Supplementary Material (ESI) for Chemical Communications

Microfluidic chemical cytometry based on modulation of local field strength

Hsiang-Yu Wang and Chang Lu

Microchip Fabrication

Microfluidic devices were fabricated based on polydimethylsiloxane(PDMS) using standard soft lithography method[1]. The microscale patterns were first created using a computer-aided design software (FreeHand MX, Macromedia, San Francisco, CA) and then printed out on high-resolution (5080 dpi) transparencies. The transparencies were used as photomasks in photolithography on a negative photoresist (SU-8 2025, MicroChem Corp., Newton, MA). The thickness of the photoresist and hence the depth of the channels was around 33 μm (measured by a Sloan Dektak3 ST profilometer). The pattern of channels in the photomask was replicated in SU-8 after exposure and development. The microfluidic channels were molded by casting a layer (~5 mm) of PDMS prepolymer mixture (General Electric Silicones RTV 615, MG chemicals, Toronto, Ontario, Canada) with a mass ratio of A:B = 10:1 on the photoresist/silicon wafer master treated with tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane (United Chemical Technologies, Bristol, PA). The prepolymer mixture was cured at 85^{o}C for 2 hours in an oven and then peeled off from the master. Glass slides were cleaned in a basic solution (H_2O: NH_4OH (27%) : H_2O_2 (30%) = 5:1:1, volumetric ratio) at 75^{o}C for an hour and then rinsed with DI water and blown dry. The PDMS chip and a glass slide were rendered hydrophilic by oxidizing them using a Tesla coil (Kimble/Kontes, Vineland, NJ) in atmosphere. The PDMS chip was then immediately brought into contact against the slide after oxidation to form closed channels.

Reagents and Cell Culture

Chinese hamster ovary (CHO-K1) cells have been employed in all our experiments. Cells were incubated at 37 ^{o}C, under 5% CO_2 in the Dulbecco's modified Eagle's medium (DMEM, Mediatech Inc., Herndon, VA) supplemented with 10% (v/v) fetal bovine serum (FBS, Sigma, St. Louis, MO), penicillin (100 units/ml, Sigma, St. Louis, MO), and streptomycin (100 μg/ml, Sigma, St. Louis, MO). Cells were diluted everyday to maintain them in the exponential growth phase (~1×10^6 cells/ml). They were harvested by adding Trypsin-EDTA (Sigma, St. Louis, MO) to the culture and centrifuged at 300g for 10 minutes to remove the supernatant. The isotonic buffer (8 mM Na_2HPO_4, 2 mM KH_2PO_4, and 250 mM sucrose , pH=7.4) was used to suspend the cell pallet for the subsequent experiment. The cell density was typically 10^6-10^7 cells/ml before flowing into the microfluidic device. To prevent clogging, the buffer was filtered by a 0.2 μm filter.

For observing the leak of intracellular materials during electroporation, cells were loaded with a fluorogenic dye, calcein AM (MW~ 995, generating calcein with spectra of 495/517 nm, Molecular Probes, Eugene, OR) at a concentration of 1μg/ml in the buffer for 15 minutes. In live cells the nonfluorescent calcein AM is converted to green-fluorescent calcein, after acetoxymethyl ester hydrolysis by intracellular esterases.

Fluorescence Microscopy

During the experiments, we observed the cells in the channel using a fluorescent microscope with phase contrast. The microfluidic device was mounted on an inverted fluorescence microscope (IX-71, Olympus, Melville, NY) with a 40X dry objective (NA= 0.40). The epifluorescence excitation was provided by a 100W mercury lamp, together with brightfield illumination. The excitation and emission from cells loaded with calcein AM were filtered by a fluorescence filter cube (Exciter HQ480/40, emitter HQ535/50, and beamsplitter Q505lp, Chroma technology, Rockingham, VT). The images of the cells were taken with a CCD camera (ORCA-285, Hamamatsu, Bridgewater, NJ) at a frame rate of 33 Hz. The electrophorograms (Figure 3 and 4) were generated by recording the fluorescence intensity at specific locations in the separation channel over time using the same CCD camera with a frame rate of 33 Hz.

Microchip Operation

Prior to the experiments, the channels were flushed with the buffer for 15 minutes to condition the channels and remove impurities. Both the inlets 1 and 2 were connected to a syringe pump (PHD infusion pump, Harvard Apparatus, MA) through plastic tubing. The electrode (a Pt wire) in the inlet 2 reservoir was inserted into the reservoir through a hole poked by a needle with a diameter slightly smaller than that of the Pt wire. The setup ensured the contact between the electrodes and the solution, in the mean time allowed the establishment of pressure-driven flows. A single high-voltage power supply (10A12-P4, Ultravolt, Ronkonkoma, NY) was used. The experiment typically lasted 3-5 minutes. A new device was used in each experiment to eliminate the effects of cell lysate adsorption on surface properties of the channel.

References

[1] D. C. Duffy, J. C. McDonald, O. J. A. Schueller, and G. M. Whitesides, *Anal Chem*, 1998, **70**, 4974.