 10 : 1 ratio of PDMS and curing agent. Electrical impedance measurements were used to determine the onset of drying inside the micro-chambers. Batch culture of *L. innocua* and *E. coli* were performed in this PDMS/silicon hybrid biochip, demonstrating the possible utility of these devices in a wide variety of biomedical sensing, diagnostics, drug screening and biotechnological applications.

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