

Supplementary Material for Soft Lithography: Masters on Demand

Mohamed Abdelgawad, Michael W.L. Watson, Edmond W.K. Young, Jared M. Mudrik,
Mark D. Ungrin, and Aaron R. Wheeler

Electrophoretic separations

Microfluidic devices used for electrophoretic separations had simple cross-patterns with 180- μm wide and 35- μm deep channels. Typically, channels were filled by submerging devices in isopropanol (IPA) under vacuum in a dessicator until all gas was removed (~5 min). Then, reservoirs were filled with run buffer which was pushed through the channels with pressure from a pipette. For pinched injections and electrophoretic separations, fluids were controlled by applying electric fields via a high voltage sequencer (LabSmith, Livermore, CA). Analytes were detected by laser induced fluorescence using an inverted microscope (Olympus IX-71) mated to an argon ion laser (Melles Griot, Carlsbad, CA). The 488-nm laser line was used for green fluorescence (fluorescein and rhodamine), and the 457-nm line was used for blue fluorescence (NDA-derivatives). The laser was focused into the channel using a 60x objective; the fluorescent signal was collected by the same lens and filtered optically (536/40-nm band pass and 488-nm notch filter for green fluorescence and a 482/35-nm band pass and 457-nm notch filter for blue fluorescence) and spatially (500 μm pinhole) and imaged onto a photomultiplier tube (Hamamatsu, Bridgewater, NJ). PMT current was converted to voltage using a picoammeter (Keithley Instruments, Cleveland, OH) which was then collected using a DAQpad A-D converter (National Instruments, Austin, TX) and PC running a custom LabView (Natl. Inst.) program.

All reagents used in electrophoretic separations were obtained from Sigma-Aldrich (Oakville, ON). Depending on the analytes, the detector was optimized (as described above) for green fluorescence (fluorescein and rhodamine 123, each 1 μ M in run buffer) or blue fluorescence (aspartic acid, histidine and phenylalanine, each 1 μ M in run buffer). In the latter case, the analytes were labeled with naphthalene 2,3-dicarboxyaldehyde (NDA) using the procedure described by Throckmorton et al.¹ In all cases, analytes were separated by micellar electrokinetic chromatography (MEKC) in run buffers containing sodium dodecyl sulfate (SDS) and acetonitrile (ACN). For separation of fluorescein and rhodamine, the run buffer contained 50 mM SDS and 30% ACN (in 10 mM sodium borate, pH 9) and the applied field was ~250 V/cm, and in separations of amino acids, the run buffer contained 100 mM SDS and 30% ACN (in 20 mM sodium borate, pH 9) and the applied field was ~115 V/cm.

Cell culture

Unless stated otherwise, chemicals and reagents for cell isolation and culture were purchased from Sigma-Aldrich Canada (Oakville, ON, Canada), fluorescent dyes were from Invitrogen (Carlsbad, CA), and all other materials were purchased from Fisher Scientific (Pittsburgh, PA). Primary porcine aortic valve endothelial cells (PAVECs) were isolated from the valve leaflets of fresh pig hearts, as described previously.² Briefly, valve leaflets were enzymatically treated in a digestion solution (60 U/mL collagenase, 2.0 U/mL dispase, 2.5 h, 37°C), followed by scraping to dislodge the cells. Isolated cells were cultured for 3-4 days in EGM-2 basal medium with SingleQuots (Cambrex Clonetics, East Rutherford, NJ) supplemented with 20% fetal bovine serum (FBS) and 1% penicillin-streptomycin (P/S), and then sorted by the MACS magnetic cell sorting system (Miltenyi Biotec, Auburn, CA) to remove non-endothelial cells. The cultures were then expanded in M199 media supplemented with 10% FBS (Fisher) and

1% P/S in flasks pre-coated with 3% (w/v) gelatin. PAVECs between passage 5-6 were used in the experiments.

Microchannels were sterilized by rinsing with 70% ethanol (10 min), followed by phosphate buffered saline (PBS) (10 min). Channels were subsequently filled with a 1 mg/mL solution of bovine plasma fibronectin and incubated (RT, 80 min) to allow passive protein adsorption onto the channel walls. PAVECs were prepared for microchannel culture by labeling with Hoechst and CellTracker Green vital dyes, dislodging in 0.05% trypsin containing 2 mM EDTA, and resuspending in complete media to a cell density of 20 million cells/mL. Cells were introduced into the microchannels via syringe, and devices were then stored in an incubator (37° C, 5% CO₂) to permit cell attachment and spreading on the channel surface. Observations were made immediately after the two-hour incubation using an inverted fluorescent microscope (Olympus IX-71), and fluorescent images were collected with a CCD camera (QImaging, Retiga 2000-R). Subsequent to live-cell observations, cells were fixed, permeabilized, and stained for an endothelial cell marker (CD31) to verify phenotype.

References

1. D. J. Throckmorton, T. J. Shepodd and A. K. Singh, Electrochromatography in microchips: Reversed-phase separation of peptides and amino acids using photopatterned rigid polymer monoliths, *Anal. Chem.*, 2002, **74**, 784-789.
2. E. W. K. Young, A. R. Wheeler and C. A. Simmons, Matrix-dependent adhesion of vascular and valvular endothelial cells in microfluidic channels, *Lab Chip*, 2007, **7**, 1759-1766.