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A microfluidic device for physical trapping and electrical lysis of bacterial cells

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In this letter, we report a simple microfluidic device that integrates the capture of bacterial cells using a microscale bead array and the rapid electrical lysis for release of intracellular materials. We study the retention of *Escherichia coli* cells with different concentrations in this type of bead array and the optimal electrical parameters for the electroporative release of intracellular proteins. Our design provides a simple solution to the extraction of intracellular materials from a bacterial cell population based entirely on physical methods without applying chemical or biological reagents.

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Due to powerful amplification techniques such as polymerase chain reaction and detection techniques such as laser-induced fluorescence, the analysis of intracellular nucleic acids and proteins at the single cell level has become routine for mammalian cells.^{1–6} However, the analysis of intracellular materials from bacterial cells, especially proteins, still has to be conducted based on a fairly large number of cells. For example, a single *Escherichia coli* cell has a length of 2 μm and a diameter of 0.8 μm which yield a volume of ~ 1 fl.⁷ This volume is about three orders of magnitude smaller than that of a typical eukaryotic somatic cell. Analysis of intracellular proteins from bacterial cells typically requires the gathering of a substantial number of cells in a relative small volume to generate the desired concentration needed for analysis. Effective concentration strategies can save valuable time spent on growing cells.

In this work, we demonstrate a simple microfluidic device that can serve as a basic unit operation for physical trapping of bacterial cells and electrical releasing of intracellular proteins. Microscale silica beads (~ 4.8 μm in the diameter) are packed in a microfluidic channel and the array of beads provides microscale matrix that filters *E. coli* cells in the solution. The packing and release of the microscale beads are achieved using a mechanical valve fabricated by multilayer soft lithography.^{8,9} Subsequent electrical pulses rapidly lyse the cells and release intracellular proteins from the cells. The green fluorescent protein (GFP) released by GFP expressing *E. coli* cells is detected using laser-induced fluorescence in the downstream of the channel. In our approach, both capturing and lysis of cells are carried out based on physical methods without introducing chemical or biological reagents. This is advantageous for analysis of bacterial proteomics by eliminating potential outside interference.

We created an array of microscale beads in a microfluidic channel by pinching the Polydimethylsiloxane (PDMS) channel using a pneumatic valve made by multilayer soft lithography.^{8,9} The depth of the fluidic channel was ~ 12 μm and the depth of the control channels was ~ 58 μm . The fluidic layer had a thickness of ~ 93 μm and the control layer had a thickness of ~ 5 mm. When we made the master

for the fluidic layer, we intentionally avoided the reflow of the photoresist under high temperature. Such reflow was critical for producing the round cross-sectional profile of the fluidic channel so that the complete closure of the channel by such a valve is possible.^{8,9} In our application, we had only the center part of the PDMS membrane in contact with the glass bottom when we actuated the valve due to the square cross section of the fluidic channel (as shown in the inset image of Fig. 1). A pressure regulator with a pressure meter was used to adjust the pressure in order to retain the beads by partially closing the channel. The partial closure of the valve allowed the flow of the fluid and the retention of the beads simultaneously. When the valve was pinched under a pressure of 16 psi, the beads with 4.8 μm diameter were retained by the partially closed valve with a flow rate of 1 $\mu\text{l}/\text{min}$ in the channel and accumulated to form a column. Due to the depth and the cross section of the channel ($\sim 200 \times 12$ μm^2), two to three layers of the beads were packed in the channel. Assuming the beads are closely packed, the gaps between the beads (potentially allowing the passage of spheres with diameters of 0.75–1.25 μm , depending on the fashion that the beads are packed) would allow possible passage of *E. coli* cells (averagely 0.8 μm in the diameter of the rod shape) with difficulty created by the trapping in the gaps.

We tested the capture of *E. coli* cells from a solution with a density of $\sim 1 \times 10^3$ cells/ml (diluted by 10^6 times from the original culture broth) at a flow rate of 1 $\mu\text{l}/\text{min}$ through the channel. GFP expressing *E. coli* was used to facilitate observation. As shown in Fig. 2(a), we observed that the cells were all captured by the bead array. The cells penetrated deeper into the array as time progressed and the cells were distributed through the array with a fairly uniform density. With the low concentration of the cells in the bead array, the number of the fluorescent spots roughly corresponded to the number of cells. The plot of the number of the cells over time [Fig. 2(b)] indicates that the capture of the cells was close to 100%. This result also confirmed that the focal volume covered the entire depth of the bead array and all the cells at different layers of beads were observed using our approach (a $10\times$ objective was used for the imaging). The clustering of cells was rare and the bypass of cells through the bead array can be prevented by increasing the bead bed length in principle.

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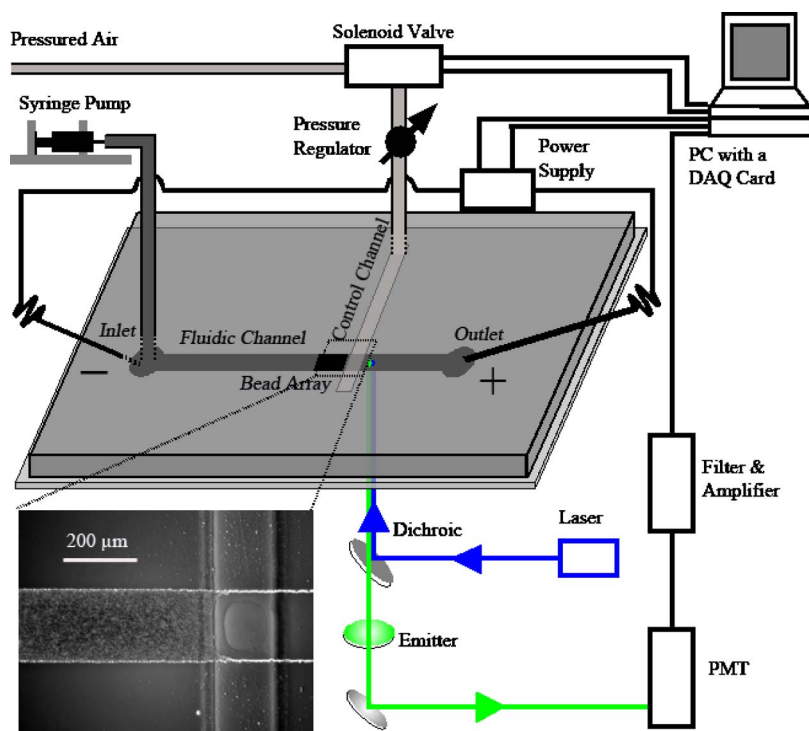


FIG. 1. (Color online) A schematic of the microfluidic device for intracellular analysis of bacterial cells. The most important parts of the device included a micro-scale bead array and a two-layer pneumatic valve that formed or removed the bead array. The width and length of the fluidic channel were $200\ \mu\text{m}$ and $10\ \text{mm}$, respectively. The width of the control channel was $200\ \mu\text{m}$. The inset image shows the formed bead array by partially closing the valve.

We further increased the density of *E. coli* cells to 1×10^7 cells/ml. The images in Fig. 3(a) show that the bead array became heavily populated with *E. coli* cells over the time. We were able to roughly estimate the cell concentration

in the bead array based on the calibration of the fluorescence intensity against fluorescent images from bacterial suspension with known concentrations in the same microfluidic channels without beads.¹⁰ (More accurate quantification may require the use of confocal microscopy.) The plot of the number of cells against the time in Fig. 3(b) shows that the concentration of cells in the array rapidly rose from the in-

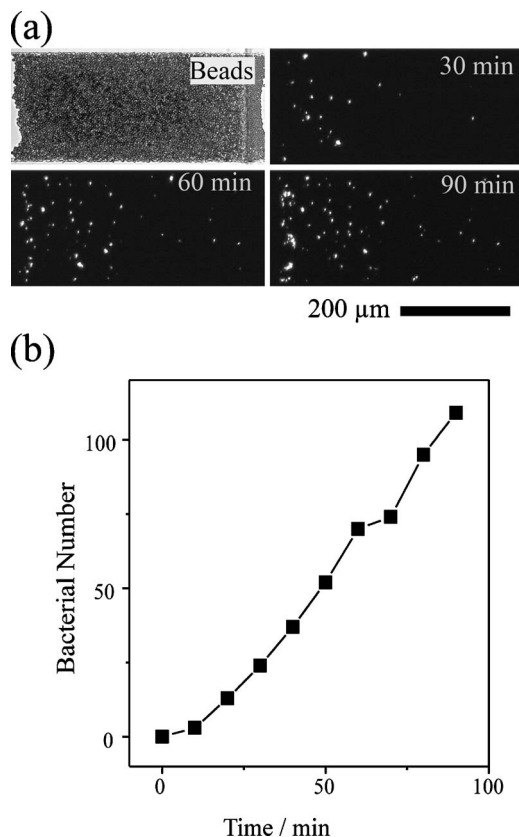


FIG. 2. The capture of *E. coli* cells at the concentration of $\sim 1 \times 10^3$ cells/ml by the bead array. The bead array was formed by silica beads with the diameter of $4.8\ \mu\text{m}$. (a) The optical image of the bead array and the fluorescent images of captured cells over time (at 30, 60, and 90 min). (b) The number of cells captured over time.

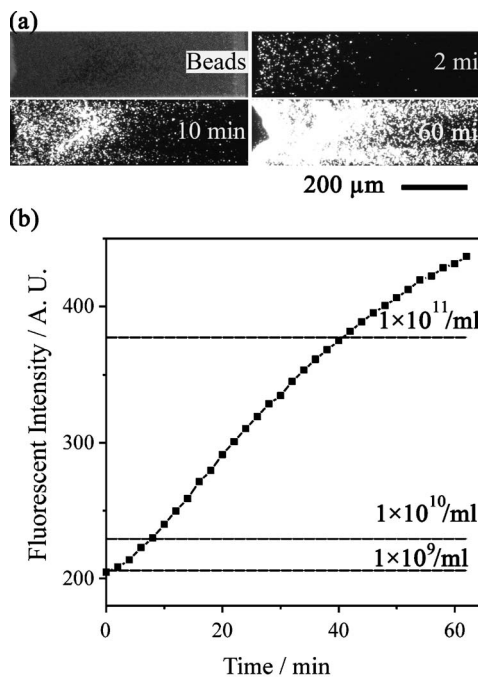


FIG. 3. The capture of *E. coli* cells at the concentration of $\sim 1 \times 10^7$ cells/ml by the bead array. The bead array was formed by silica beads with the diameter of $4.8\ \mu\text{m}$. (a) The optical image of the bead array and the fluorescent images of captured cells over time (at 2, 10, and 60 min). (b) The variation in the cell concentration in the bead array observed over time. The cell concentration at different times (indicated by the horizontal lines) was estimated by comparing the fluorescence intensity of the images to those of the images with known concentrations.

coming concentration of 1×10^7 to around 1×10^9 cells/ml within the first 2 min and the array was able to concentrate the cells by a factor of 10^4 (1×10^7 to 1×10^{11} cells/ml) within 40 min. Based on our data, the $4.8 \mu\text{m}$ bead array with the cross section of $200 \times 12 \mu\text{m}^2$ can accommodate up to $\sim 1 \times 10^5$ cells/100 μm length.

The retention of the *E. coli* cells is due to physical trapping in the microscale structures in the bead matrix when the gaps in the $4.8\text{-}\mu\text{m}$ -diameter-bead matrix ($>0.75 \mu\text{m}$) were slightly larger than the cell size. The dynamic movement of *E. coli* cells in the matrix was observed while the overall occupancy by the cells gradually increased over time. This mechanism is purely physical and very different from commonly used immunoseparation based on antigen-antibody interaction.^{11,12} Since the identity of the bacterium in proteomic studies is often known, our approach offers a simple alternative for bacteria capturing and also avoids interference with the results due to introducing an antibody.

The microfluidic bead array provides an ideal platform for the incorporation of rapid lysis methods such as electrical lysis after cell capture. Electrical lysis is generally considered to be the most rapid lysis method and does not require chemical or biological reagents which may interfere with subsequent assays. Electrical lysis of bacterial cells has been applied to flowing cells by us and other researchers.^{13,14}

However, in these settings, it was difficult to generate cell lysate with substantial concentration for proteomic analysis since the flowing cells could not be easily concentrated. In this study, we accumulated GFP-expressing *E. coli* cells with an original concentration of $\sim 10^7$ cells/ml concentrated in the $4.8 \mu\text{m}$ bead array for 40 min at a flow rate of $1 \mu\text{l}/\text{min}$. We focused a laser in the downstream of the bead array and applied periodic electrical pulses (3 s duration for each pulse with 10 s intervals between pulses) in the channel with different field intensities after such cell capture. We were able to detect fluorescence bursts after the pulses when the field intensity was higher than 1000 V/cm (Fig. 4). This threshold is similar to what we observed for electrical lysis of flowing cells.¹⁴ The fluorescence burst indicates the release of intracellular materials (GFP and other intracellular materials generating autofluorescence). Such release was mostly finished within the first electrical pulse when the field intensity was at 1250 V/cm and occurred slower (within the first six to seven pulses) when the field intensity was at 1000 V/cm. In this case, the elution of molecules through the bead array was due to electrophoresis during the high-intensity pulses which involved effects both electrophoretic mobility of protein molecules and electroosmotic flow. In principle, a complete elution of the intracellular molecules from the bead array can also be achieved by applying a low-intensity long pulse following the electrical lysis. The cells would be dead when electrical lysis occurs as we showed in previous work.¹⁴ These results reveal that when combined with electrical lysis, the device can produce cell lysate from bacterial cells after the capture of cells using the bead array. It needs to be noted that the field intensities described above were calculated based on the channel length and the applied voltage. The actual field intensity inside the bead array should be higher

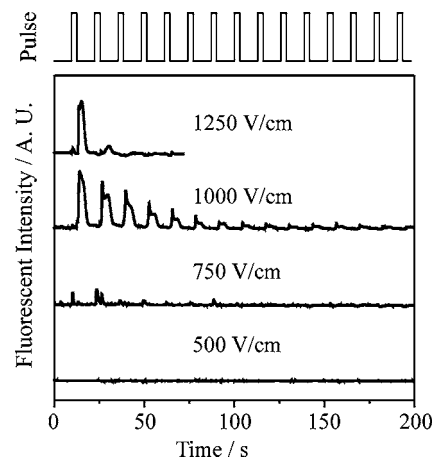


FIG. 4. The fluorescence intensity detected in the downstream of the bead array under different lysis field intensities when the series of electrical pulses were applied. Each trace was generated by a fresh bead array (formed by $4.8 \mu\text{m}$ beads) and captured cells ($\sim 1 \times 10^7$ cells/ml solution flowing for 40 min at a flow rate of $1 \mu\text{l}/\text{min}$). The pulse sequence consisted of 15 pulses of 3 s duration with 10 s intervals in between for pulse intensities at 500, 750, and 1000 V/cm and five pulses of the same pattern at 1250 V/cm. The detection of the released GFP from bacterial cells after electrical lysis was carried out by focusing a laser focal volume in the downstream of the bead array ($\sim 300 \mu\text{m}$ away from bead array).

than the calculated value due to the fact that the beads are insulators. One important feature of the device is its reusability after cell lysis. The bead array together with lysed cells can be flushed out when the mechanical valve is open after one run. The bead array can be formed again and the device can be reused after that.

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