DEVELOPMENT OF CONTINUOUS CELL LYSIS AND SEPARATION DEVICE USING REPULSIVE FORCE GENERATED BY ION CONCENTRATION POLARIZATION

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ABSTRACT

In this paper, we report a novel continuous cell lysis and separation device using the electrical repulsion in the depletion region formed by ion concentration polarization (ICP). In the depletion region, strong electrophoretic force is applied to charged materials due to the concentrated electric field. Using the strong electrophoretic force, we lysed cells and separated molecule dyes whose size range is order of 1 nm in the same device. Therefore, the developed multifunctional microfluidic chip is expected to be a very useful tool as an integrated system for analyzing biomolecules in cells such as DNAs and proteins.

KEYWORDS: Ion concentration polarization, Cell lysis, Separation, Integrated analysis system

INTRODUCTION

For biochemical analysis and molecular detection utilizing microfluidic devices, cell lysis and on-chip separation are essential and important steps [1]. Although many research groups have introduced various lysis and separation methods, development of an integrated device which can perform lysis and separation simultaneously is still a challenging and important issue to the biochemist in terms of speed, cost, and automation. In this research, based on ion concentration polarization (ICP), we proposed a novel microfluidic chip which can be used as an integrated system for lysing cells and separating biomolecules in the cells simultaneously.

When applying external electric field across an ion perm-selective membrane, ion-depletion and ion-enrichment regions are generated around the membrane, which refers to ICP [2]. In the depletion region, due to the low ionic concentration, the electric field is concentrated and amplified. In turns, electrokinetic motions including electroosmosis and electrophoresis are significantly amplified.

In our previous works, we proposed a novel separation device using the electrical repulsion in the depletion region formed by ICP, and separated micro- and nano-sized particles [3]. Here, based on the researches, we performed proof-of-concept experiments on cell lysis and separation of molecule dyes using the developed separation device. The results shows that lysis of bacterial cells and separation of nano-size molecule dyes can be achieved in the same device, which represents a great potential of the device as an integrated analysis system.

EXPERIMENTAL

Figure 1 shows the schematic diagram of the integrated device and operating process. We fabricated a polydimethylsiloxane (PDMS) microfluidic device using conventional photolithography (microchannel height: 40 µm). As shown in Figure 1, two microchannels, separation and buffer channels, were connected by a Nafion membrane (nanojunction) with strong cation selectivity. The Nafion membrane was patterned on a glass substrate using a Nafion perfluorinated ion-exchange resin (20 wt%; Sigma-Aldrich) via the microflow patterning method [4]. To reduce the flow instability caused by the vortex in the depletion region, the Nafion membrane was inclined at an angle of 45° (Figure 1).

To apply an electric field to the device, anode and cathode electrodes were connected to the metallic syringe tube of Inlet 1 and reservoir of the buffer channel, respectively. The electric field applied across the membrane induced an ion-depletion region (red circle in Figure 1) on the anodic side due to its strong cation selectivity. The flow rates of Inlet 1 and 2 were regulated by using two syringe pumps (Pump 11 Elite, Harvard Corp.). A flow-focusing method [5] was used to drive the particles to the lower side wall.
of the separation channel, causing the particles to pass through the depletion region, where the electrical force was strong.

**RESULTS AND DISCUSSION**

When applying external electric field across the Nafion membrane, depletion region is formed around the membrane of separation channel, and very strong electric field is applied to charged materials in the region. Figure 2 represents the continuous cell lysis in the depletion region. E. coli cell which expresses green fluorescent protein (GFP) was used for experiments. As shown in Figure 2, bacterial cells are deflected in the normal direction to the stream by the electric force and some of them are lysed in the depletion region so that GFPs within the cells were extracted; represents slightly green fluorescence. Although the extracted GFPs and cells were not perfectly separated, we observed that repulsion of GFPs was greater than cells.

**Figure 2: Continuous lysis of bacterial cells (flow rate of Inlet 1: 0.3 µL/min; flow rate of Inlet 2: 30 nL/min; applied voltage: 100 V).**

We performed separation of molecule dyes whose size range is order of 1 nm to show feasibility of separating nano-sized biomolecules in cells such as DNAs and proteins. The molecule dyes we used were 4,4-difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-s-indacene-2,6-disulfonic acid, disodium salt (BODIPY<sup>2−</sup>, Invitrogen) and 1,3,6,8-pyrene tetrasulfonic acid (PTS<sup>4+</sup>, Sigma-Aldrich) which have different
electrophoretic mobilities, $\text{PTS}^+ > \text{BODIPY}^2-$, and 1 mM dibasic sodium phosphate (DSP) was used as the electrolyte. When applying external electric field across the Nafion membrane, dyes are deflected in the normal direction to the stream by the electric force in the depletion region. We observed repulsion of two molecule dyes simultaneously with different fluorescent filters. As shown in Figure 3, $\text{PTS}^+$ dye experienced greater deflection from the fluid flow due to its higher electrophoretic mobility, compared with $\text{BODIPY}^2$. The results shows that it is possible to separate nano-sized bio-molecules including DNAs and proteins using the developed microfluidic chip.

![Figure 3: Continuous separation of molecule dyes; (a) BODIPY$^2$; (b) PTS$^+$ (flow rate of Inlet 1: 1.0 µL/min; flow rate of Inlet 2: 50 nL/min; applied voltage: 100 V).](image)

CONCLUSION

In this research, we proposed a novel microfluidic chip based on ICP for cell lysis and separation of nano-sized biomolecules in the cells. The results shows that lysis of bacterial cells and separation of nano-sized molecule dyes can be achieved in the same device using the strong electrophoretic force in the depletion region formed by ICP. Although we have not combined those processes, lysis and separation, the results represents a great potential of the device as an integrated analysis system. We expect that the developed microfluidic chip can be a useful tool for analyzing biomolecules in cells due to its multifunctionality.

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