Supplementary Information

Multiplexed surface micropatterning of proteins with a pressure-modulated button membrane

Jose L. Garcia-Cordero[1] and Sebastian J. Maerkl[1]

[1]Institute of Bioengineering, School of Engineering and School of Life Sciences, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland.

Correspondence should be addressed to S.J.M. (sebastian.maerkl@epfl.ch)

Experimental details

Microfluidic chip fabrication and operation. The microfluidic device consisted of a flow and control layers. Molds for each layer were fabricated using standard lithography techniques on 4" silicon wafers. The control and flow layer molds were patterned with SU8 phothoresist to a height of ~30 µm, and with AZ9260 photoresist to a height of ~10 µm, respectively. The flow mold was baked at 190°C for one hour in a convection oven to obtain a round channel profile. Devices were cast in polydimethylsiloxane (Sylgard 184, Dow Corning) using the techniques of multilayer soft lithography. Briefly, PDMS was prepared at a 20:1 ratio and spin-coated on the flow layer mold at 1900 rpm. PDMS at a 5:1 ratio was cast on the control layer mold to a thickness of 5 mm. Both layers were baked at 80°C for 30 min. The control layer was peeled off from its mold and manually aligned to the flow layer mold, followed by a baking step at 80°C for 90 min. Pressure for flow channels was set to 10 kPa using an analog pressure gauge.

Preparation of epoxy-silane glass slides. 720 mL of milli-Q water and ammonia solution (NH_4OH 25%) in a 5:1 ratio, respectively, was heated to 80°C. Next, 150 mL of hydrogen peroxide (H_2O_2 30%) was added to the mix. Glass microscope slides were bathed in the solution for 30 min. The slides were then rinsed with milli-Q water and dried under a stream on nitrogen. Next, the slides were incubated in a solution of 1% 3-Glycidoxypropyl-trimethoxymethylsilane (97% pure) in toluene for 20 min, after which they were rinsed with toluene and dried. Next, the glass slides were placed in a convection oven for 30 min at 120°C, followed by a sonication step in toluene for 20 min, rinsed with isopropanol, and dried. Glass slides were stored in vacuum at room temperature.

Biotin-neutravidin assay. All reagents were aspirated into Tygon tubing (0.020" ID, AAQ02103, Coler-Parmer). Microfluidic control channels were primed with dH_20. The button membrane was initially actuated at 170 kPa. 1% bovine serum albumin (BSA) and 1% casein in PBS were flowed through the channels for 20 min to block the surface. Channels were cleaned with washing buffer (PBS/Tween 0.005%) for 5 min. Pressure on the button was released and biotin-BSA (29130, Thermo Fisher Scientific) at a concentration of 2 mg/mL

Supplementary Information

flowed for 20 min and washed for 5 min. The button membrane was actuated at 62 kPa and the first DyLight 650 conjugated neutravidin (84607, Thermo Scientific) at a concentration of 20 µg/ml flowed through the chip for 20 min, followed by a 10-min washing step. Next, the button was actuated at 27 kPa, and the second DyLight 550 conjugated neutravidin (84606, Thermo Scientific) at a concentration of 10 µg/ml flowed through the chip for 20 min, followed by a 10-min washing step. Finally, the pressure on the button was released and the third DyLight 488 conjugated neutravidin (22832, Thermo Scientific) at a concentration of 10 µg/ml flowed through the chip for 20 min, followed by a 10-min washing step. The chip was scanned using a fluorescent microarray scanner (ArrayWorx e-Biochip Reader, Applied Precision, USA) equipped with a Cy3 filter (540/25 X, 595/50 M), a Cy5 filter (635/30 X, 685/40 M), and an Alexa 488 filter (480/30 X, 530/40 M). Devices were scanned with an exposure time of 1 sec at the highest resolution of 3.25 µm. Stitched images were exported as a 16-bit TIFF file, **Fig. 3a**. The same chip was imaged with an inverted epi-fluorescence microscope (Eclipse Ti-E, Nikon Instruments) using a 20x achromat LWD objective (MRP00202, Nikon Instruments). The microscope was equipped with an LED based system for fluorescence illumination with three different filter cubes (Ex 460 500/DM505/BA 510-560, CY3-NX/MXU9621/C105216, CY5-MXU96214/C104453, Nikon Instruments). Images were acquired using a back-illuminated cooled CCD camera (Ixon DU-888, Andor Technology), **Fig. 3b**.

Sandwich immunoassay. Standard proteins and antibodies were purchased from eBioscience (San Diego, USA), and are summarized in the table below. Biotinylated antibodies and fluorescently-labeled antibodies were diluted in 1% casein in PBS (37528, Thermo Scientific) to a concentration of 2 µg/mL and 5 ng/mL, respectively.

	Mouse recombinant protein	Capture antibody Biotin	Detection antibody Fluorescent
IL6	39-8061-60	36-7062-85	12-7061-41 (PE)
TNFα	39-8321-60	13-7341-81	19-7321-81 (Cy5)
IL12 p70	39-8121-60	14-7122-85	12-7123-41 (PE)

Biotin-BSA was flowed through the chip for 20 min and washed for 5 min. 15 µL of neutravidin (31000, Thermo Fisher Scientific) at a concentration of 0.5 mg/mL was then flowed for 20 min and washed for 5 min. The button was actuated at 170 kPa and biotin-BSA flowed again for 20 more min, followed by a washing step for 10 min. Biotinylated anti-IL6 was flowed for 20 min, washed for 10 min. Next, the pressure was decreased to 62 kPa and biotinylated anti-TNFα flowed for 20 min and washed for 10 min. Then, the pressure on the button was released and biotinylated anti-IL12p70 flowed for 20 more min and washed for 10 min. A solution containing the three cytokines, each at a concentration of 100 pM, was introduced in the chip for 20 min, followed by a washing step. Finally, a solution of the three

Supplementary Information

detection antibodies was loaded in the chip for 20 min and washed for an additional 10 min. The chip was then scanned using the same fluorescent microarray scanner.