ELECTRO-ACTIVE MICROWELL ARRAY FOR TRAPPING AND LYSING SINGLE ESCHERICHIA COLI CELLS

Soo Hyeon Kim[1], Takatoki Yamamoto[2], Yannick Rondelez[3], Dominique Fourmy[3] and Teruo Fujii[1,3]

[1]*Institute of Industrial Science, The University of Tokyo, Tokyo, JAPAN*
[2]*Department of Mechanical and Control Engineering, Tokyo Institute of Technology, Tokyo, JAPAN*
[3]*LIMMS/CNRS-IIS, The University of Tokyo, Tokyo, JAPAN*

ABSTRACT

We propose a device to electrically trap and lyse single bacterial cells in an array format for high-throughput analysis. The applied electric field is highly deformed and concentrated toward the inside of the microwell structure patterned on the plane electrode, which effectively generates dielectrophoresis (DEP) to attract a single cell to a nearby microchamber. We sucsessfully trapped single cells (*Escherichia coli*) into the microwell array by DEP and lysed the trapped cells by applying high electric pulse.

KEYWORDS: cell trapping, cell lyse, single cell array, *Escherichia coli*, dielectrophoresis

INTRODUCTION

Single-cell manipulation and analysis hold great promise for studying diverse biological functions. For instance single-cell analysis of gene expression, transcriptome, proteome is of great importance, but impossible by conventional bulk assays [1]. Recently, cell-patterning methods have been developed for single-cell arrays using chemical surface treatment, hydrodynamic force, gravity and electric force. Using the electric force for cell-pattering has several advantages such as high sensitivity, stable trapping and short response time. However, for individual cell operation, the trapping electrode should be discretely patterned [2]. Here, we propose a single *E. coli* cell trapping and lysing device by using electro-active microwell array, which does not need electrode patterning, for high-throughput individual cell analysis.

THEORY

The design of the device takes advantage of a size effect of a miniaturized cell-trapping well located in a relatively larger space as shown in Figure 1. In this geometry, an applied electric field between the top and the bottom boundary is highly deformed and concentrated around the microwell, which effectively generates dielectrophoresis (DEP) to attract a single cell to a nearby microchamber. The key to successful traping is the shape of the microwell edge. As shown in the Figure 1(a), the applied electric field is mostly concentrated at the edge of the microwell in the case of microwell having sharp edges [3]. On the contrary, in the case of the one

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having rounded edges, the electric field is concentrated at the inside of microwell rather than its edges as shown in the Figure 1(b).

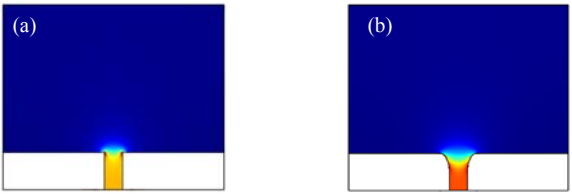

Figure 1. Finite element method simulation for (a) sharp edges and (b) rounded edges. Rounded one shows the highly localized electric field in the microwell structure when the electric field is applied between top and bottom surfaces. Color scheme shows the strength of gradient of electric field from blue (lowest) to red (highest). White areas represent the chamber structure.

Because the size of the microwell is designed to fit the size of *E. coli* cell, no more cell is trapped if a microwell has already occupied by one cell. The previously trapped *E. coli* physically excludes the second one (no more space), but also weakens the electric field by the cell body itself (no more attraction).

EXPERIMENTAL

The microwell array was fabricated on an indium tin oxide (ITO) coated glass using a positive photoresist (Microposit S1813). The thickness of the well structure was 2 μm and the diameter was 2 or 3 μm. The solution containing *E. coli* labelled with SYBR green was dropped on the microwell array, then it was covered by ITO coated cover-glass as shown in Figure 2. Because of the capillary force, the cover glass floats on the solution. The distance between the top and the bottom electrodes is about 100 μm.

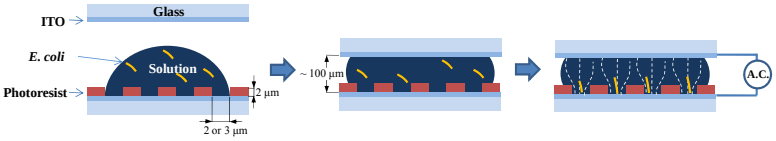

Figure 2. Schematic image of the single-cell trapping and lysing device.

RESULTS AND DISCUSSION

Figure 3(a) shows the trapped cells inside the microwell array by applying 10 Vp-p at 1 MHz AC voltage to the electrodes. Trapped *E. coli*, which has a cylindrical shape, are detected as fluorescent circles due to their orientation with their long axis parallel to the electric field lines. Figure 3(b) displays time-lapse images of a single *E. coli* trapped in the microwell. Sometimes, two cells were trapped in 3 μm diameter microwell, whereas only a single cell was trapped in a 2 μm diameter microwells. In order to lyse *E. coli* cells, we applied 30 Vp-p at 1 MHz AC voltage to the electrode. The applied electric field strength (about 3kV / cm) is higher than the threshold field strength for the *E. coli* lysis (about 1kV / cm) [4].

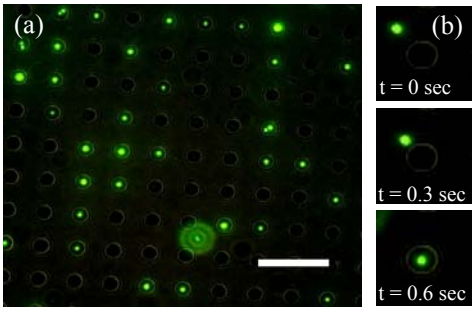

Figure 3. (a) Trapped E. coli array in 3 μm diameter microwells and (b) time lapse images during single E. coli trapping. Scale bar is 10μm.

As shown in Figure 4, the fluorescent intensity of individual cells decreased rapidly with time. It likely represents leakage of the cytoplasmic material after cell lysis.

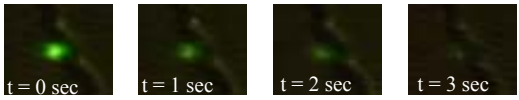

Figure 4. Time lapse image during single E. coli lysis

CONCLUSIONS

We developed a device for electrical trapping and lysing of single bacterial cells in an array format. This device can be used for high-throughput analyses of intracellular material of single cells, for example, gene expression, transcriptome and cellular proteome analyses.

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