Supplementary Material (ESI) for Lab on a Chip

Supplementary Information

for

A Microfluidic Platform for Complete Mammalian Cell Culture

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DEVICE FABRICATION AND OPERATION

Silane A174 and Parylene-C dimer were from Specialty Coating Systems (Indianapolis, IN) and Teflon-AF was from DuPont (Wilmington, DE) and all other reagents were from Sigma-Aldrich (Oakville, ON). Digital microfluidic devices were fabricated using conventional methods in the University of Toronto Emerging Communications Technology Institute (ECTI) cleanroom facility, using a positive-film photomask printed at the Pacific Arts & Designs (Markham, ON). Glass devices bearing patterned chromium electrodes were formed by photolithography and etching as described previously.[1] After forming electrodes, devices were exposed to Silane A174 solution (0.5 % A174 in 50/50 isopropanol/DI water, 30 min). After rinsing and drying, devices were coated with 2.5 μm of Parylene-C (vapor deposition) and 50 nm of Teflon-AF (spin-coating, 1% wt/wt in Fluorinert FC-40, 2000 rpm, 60 s), and then post-baked on a hot-plate (160 °C, 10 min). The polymer coatings were removed from contact pads by gentle scraping with a scalpel to facilitate electrical contact for droplet actuation. In addition to patterned devices, unpatterned indium-tin oxide (ITO) coated glass substrates (Delta Technologies Ltd, Stillwater, MN) were coated with Teflon-AF (50 nm, as above).

Most devices used here had geometry identical to that shown in Figure 1 in the main text. The 89 actuation electrodes (4.8 mm^2 ea.) had 12-sided irregular-polygon shapes to promote droplet transfer and were connected to 7 square reservoir electrodes (5 × 5 mm ea.), with inter-electrode gaps of 30 μm. Six of the actuation electrodes had patterned square openings (0.8 x 0.8 mm), which served as transparent windows for monitoring cell culture and growth. Prior to use, devices were sterilized in 70% ethanol, and adhesion pads were formed by pipetting 500-nL aliquots of fibronectin (33 μg/mL in DI water) onto the patterned openings and allowing the solutions to dry. The resulting spots were circular (~1 mm^2) and contained ~0.0165 μg of protein.

Devices were assembled with an unpatterned ITO-glass top plate and a patterned bottom plate separated by a spacer formed from two pieces of double-sided tape (~150 μm thick). Unit droplet and reservoir droplet volumes on these devices were ~1 μL and ~4 μL, respectively. Upon device assembly,

reservoir droplets were placed onto partially exposed reservoir electrodes, from which the unit droplets were dispensed and actuated by applying driving potentials between the top electrode (ground) and sequential electrodes on the bottom plate (Figure 2a in the main text) via the exposed contact pads. Driving potentials (80-100 V_{RMS}) were generated by amplifying the output of a function generator (Agilent Technologies, Santa Clara, CA) operating at 15 kHz. Droplets and cells were monitored and recorded by microscopy with fluorescence illumination (Olympus SZX12 stereomicroscope, Olympus Canada, Markham, ON, and Leica DM2000 upright microscope, Leica Microsystems Canada, Richmond Hill, ON).

In most experiments, droplets on digital microfluidic devices were delivered to adhesion pads by means of a technique called passive dispensing (Fig. 3 in the main text). In this method, a small sub-droplet splits from a larger source droplet as a consequence of differences in surface hydrophilicity. In the devices used here, source droplets were 1 μL and passively dispensed droplets were 150 nL. As depicted in Figure 1S, this process was used to deliver droplets to dry adhesion pads as well as to implement solution exchange on pads already bearing droplets. The efficiency of solution replacement via passive dispensing was evaluated by replacing a 150-nL droplet of fluorescein solution (0.5 nM in PBS) from an adhesion pad using five consecutive source droplets of PBS. Fluorescence of a dispensed droplet of fluorescein and fluorescence persisting after each source droplet wash was measured using a PHERAstar microplate reader (BMG Labtech, Durham, NC). This experiment was carried out in triplicate, and the data was normalized relative to the fluorescence before washing.

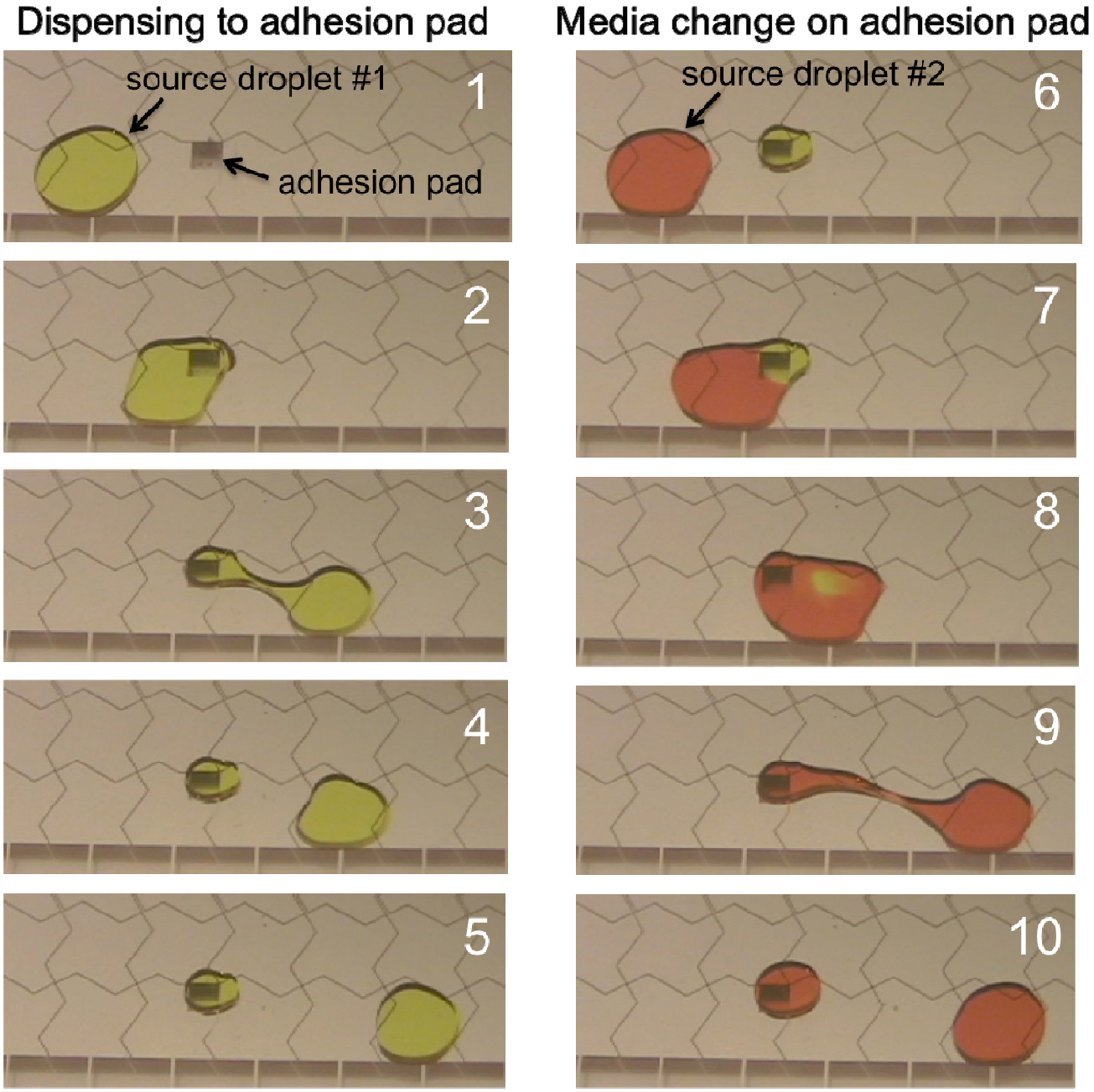

Figure 1S: Frames from a movie depicting passive dispensing onto an adhesion pad. In frames 1-5, a yellow droplet is dispensed onto a dry pad, and in frames 6-10, the yellow droplet is replaced with a red droplet. The source and dispensed droplet volumes were 1 μL and ~150 nL.

REFERENCES

1. I. Barbulovic-Nad, H. Yang, P. S. Park and A. R. Wheeler, *Lab Chip*, 2008, **8**, 519-526.