On-Chip Transformation of Bacteria

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On-chip transformation of *Escherichia coli* cells was accomplished for the first time using a microbial array chip. The continuous *E. coli* transformation procedures were performed on a chip in which the microcompartment was composed of PDMS microfluidic channels and a silicon substrate predeposited with different plasmid DNAs. The PDMS microfluidic device enabled the parallel transformation of *E. coli* cells with various plasmid DNAs by separating each transformation area. The phenotypic differences reflecting different plasmid DNAs were identified by various approaches such as colorimetry, fluorometry, and electrochemical methods. This microbial array chip could become a versatile tool for many cell biological applications.

The availability of complete genome sequences has accelerated the study of gene functions on a genomewide scale. DNA microarrays have enabled the survey of the intracellular gene network by comparing genomewide transcriptional profiling in response to environmental stimuli. While this method provides discontinuous results of gene expression changes, it is not ideal for investigating time-dependent behavior. Since genetic information encoded in the genome is ultimately manifested at the cellular level, global approaches for analyzing cell phenotypes would facilitate our understanding of cellular functions of genes. The currently developed cell-based microarrays enable comprehensive analysis of gene functions on a genomewide scale from phenotypes of living cells. In 2000, Fujita et al. suggested a new cell microarray for the on-chip DNA transfection of living cells. The living cells were captured in microchamber arrays, and the local electroporation of the cells was performed to introduce plasmid DNA delivered though the microcapillary fabricated bottom of the microchamber. While this is an advanced concept for high-throughput analysis of cell microarrays, this process requires molecular biology techniques and long preparation times.

In this paper, we report the first study of the fabrication and characterization of a microbial array chip composed of PDMS channels and a silicon substrate, simultaneous realization of the introduction of plasmid DNA into a bacterial host, and the monitoring of gene expression in the phenotypes of bacteria during the transformation process.

MATERIALS AND METHODS

Bacterial Strains and Plasmid DNAs. *Escherichia coli* JM109 competent cells were purchased from Nippon Gene. Two types of plasmid DNA, a cloning vector pUC19 (Nippon Gene) and pUC-GFP, were used (Figure 1).

pUC-GFP was constructed by inserting the fragment of the *gfp* gene from pQBI T7 GFP (Nippon Gene) into the restriction site of pUC19 located within the *lacZ* gene sequence. A fragment containing the Shine–Dalgarno sequence and the *gfp* gene was PCR amplified from pQBI T7 GFP. *EcoRI* and *HindIII* restriction sites were inserted at the ends during amplification. The following primers were used for the PCR: (1) 5′-GAATTCTTAGCAGCCG-GATCCTCAGTT-3′; (2) 5′-AAGCTTATTCTTGTGAATTACC-3′.

The underlined bases encode for the restriction enzyme cleavage site, *EcoRI* in (1) and *HindIII* in (2). The PCR product was cloned between *EcoRI* and *HindIII* restriction sites of the plasmid pUC19 to yield the plasmid pUC-GFP. The new plasmid

**REFERENCES**

contains the \textit{amp}R gene that confers resistance to ampicillin, the \textit{gfp} gene with the regulatory system lacP O/P region (Figure 1).

Restriction digestion analysis with \textit{EcoRI} and \textit{HindIII} enzymes followed by a 0.8% agarose gel electrophoresis was employed to confirm the presence of plasmid pUC-GFP. Plasmid pUC-GFP was dissolved in Tris-EDTA (pH 8.0) and stored at −30 °C until further use.

**Fabrication of the Pyramidal Hole Arrays on the Silicon Wafer.** A (100) silicon wafer (230 μm thick; optically polished on both sides) was immersed into the piranha solution consisting of a 3:1 mixture of 30% H2O2 and H2SO4 for 2 h to clean the surface. After that, the silicon wafer was oxidized in a wet oxygen atmosphere at 1000 °C for 10 h to produce a 1.5-μm-thick oxide layer. Quadratic windows (400 × 400 μm²) were photolithographically patterned in the oxide layer to define the large openings of the microhole. Using anisotropic etching in 25% (CH3)4NOH at 80 °C, pyramidal holes were etched into the wafer. The resulting small openings of the pyramidal holes had an area of 100 × 100 μm². The silicon wafer was then immersed into the HF/NH4F solution (50 wt % HF:50 wt % NH4F = 12:100) to remove the oxide layer and then washed with distilled water.

**Fabrication of the Poly(dimethylsiloxane) (PDMS) Microfluidic Channels.** PDMS microfluidic channels were fabricated by curing the prepolymer on glass substrate with a master. The master was photolithographically patterned using SU-8 negative photoresist (Micro Chem). The master had four parallel protruding features (length 5.0 mm; width 500 μm; height 25 μm), which resulted in PDMS replicas with the opposite sense. A 10:1 mixture of 30% H2O2 and H2SO4 for 2 h to clean the surface.

**Detection of GFP expressed from plasmid pUC-GFP was performed using fluorescence microscopy.**}

**Colorimetric Detection of β-Galactosidase Activity with X-Gal.** 5-Bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal) was purchased from Wako Pure Chemicals and used without further purification. After incubation with SOC medium, the microbial array chip was immersed into the medium solution containing 1.0 mg/mL X-Gal, 1.0% dimethyl sulfoxide, 1.0 mM IPTG, and 50 μg/mL PAPG. The microbial chip was incubated at 37 °C for 16 h and further purification. After incubation with SOC medium, the microbial array chip was immersed into the medium solution containing 1.0 mg/mL X-Gal, 1.0% dimethyl sulfoxide, 1.0 mM IPTG, and 50 μg/mL PAPG. The microbial chip was incubated at 37 °C for 16 h. Following this, optical microscopy revealed that the colony color changed to blue, indicating β-Gal expression.

**SECM Measurement of β-Galactosidase Expression in Transformed \textit{E. coli} Cells.** Amperometric measurement of β-Gal expression was conducted using scanning electrochemical microscopy (SECM). A Pt microdisk electrode (Pt radius, 10 μm) was used as a probe. The potential of the microelectrode tip was maintained at 0.30 V versus Ag/AgCl to detect the p-aminophenol produced by the β-aminopropionyl β-D-galactopyranoside hydrolysis reaction of intracellular β-Gal. The time required for imaging an area of 2000 × 600 μm² (spatial resolution, 30 μm) was ~30 min. The SECM measurements were performed in a PBS (−) buffer solution containing 2.0 mg/mL PAPG.

**Detection of GFP Expression with Fluorescent Imaging.** Detection of GFP expressed from plasmid pUC-GFP was performed using fluorescence microscopy. \textit{E. coli} cells immobilized on the silicon wafer were excited by irradiation with light from a
RESULTS AND DISCUSSION

Figure 3 shows a micrograph of the blue and red coloring stream in PDMS microfluidic channels attached on the slide glass (A) and pyramidal hole arrays on the silicon wafer filled with each color solution using PDMS microfluidic channels (B). By utilizing this PDMS microfluidic device, parallel transformation of *E. coli* with various plasmid DNAs was accomplished.

Figure 4 shows a micrograph of the microbial array chip demonstrating the on-chip transformation of *E. coli* with pUC19. The transformed *E. coli* cells were identified by colorimetric measurement using X-Gal, a chromogenic substrate of β-Gal. All of the arrayed *E. coli* cells expressed β-Gal as indicated by the blue staining, suggesting that on-chip transformation of *E. coli* was conducted within the microcompartment arrays.

Next, the simultaneous transformation of *E. coli* cells with two different plasmids, pUC19 and pUC-GFP, was performed. Identification of the transformed *E. coli* was confirmed by GFP fluorescence measurement expressed from pUC-GFP and SECM measurement of β-Gal activity expressed from pUC19. Figure 5 shows a micrograph of the microbial array chip with two spots of *E. coli* transformed with pUC19 (right spot) and pUC-GFP (left spot), along with a fluorescent and an SECM image of the same areas. No GFP expression was detected in *E. coli* cells transformed with pUC19, whereas a high current response reflecting β-Gal expression was observed. On the other hand, *E. coli* transformed with pUC-GFP lost β-Gal activity attributed to the insertion of the *gfp* gene into the *lacZ* gene of pUC19. The microcompartment composed of the silicon substrate and PDMS microfluidic channel prevents cross-contamination between cells and plasmid DNA. The phenotypic differences caused by different plasmid DNAs can be clearly identified from the fluorescent and SECM images. Since known plasmid DNAs are immobilized on defined areas, it is possible to directly identify the gene functions of interest from the phenotypes.

In the reverse transfection method proposed by Ziauddin and Sabatini, the cells must be attached to the plasmid DNA spots to take them up as plasmid DNAs were immobilized on the glass substrate with gelatin. On the contrary, our method has a wider...
application in the introduction of DNA into the swarming cells such as E. coli or nonadherent planktonic cells, because the gene introduction was performed in the microcompartment filled with collagen solution.

The transformation efficiency on the chip was quite different between pUC19 and pUC-GFP despite the same transformation protocol. The transformation efficiency means the ratio of the transformed E. coli spots to all of the E. coli spots on the chip, suggested by blue staining or green fluorescence. When pUC19 was used, more than 80% of the E. coli spots were transformed reproducibly. On the other hand, in case of pUC-GFP, less than 10% of the E. coli spots were transformed. This difference in transformation efficiency may be attributed to differences in plasmid DNA length or structure.

As Ziauddin and Sabatini pointed out, each plasmid DNA introduced into host cells must be purified and immobilized on a certain address. This process still requires many types of plasmid DNA expression constructs. Furthermore, the colony formed in each pyramidal hole is not always derived from a single E. coli cell, resulting in the formation of a heterogeneous colony. The conventional agar plating technique provides colonies obtained from a single cell, resulting in the isolation of a specific clone. This problem can be overcome by estimating the E. coli cell concentration in the pyramidal hole from transformation efficiency to transform a single E. coli cell per well, without modifying the device itself.

On-chip transformation could become a fundamental technique for analyzing the gene function on a genomewide scale in bacterial cells. The microbial array chip can also be applied to microbial cell-based protein arrays by constructing various recombinant protein expression systems using microbial hosts on the chip. In our previous work, electrochemical mutagen screening was performed by recombinant Salmonella typhimurium immobilized on the chip. On-chip construction of recombinant bacterial sensor arrays enables facultative modification of bacterial specificity for analytes.

CONCLUSION

On-chip transformation of E. coli cells with microbial array chip was developed. Continuous E. coli transformation procedures were performed within the compartment composed of PDMS microfluidic channels and the silicon wafer immobilized with different plasmid DNAs. Simultaneous transformation of E. coli cells with two different plasmids, pUC19 and pUC-GFP, was performed. The phenotype differences caused by different plasmid DNAs can be clearly identified from the fluorescent and SECM images. This microbial array chip has various applications for cell-based protein array and recombinant microbial biosensor chip.

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