Electronic Supplementary Information for:

Biofunctionalization of electrowetting-on-dielectric digital microfluidic chips for miniaturized cell-based applications

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Reagents

Poly-L-Lysine hydrobromide (PLL), FITC-labeled PLL (PLL-FITC), boric acid, Pluronic F68, Calcein AM (CAAM), and propidium iodide (PI) were purchased from Sigma Aldrich (Oakville, ON, USA). Both PLL and PLL-FITC were diluted in borate buffer (pH 8.5, 0.1 M) to a final concentration of 0.5 and 1.0 mg mL^{-1} respectively. Fluorinert FC-40 was obtained from 3M (St. Paul, MN, USA). Reagents used for photolithography included S1818 and SPR220-7 positive photoresist, and 351-developer (Rohm and Haas, Marlborough, MN, USA). Parylene-C dimer and silane A174 adhesion promoter were purchased from Plasma Parylene Coating Services (Rosenheim, Germany), and Teflon-AF® was obtained from Dupont (Wilmington, DE, USA). All cells were obtained from the American Type Culture Collection (Manassas, VA, USA). Cell culture media, trypsin-EDTA, fetal bovine serum (FBS), and HEPES were purchased from Invitrogen (Merelbeke, Belgium).

Cell culture

HeLa cells were cultured in Dulbecco's Modified Eagle's medium (DMEM), supplemented with 10% FBS, 2% HEPES, 1% sodium pyruvate and 2 mM L-glutamine in a humidified tissue culture incubator at 37 °C and 5% CO_2. ATDC5 cells were cultured in DMEM-F12 supplemented with 5% FBS, human transferrin, selenite, 1% antibiotics, 2 mM L-glutamine and 1% sodium pyruvate. hPDC TE Pooled (P7) stem cells were cultured in DMEM supplemented with 10% FBS, 1% antibiotics, 2mM L-glutamine and 1% sodium pyruvate. Cells were grown to confluency before being harvested with trypsin-EDTA, and concentrated to ~5×10^5 cells mL^{-1} before being used in experiments.

Chip fabrication and operation

Digital microfluidic chips were fabricated at the cleanroom facilities of the Katholieke Universiteit Leuven (ESAT-MICAS). In summary, glass wafers were cleaned in acetone and isopropyl alcohol for 5 min. A thin layer of chromium (100 nm) was then deposited by magnetron sputtering (Balzers BAE 370, Pfäffikon, Switzerland). The chromium layer was patterned by standard photolithographic processes using S1818 positive photoresist, chrome-on-glass photomasks, and wet etching using Cyantec CR-7 chromium etchant. Next, chips were cleaned in O_2-plasma (150 mtorr, 100W) and primed with silane A174 before being coated with a layer of Parylene-C (3 μm) which was deposited using chemical vapor deposition (AL 200, Plasma Parylene Coating Services, Rosenheim, Germany). A thin layer of Teflon-AF® (~200 nm thickness, 3% w/w in Fluorinert FC-40) was subsequently spincoated (1200 rpm) on top of the Parylene-C layer, and baked for 5 min at 110 °C, and 5 min at 200 °C. Crenellated actuation electrodes of 1.4 mm × 1.4 mm were used for actuation of individual droplets of 0.8 μL. The upper plate of the digital microfluidic device was fabricated by spincoating Teflon-AF® (as above) on top of indium tin oxide-coated glass slides (Delta technologies Ltd, Stillwater, MN, USA). Tape of 100 μm thickness was applied on the bottom plate of the chip as a spacer before closing the 2 plates in order to assemble the microfluidic device. All reagents were supplemented with 0.1% (w/v) Pluronic F68 before being manually dispensed as droplets (0.8 μL) onto actuation electrodes of the chip before placing the upper plate on the chip surface. Droplets were actuated by a pulsed DC-voltage of 120 to 140 V, an activation time of 300 ms, and a relaxation time of 40 ms. Pulsing of the DC-actuation voltage was realized by a function generator operating at 7 kHz (GFG-8216A, ISO-TECH, England). The chip was placed in a specially designed chip holder that allowed for the visualization from the bottom side of the chip. The actuation sequence of electrodes was controlled by a custom LabVIEW program (National Instruments, Austin, Texas, USA). The optimization of the different chip actuation parameters can be found in previous work[1].

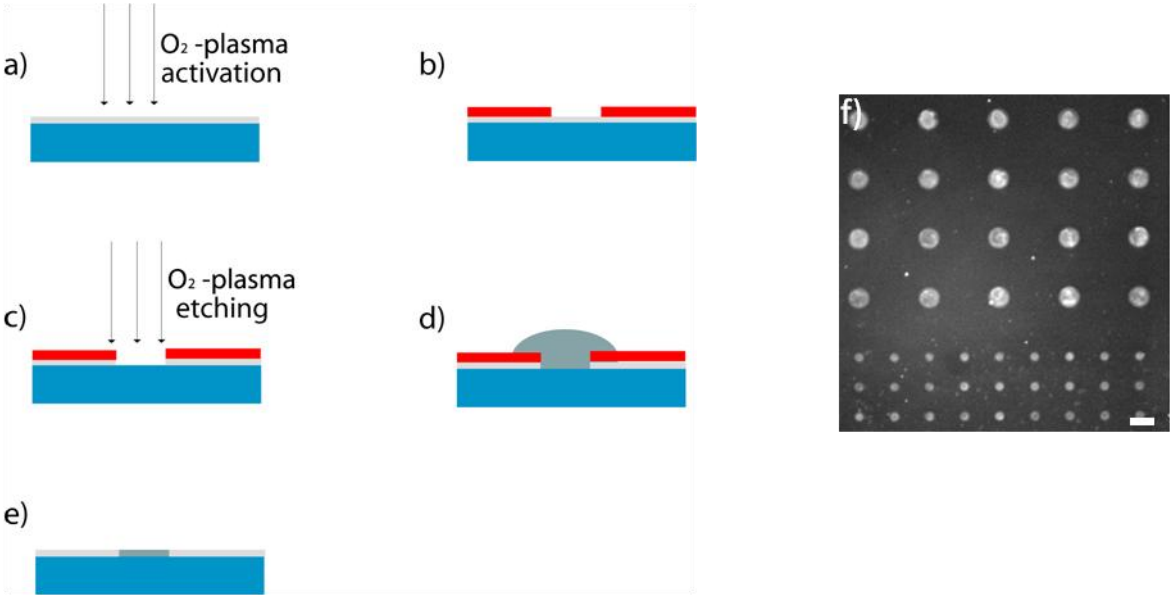

Figure S1 Schematic drawing of the wet lift-off method for functionalizing of EWOD digital microfluidic chips: a) O_2-plasma activation of the Teflon-AF® surface, b) photolithography, c) O_2-plasma etching of exposed Teflon-AF®, d) seeding biomolecules, e) lift-off of sacrificial photoresist in ethanol, and f) fluorescent image of PLL-FITC micropatterns in a Teflon-AF® coated substrate as generated with the WLO method (scale bar = 50 µm).

Table S1 Static contact angle measurements for different Teflon-AF® treated surfaces. DLO = dry lift-off, WLO1 = wet lift-off without post-process bake, WLO2 = wet lift-off followed by post-process bake. Standard deviations for 3 replicates are given.

Teflon-AF® treatment	Control	DLO	WLO1	WLO2
Contact angle (°)	121.8±1.2	122.0±0.5	104.1±1.1	118.8±0.6

References

1 Vergauwe, N., Witters, D., Atalay, Y.T., Verbruggen, B., Vermeir, S., Ceyssens, F., Puers, R. and Lammertyn, J. *Microfluid Nanofluid*. DOI 10.1007/s10404-011-0769-6.