

Antigen-Responsive, Microfluidic Valves for Single Use Diagnostics

Electronic Supplemental Information

Materials

Glass microscope slides (Corning), glass coverslips (FisherFinest), sulfuric acid, hydrogen peroxide, absolute ethanol, methanol, triethanol amine, phosphate buffered saline (PBS, 10x), β -D-glucose (glucose, anhydrous), and Evans Blue were purchased from Fisher Scientific (Waltham, MA) and used as received. Glucose oxidase (GOx, *Aspergillus niger*), iron sulfate, pentaerythritol tetra(3-mercaptopropionate) (PETMP), poly(ethylene glycol) diacrylate (PEGDA, M_n 575 Da), Ethylenediaminetetraacetic acid, bovine serum albumin (BSA), biotinylated bovine serum albumin, and monoclonal anti-cytokeratinpeptide 18 antibody produced in mouse were purchased from Sigma-Aldrich. Irgacure 184 (I-184) was purchased from Ciba Specialty Chemicals. 2-Iminothiolane (Traut's Reagent) was purchased from Pierce (Rockfield, IL). Rabbit Polyclonal Antibodies against human von Willebrand Factor were purchased from Dako. Human Transforming growth factor β 1 (TGF β) was purchased from Cell Signaling Technologies. Capture and biotinylated detection antibodies against human TGF β were purchased from eBioscience. Biotinylated goat antibodies against rabbit IgG, and Biotinylated goat antibodies against mouse IgG, and Glucose oxidase-avidin (GOx-Av) were purchased from Vector Labs. (3-Acryloxypropyl) methyltrimethoxysilane was purchased from Gelest. 2-(N-morpholino)ethanesulfonic acid buffer (MES) was purchased from Teknova. Methacryloxyethyl thiocarbonyl rhodamine B (rhodamine b methacrylate, excitation/emission: 548/570) was purchased from Polysciences. Tubing (high purity perfluoroalkoxyalkane, 360 μ m O.D.) and luer-lock tubing adapters were purchased from IDEX Health and Sciences.

Methods

Acrylate Functionalization of Glass

Glass substrates (microscope slides and coverslips) were exposed to a solution (Caution: hazardous material) of approximately 70% sulfuric acid and 30% hydrogen peroxide by volume for one hour. Critically, the substrates were rinsed three times in DI water and dried in a nitrogen stream. The substrates were then placed immediately in a solution of 0.1 % (3-acryloxypropyl) methyltrimethoxysilane in absolute ethanol overnight. The resulting substrates are assumed to be coated with acrylate functionality, and offered improved polymer adhesion when compared to unfunctionalized glass substrates.

Photopolymerization of Microfluidic Devices

Devices were manufactured on a mask alignment system constructed by OAI. Functionalized microscope slides were cut in half to the approximate dimensions of 4 cm by 2.5 cm and placed on the stage. A monomer mixture of pentaerythritol tetra(3-mercaptopropionate) (PETMP) and poly(ethylene glycol) diacrylate (PEGDA) was formulated in 1:1 stoichiometry between the thiol and acrylate groups and 0.05 wt % I-184. Monomer was placed on top of the slide section, and covered with an acrylate functionalized coverslip and photomask (Estey Printing, Boulder, CO). The distance between the coverslip and slide was adjusted to approximately 400 μ m, and the sample was irradiated with collimated light at 35 mW cm⁻², 320-500 nm for 7 s. Unreacted monomer was cleared from channels with rinsing with methanol and compressed air. This device manufacturing technique is based on the CLiPP methodology and has been described in detail previously.¹⁵

Biodetection Procedure

In general, a capture material was conjugated to the device walls through thiol-acrylate Michael addition. Capture antibodies were thiolated using a 4-fold excess of Traut's Reagent in a previously published procedure.¹⁸ After desalting, phosphate buffered saline (PBS) was added to the thiolated proteins and the pH was adjusted to 8 with triethanol amine. The pH adjusted capture antibody solution was then added to the microfluidic channel in a humidified chamber for 1 h at room temperature.

Samples were rinsed by adding PBS with 0.1 wt % BSA to one side of the device and drawing the reagent through the device with capillary action using KimWipes. Additional detection reagents were added in an aqueous solution of PBS with 0.1 wt % BSA. All incubations and rinses were 5 minutes unless otherwise specified. Different antibodies and detection probes were used for each detection scheme and are described in the body of the text. Universally, biotin functionality was used to specifically bind a GOx-Av conjugate. After rinsing away unreacted GOx-Av, a monomer

solution was introduced to the channel for 5 minutes of polymerization based amplification. The monomer solution is static during the polymerization process. The monomer solution consisted of 250 mM FeSO₄, 512mM b-D-glucose, 35 wt. % poly(ethylene glycol) diacrylate, and 20mM 2-(N-morpholino)ethanesulfonic acid buffer stabilized at pH 4.5 in water. For enhanced visual detection, 800 µg mL⁻¹ Evans Blue was added to the monomer solution, resulting in a dark blue color. Copolymerization with 35 mM methacryloxyethyl thiocarbamoyl rhodamine B enabled fluorescent detection of the polymer. For visual and fluorescent detection, unreacted monomer was rinsed away with deionized water.

Visual Detection of Antibody Pairs

The devices in Figure 2A-C were fabricated as described above using the photomask pattern in Figure ESI 1, where light areas were irradiated to form polymer walls of the device. For all incubations in this section, the solution was added to the glass slide adjacent to the channel inlet at one side of the device and a dry Kimwipe was applied to the opposite side of the device. The capillary action of the Kimwipe was used to draw the solution into and across the device. No tubing or pumping was used. Antibodies raised in mouse and rabbit were thiolated in a 10 fold excess of Trauts reagent in PBS, desalted and rebuffed in PBS. Thiolated antibodies in PBS were brought to pH 8 with triethanol amine and added to the appropriate device channels for 1 hour, where channel 1 was exposed to thiolated mouse antibodies, and channel 2 was exposed to thiolated rabbit antibodies. Devices were rinsed and blocked with 0.1% BSA in PBS for at least 15 minutes. Devices were exposed to 1:250 dilution of either biotinylated anti-mouse or anti-rabbit secondary antibodies. Devices were rinsed with 0.1% BSA in PBS for 5 minutes. Devices were exposed to 50 µg/mL GOx-Av for 5 minutes. Devices were rinsed with 0.1% BSA in PBS for 5 minutes. Devices were exposed to a monomer solution consisted of 250 mM FeSO₄, 512mM b-D-glucose, 35 wt. % poly(ethylene glycol) diacrylate, and 20mM 2-(N-morpholino)ethanesulfonic acid buffer stabilized at pH 4.5 in water with 800 µg mL⁻¹ Evans Blue. After 5 minutes, the exterior of the device was rinsed with water and capillary action (as before) was sufficient to clear unreacted monomer from channels in the absence of specific binding. Polymerized channels (biorecognition) are clearly observed by the blue dyed polymer remaining in the device, and unpolymerized channels (no biorecognition) contained un-dyed rinse water. Some delamination of the devices (Figure 2A-C) was observed and is likely a result of incomplete acrylation of this batch of the glass substrates. Delamination is not observed in any of the other experiments (Fig 2D, 3B, 3C, and 3D) The use of on-chip negative controls in Fig 2A and 2B, indicates the specificity of the system even when delamination is present.

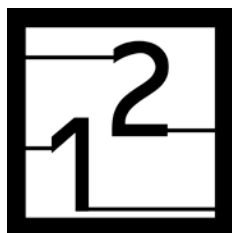


Fig ESI 1. Photomask used for the creation of devices for Fig 2 A-C.

Flow Diversion

Tubing was cut to 100 mm lengths and inserted into the device channel 5 mm, approximately. The tubing was sealed in place using a Loctite Plastic Bonder Epoxy (Home Depot) and left overnight. Each reagent and rinsing solution was introduced through a hand actuated syringe connected to the tubing through a luer lock tubing adapter (IDEX). Following 5 minutes of polymerization amplification, a solution of green food coloring in deionized water was introduced to the device by syringe. See Figure ESI 2 for the experimental set up.

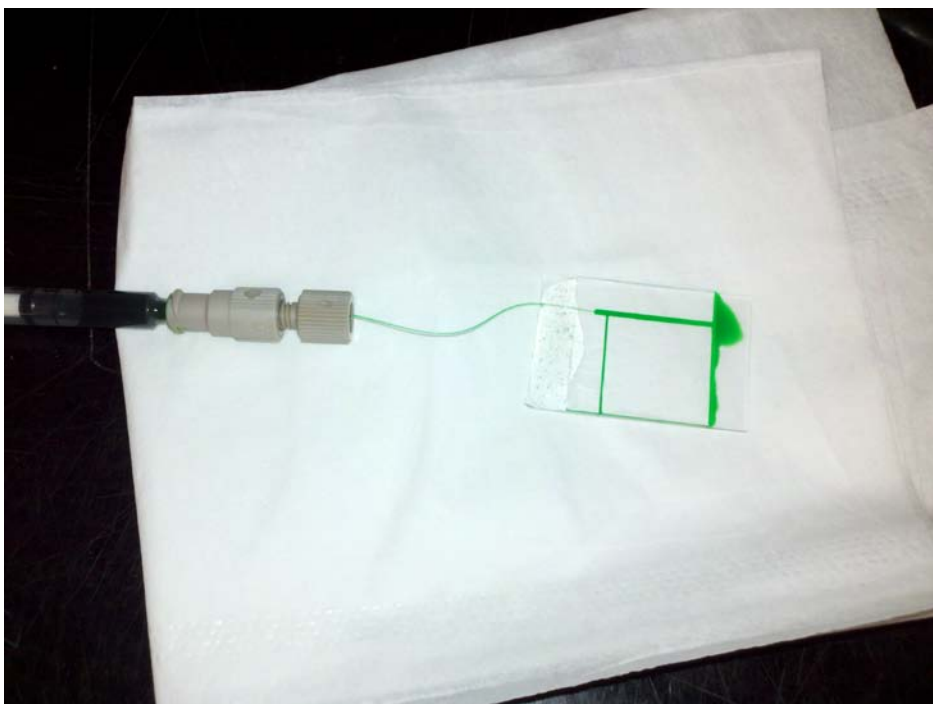


Fig ESI 2. Experimental set up for flow diversion experiments

Pressure Monitoring

Tubing was connected to the device as in the flow diversion experiments. After a 5 minute exposure to each of the appropriate biodetection reagents and the monomer solution, the device tubing was connected to a syringe pump (Harvard Apparatus 44), and a pressure transducer was connected to the tubing through a Y-connection. Up to 50 mL of air was delivered at $50 \mu\text{L s}^{-1}$, and the voltage signal from the pressure transducer (Honeywell, 140PC15D) was recorded every 0.1 s using LabView Signal Express for DAQ (National Instruments). Voltages were correlated to system backpressure with calibration curves. Figure ESI 3 Shows the experimental arrangement.

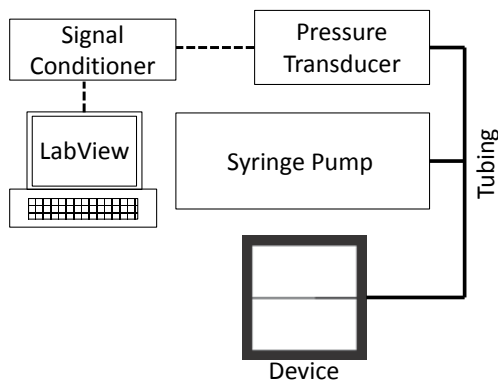


Fig ESI 3. Experimental set up for back pressure monitoring experiments. Tubing is glued into the right side of the device (width $450 \mu\text{m}$), and flow across the device is from right to left.