DNA transformation via local heat shock

Sha Li
Berkeley Sensor and Actuator Center, Department of Mechanical Engineering, University of California at Berkeley, Berkeley, California, 94720

L. Meadow Anderson
Department of Chemistry, University of California at Berkeley, Berkeley, California, 94720

Jui-Ming Yang[a] and Liwei Lin
Berkeley Sensor and Actuator Center, Department of Mechanical Engineering, University of California at Berkeley, Berkeley California, 94720

Haw Yang
Department of Chemistry, University of California at Berkeley, Berkeley California, 94720

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This work describes transformation of foreign DNA into bacterial host cells by local heat shock using a microfluidic system with on-chip, built-in platinum heaters. Plasmid DNA encoding ampicillin resistance and a fluorescent protein can be effectively transformed into the DH5α chemically competent *E. coli* using this device. Results further demonstrate that only one-thousandth of volume is required to obtain transformation efficiencies as good as or better than conventional practices. As such, this work complements other lab-on-a-chip technologies for potential gene cloning/therapy and protein expression applications. © *2007 American Institute of Physics*. [DOI: 10.1063/1.2754648]

DNA transformation is a process by which foreign DNA is taken up by a host cell and the genes encoded on it are expressed.[1] It is an indispensable procedure in modern research, used in disciplines ranging from molecular and cell biology to microbial engineering. For bacterial transformation, the foreign DNA must penetrate the protective membrane so that the intracellular machinery can decode the DNA sequence and express the desired biomolecules.[2] The current methods to increase the permeability of the cell membrane to facilitate such a process include electroporation using an externally applied electrical field[3,4] and heat shock by a rapid increase in temperature.[5,6] Like many other biochemical procedures that have been implemented on the microscale using the microelectromechanical system (MEMS) technologies, on-chip DNA transformation could help advance areas in microfluidics systems integration and low-cost, high-volume parallel processing.[7–9] Recently, on-chip electroporation has been demonstrated.[10–12] For bacterial transformation, however, exposing cells to the high voltages required for successful electroporation may be undesirable because of the high cell mortality rate at these voltages.[13] Heat shock, on the other hand, is a milder procedure and may serve as a viable alternative. The feasibility of carrying out this method of DNA transformation on the chip level, however, has not been examined. This work demonstrates on-chip DNA transformation of *E. coli* cells with local heat shock, complementing other efforts for miniaturization of instrumentation.

It has been shown that both the temperature and the duration of the heat shock step affect the final transformation efficiency significantly.[14] Figure 1(a) illustrates schematically how the local heat shock compares with a conventional heat shock process. Typically 100 μl of the transformation mixture is used in bulk heat shock while the local heat shock experiments demonstrated in this work have a volume of about 0.1 μl, three orders of magnitude smaller. Commercially prepared competent cells are expensive, so using smaller quantities of sample can significantly reduce costs. In conventional bulk heat shock, the sample is heated by immersing the tube into a 42 °C water bath, whereas the heating of local heat shock is provided by resistive Joule heating from a patterned microheater at the bottom. Resistive joule heating is used to raise the temperature of a $1200\times1480\times50$ μm^3 microchamber from 0 to 50 °C within 5 s and maintain at this temperature for a period of 90 s. Because the

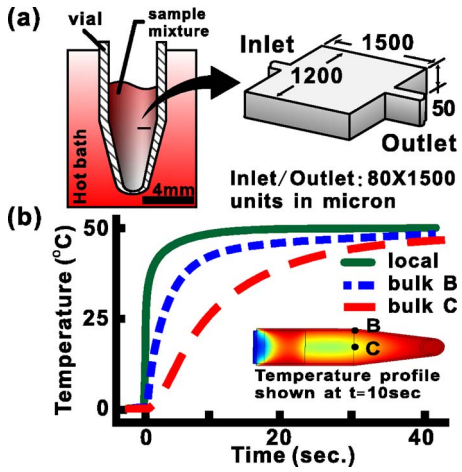

FIG. 1. (Color online) Schematic diagram of macro- and local heat shock. (a) The working volume of the local heat shock device is a very small portion of that of the conventional one. The black line inside the vial represents the volume used in local heat shock experiment. (b) Local heat shock produces fast and uniform heating profile as compared with that of conventional heat shock in the first 40 s out of a total, 90 s heating process. Inset shows the simulated temperature distribution of the sample mixture in vial 10 s after heat shock treatment.

[a]Electronic mail: juimingy@berkeley.edu

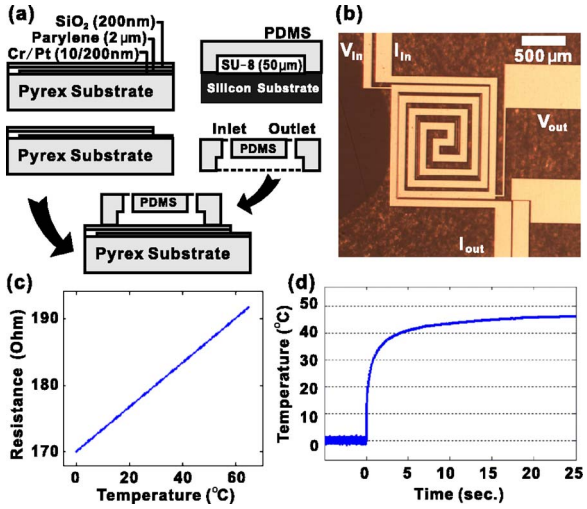

FIG. 2. (Color online) On-chip local heat shock device and its temperature characterization. (a) Fabrication process of the on-chip local heat shock device. (b) Optical image of a fabricated microheater with double-spiral design. Two pairs of electrodes, V_{in}/V_{out} and V_{in}/V_{out}, are used for four wire measurement. (c) Resistance of the microheater as a linear function of temperature. (d) Temperature profile of the local heat shock process in the first 25 s. The noise level before 0 s serves as a guide for the measurement uncertainties.

thickness of the local heat shock chamber is only 50 μm, temperature in the chamber responds rapidly to the adjustable heating power. On the other hand, thermal simulations using finite element software show a significant time delay for the bulk heat shock experiment, as illustrated in Fig. 1(b). Locations B and C are sampled at the boundary and center of the polypropylene tube as shown.

Experimentally, two key elements are used in DNA transformation by heat shock:[15] (1) chemically competent bacterial cells, which have been treated with $CaCl_2$ to make the cell membrane more permeable; and (2) recombinant plasmid DNA, a circular DNA with the target gene to be transformed inside the cells. Plasmids typically encode an antibiotic resistance gene for selection purposes; cells with transformed DNA can survive under antibiotic treatment, but host cells that have not taken up the plasmid are not able to grow and divide.

In a typical bulk heat shock experiment, a 100 μl mixture of competent cells and the recombinant plasmid are mixed at 0 °C for 30 min. Afterwards, the mixture is treated by a heat pulse in a 42 °C bath for 90 s, and cooled on ice for 2 min. After adding cell culturing medium, LB (Luria-Bertani), the solution is placed in a 37 °C incubator for 45 min to allow expression of antibiotic resistance genes. The resulting culture is then spread on LB agar plates containing the appropriate antibiotic—in this case, ampicillin—and incubated at 37 °C for 10–20 h to allow colonies to grow from transformed cells. Colonies able to form in the presence of antibiotics are counted as instances of successful plasmid DNA transformation.

The on-chip local heat shock device was fabricated by micromachining processes. Figure 2(a) illustrates the two major parts of the MEMS heat shock device, a fluidic cap using molded polydimethylsiloxane (PDMS) to construct the microchamber along with channels and a heating substrate of resistive microheaters covered with an electrical insulation layer. First, 200-nm-thick platinum heaters were deposited using the lift-off process on a Pyrex substrate with a 10-nm-thick chromium adhesion layer. Platinum was chosen for its chemical inertness and its linear resistance-temperature relationship over a wide range of temperature. A double-spiral heater design with four measurement wires was used in the prototype device. Afterwards, Kapton (polyimide) tape was used to cover the contact pads to protect them from being coated by the insulation layer; this way, an etching step to open contact areas could be omitted. A 2-μm-thick parylene C film was deposited, followed by coating a 200-nm-thick plasma-enhanced chemical vapor deposit SiO_2 as an insulation layer to facilitate the bonding with the PDMS cap. Afterwards, the Kapton tapes were peeled off to expose the contact pads, in which any remaining tape residue was removed with acetone. The PDMS cap was fabricated by spinning and patterning a 50-μm-thick SU-8 layer on a bare silicon wafer as the mold structure. The PDMS cap was later cast to construct the microchamber and channels and the inlet and outlet holes were punched open mechanically. The PDMS cap and the microheater on Pyrex were finally aligned and bonded together using a 40-s, 40 W oxygen plasma treatment on the two complementary interfaces. Figure 2(b) shows a fabricated double-spiral platinum microheater on the Pyrex substrate. Fabricated chamber and channel sizes were $1200\times1480\times50$ and $80\times1500\times50$ μm^3, respectively. The thickness of the microchamber, 50 μm, was much smaller than the size of the commonly used biological test tubes. Therefore, the heat transfer process inside the sample solution is faster than that of the conventional process. The platinum microheater also served as an on-chip thermometer to measure the chamber temperature using the excellent responses of temperature coefficient of resistance of platinum. The four-wire measurement scheme is designed to have two pairs of current and voltage electrodes, namely, I_{in}/I_{out} and V_{in}/V_{out}, as shown in Fig. 2(b), to eliminate errors from contact resistances. The resistance of the microheater was measured and calibrated using external thermocouple, as shown in Fig. 2(c), with excellent linearity between 0–65 °C. Using this four-wire measurement scheme, the measurement error of the temperature profile was less than 0.03 °C.

All competent cells were prepared using the Inoue method[16] and stored at −80 °C. The local heat shock device was first sterilized with absolute ethanol and placed on ice to cool to 0 °C for cell incubation. A 100 μl aliquot of frozen DH5α chemically competent *E. coli* was thawed on ice and mixed with 0.5 μl of 240 μg/ml ice-chilled plasmid DNA, which contained the Venus (a green fluorescent protein) gene[17] and an ampicillin resistance gene. This mixture was incubated on ice for 30 min in a 500 μl polypropylene microcentrifuge tube. Afterwards, 0.3 μl of the mixture was directly loaded into the inlet of the ice-chilled device with a sterilized micropipette. Only about 0.1 μl of mixture filled up the microchamber with the remaining 0.2 μl spreading into the input/output ports and channels. The local heat shock was conducted by continuously applying constant current (21.2 mA) to the microheater to heat the microchamber to 50 °C for 90 sec. The heating profile of the initial 25 s is shown in Fig. 2(d), which is a rapid response compared to the macroheat shock setup as illustrated in Fig. 1(b). Afterwards, the device was cooled on ice for two more minutes and the cell sample was washed out by 300 μl LB culture medium. For comparison purposes, a conventional heat shock experiment was also conducted using the same batch of cell and DNA mixture. After the 30 min incubation on ice,

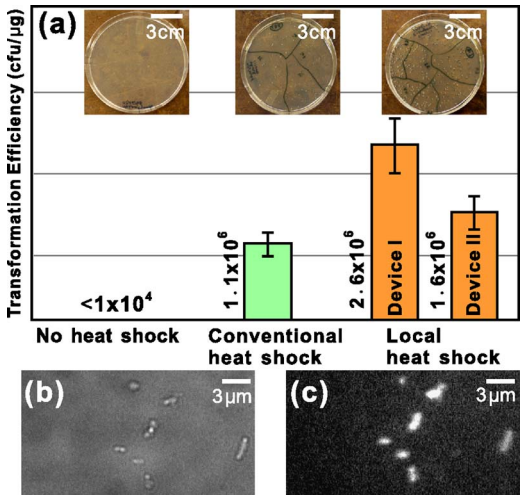

FIG. 3. (Color online) DNA transformation by heat shock. (a) The transformation efficiency of *E. coli* cells: (left) without heat shock, (center) with conventional heat shock, and (right) with local heat shock. Insets: corresponding bacterial cultures, where white dots represent colonies. (b) Bright-field and (c) fluorescence mode pictures of *E. coli* cells after local heat shock of the plasmid DNA with Venus gene. Error bars were calculated according to Poisson statistics as the square root of the plate counts.

the tube containing the transformation mixture was transferred to a 42 °C water bath for 90 s, then the tube was returned to cool on ice for 2 min. After cooling, a 0.3 μl volume was withdrawn from the 100 μl heat shocked sample and transferred to 300 μl of LB medium for recovery. Both conventional and local heat shock samples were cultured in a 37 °C incubator for 45 min before they were diluted tenfold with LB medium. Then 100 μl of each dilution was spread and cultured on an LB agar plate that contained 50 μg/ml ampicillin to prevent the growth of nontransformed cells. The transformation efficiencies of each method were determined by counting the colonies present on the antibiotic selective plate after 18 h of incubation at 37 °C.

These experiments were conducted using two devices of the same design that were fabricated to examine the feasibility of the approach. Figure 3(a) compares the transformation efficiency of the conventional and local heat shock experiments. Transformation efficiency is defined as the number of colony-forming units produced per microgram of plasmid DNA. Each colony on the LB ampicillin plate grew from a single cell in the original mixture and represents a bacterial cell that was transformed with plasmid DNA and became antibiotic resistant. Control experiments showed that no transformation was detected without the heat shock procedure. Experimental results on LB ampicillin plates are also shown after incubation for each corresponding experiment. Each small dot on the plate is a cell colony grown from a transformed cell. Since the inserted plasmid DNA carries the Venus gene, the effectiveness of the DNA transformation and protein expression was further verified by observing the expressed green fluorescent protein in transformed cells using a fluorescence microscope. Cells from colonies grown after the local heat shock were selected at random and examined using a 100× oil-immersion objective with a mercury lamp as the excitation light source and the Endow green fluorescent protein (GFP) filter set (Chroma Technology, Rockingham, VT). Figure 3(b) shows the bright field picture of the sample while Fig. 3(c) is the fluorescent picture of the same field of view. All the cells from the selective plates that were observed expressed Venus, confirming successful transformation with plasmid DNA.

The two microdevices achieved transformation efficiencies that were comparable to or greater than the conventional heat shock approach. It has been reported that the larger the volume of the transformation mix, the longer the time that is needed to achieve the optimal transformation efficiency.[18] This indicates that the optimal heat pulse time for the microfluidic local heat shock chip could possibly be much shorter than the one used in our experiment, which has been optimized for the macroscale protocol. We believe an optimization of parameters for the local heat shock chip can increase the transformation efficiency reported here.

A local heat shock device with an embedded microresistive heater has been implemented to demonstrate DNA transformation experiments on competent *E. coli* cells. Using only a small volume of sample, it achieves reasonable transformation efficiencies and has good potential for further optimization. The device presented here serves as a module that can be integrated into a larger lab-on-a-chip device to streamline the processes in gene cloning, which would be useful for increasing sensitivity, lowering costs, and allowing higher throughput for genetic engineering processes.

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