MICROFLUIDIC ARCHITECTURES FOR INTEGRATED CELL LYSIS, LYSATE DIALYSIS AND CELL STIMULUS

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Abstract

Microfluidic architectures suitable for cell lysis, lysate dialysis, and cell stimulus are proposed. Laser-patterned nanoporous polymer membranes are fabricated in microchips using projection lithography and are used to separate cell lysate based on size and concentrate cytosolic proteins by electrophoretic concentration. Cell stimulus is achieved by rapidly changing the cell environment with aid of in-line, electrokinetic flow modulation.

Keywords: cell lysis, dialysis, polymer membrane, cell stimulus

1. Introduction

Proteomic measurements of cellular response to stress or other stimulus are typically performed by exposing macroscopic cell suspensions to stimulus and taking time course data via periodic lysis of cell solutions followed by analysis of the lysates. Microchip platforms are ideally suited for generation of highly time-resolved data by speeding the process of cell stimulus and lysis. Here we demonstrate microfluidic architectures for separation of cytosolic contents from membrane fragments and geometry-independent fluidic forcing techniques for rapidly establishing changes in cellular environment. A cascade of nanoporous polymer membranes is used to separate the cytosolic contents from the cell membrane fragments. Removal of membrane fragments and organelles greatly increases reliability of CE and other analytical techniques. Rapid homogeneous application of chemical or other stresses enable measurement of fast cellular responses.

2. Materials and Methods

Fused silica microchips are fabricated using conventional wet-etch techniques, and the internal surface is coated with linear polyacrylamide [1] to inhibit cell adhesion and suppress EOF. To allow for cell trapping and size-based separation of cell lysates,

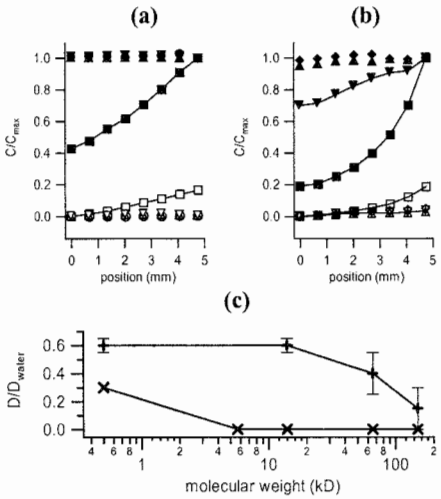


Figure 1. Counterflow mass transport. Top: concentration profiles with membranes for (a) low MWCO (b) high MWCO. Closed symbols: sample channel, flow enters at L=5mm and exits at L=0mm. Open symbols: perfusion channel, flow enters at L=0mm and exits at L=5mm. ■ Rhodamine 560 (0.5 kD); ● monomeric insulin (5.7 kD); ▼ lactalbumin (14 kD); ♦ BSA (66 kD); ▲ anti-biotin (150 kD). Bottom: (c) specific diffusivity through membrane as a function of analyte molecular weight. × low MWCO, + high MWCO

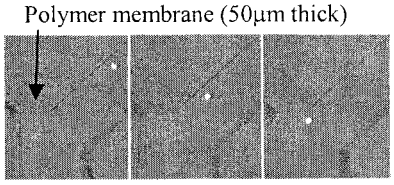


Figure 2. Electrophoretic cell trapping at laser-patterned nanoporous membrane. Cell is IL-2-dependent murine T cell in PBS. Microchip substrate is glass coated with linear polyacryamide to suppress protein adhesion and EOF. The images were taken at a 100 ms interval as voltage is applied across the membrane. The cell moves toward anode.

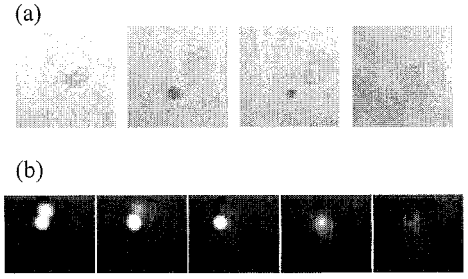


Figure 3. Time series of lysed cell in a microchannel. (a) optically with 7ns, 100 μJ, 532nm laser pulses (b) chemically with 2% CHAPS in PBS

nanoporous polymer dialysis membranes are laser-patterned in microchip using projection lithography[2]. Control of pore sizes of the dialysis membranes is achieved via chemical formulation. The size is determined by measurements of diffusive transport properties, which are quantified via counterflow mass exchange (Fig. 1).

Interleukin-II-dependent murine T cells are washed and suspended in PBS, labeled with 5-(and-6)-carboxyfluorescein diacetate, succinimidyl ester, and loaded on chip. Cells are manipulated in microchannels with pressure and voltage applied at reservoirs, and when desired, could be trapped at dialysis membranes via voltage applied across the membrane (Fig. 2). Cell lysis in microchannels is achieved chemically (2% CHAPS in PBS) or optically (7 ns, 100 uJ, 532 nm laser pulses) (Fig 3) [3,4].

3. Results and Discussion

Chip-level integration of laser-patterned nanoporous polymer membranes allows on-chip processing of nascent analytes such as cell lysates. Electrophoretic force is used to hold cells in place against the dialysis membrane while chemical or optical means are used to lyse them. To separate the cell lysates based on size, a cascade of dialysis membranes with two different molecular weight cutoffs (MWCO) is proposed. The high MWCO membrane filters cell membrane and organelle fragments while cytosolic proteins pass through the dialysis membrane. Then the low MWCO membrane stops, collects and concentrates cytosolic proteins. Finally, the concentrated proteins are eluted and injected downstream for further analysis.

The concentration of cytosolic proteins is possible due to the attractive electrical properties of the dialysis membrane. The low MWCO membrane has pores small enough to prevent protein transport but large enough to allow small-ion transport and current conduction [5]. Upon application of voltage, proteins electrophorese to the membrane and stack on the membrane surface. The electrical properties have been quantified by measuring the temporal stability of current fluxes in microchannels with and without the membrane. No measurable resistance was observed through the membrane, and voltage could be applied at length. Electrophoretic concentration of proteins at the nanoporous dialysis membrane provides more than 100-fold concentration that is linear in time and to voltage, indicating there is no dielectric breakdown or ion depletion in the system (Fig. 4).

Rapid cellular stimulus requires rapid change and homogenization of cellular environment (carbon source, gas concentration, pH, temperature) without geometries that lead to clogging. Rapid control of fluids in standard channel junctions facilitates the rapid cellular stimulus in

microchip. In-line flow modulation using electrokinetic pressure generation can introduce a series of fluids into a microchannel or microchamber and be programmed. The in-line flow modulation is achieved straightforwardly and inexpensively by applying AC voltage waveforms with a DC offset across in-line silica fiber mesh syringe filters. For example, a low-frequency modulation injects small plugs of two different fluids into a channel (Fig 5, left). When rapid mixing is required, the modulation can be performed at high frequency because the bandwidth of electrokinetic pressure generation can be as high as >1 kHz and the pressure modulation drives naturally dispersive parabolic channel flow in the microchannels. The predicted mixing length can be reduced by four orders of magnitude simply by changing the voltage waveform (Fig 5, right).

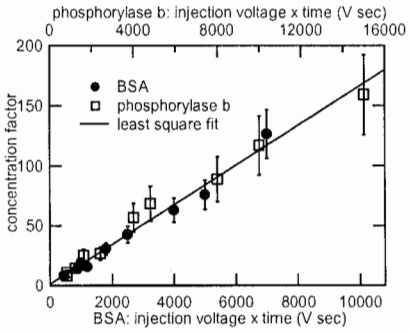
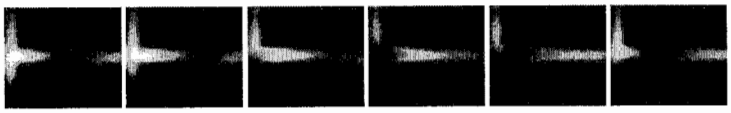


Figure 4. Linear concentration of two proteins by electrophoresis. Proteins are concentrated by over two orders of magnitude. Linear response facilitates metered concentration and evinces efficient small-ion transport.

5. Summary

Microfluidic architectures for cell lysis, lysate dialysis, and cell stimulus in microchips have been proposed. Nanoporous polymer membranes laser-patterned in the microchips are used to separate cell lysates based on size and concentrate cytosolic proteins on the membrane surface. In-line electrokinetic flow modulation promises rapid cell stimulus in microchips by rapidly changing the cell environment. In future work, we expect to integrate the cell lysis, stimulus, and lysate dialysis in a microchip.



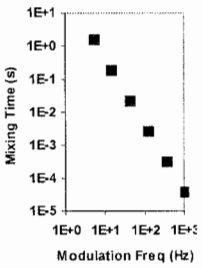


Figure 5. Pressure-driven flow modulation with AC driving from in-line electrokinetic pump fabricated simply and inexpensively from a syringe filter and 100V applied voltage. Images are at 66 ms intervals. Inlets: top (dye) and bottom (water); outlet at right. 2.5 Hz forcing is shown here for clarity; higher frequencies enhance mixing. Right: mixing times as function of modulation frequency shown for addition of pH stressor.

References

[1] B.J. Kirby, A.R. Wheeler, R.N. Zare, J.A. Fruetel, T.J. Shepodd, *Lab Chip*, **3**, pp. 5-10 (2003).

[2] S. Song, A.K. Singh, T.J. Shepodd, B.J. Kirby, *Anal. Chem.*, **76**, pp 2367-2373 (2004).

[3] N. Munce, J. Li, P. Herman, and L. Lilge, *Proc. SPIE*, **4982**, pp. 28-36 (2003).

[4] C. E. Sims, G. D. Meredith, T. B. Krasieva, M. W. Berns, B. J. Tromberg, and N. L. Allbritton, *Anal. Chem.*, **70**, pp. 4570-4577 (1998).

[5] S. Song, A.K. Singh, B.J. Kirby, *Anal. Chem.*, In press (2004).