Supplementary Information

Integrated microfluidic devices for the combinatorial cell-based assays

Zeta Tak For Yu, Ken-ichiro Kamei, Hiroko Takahashi, Chengyi Jenny Shu, Xiaopu Wang, George Wenfu He, Robert Silverman, Caius G. Radu, Owen N. Witte, Ki-Bum Lee, and Hsian-Rong Tseng

Department of Mechanical and Aerospace Engineering, University of California, Los Angeles, CA, 90095, USA

Crump Institute for Molecular Imaging, University of California, Los Angeles, CA, 90095, USA

Department of Molecular and Medical Pharmacology, University of California, Los Angeles, CA, 90095, USA.

Department of Microbiology, Immunology and Molecular Genetics, University of California, Los Angeles, CA, 90095, USA

The Howard Hughes Medical Institute, University of California, Los Angeles, CA, 90095, USA.

Department of Chemistry and Chemical Biology, Institute for Advanced Materials, Devices and Nanotechnology, The Rutgers Stem Cell Research Center, Rutgers, The State University of New Jersey, Piscataway, NJ 08854, USA.

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Supplemental Methods

General information

PDMS pre-polymers (Sylgard 184, AB kit) were purchased from Dow Corning. Photoresist SU-8 2100 and SU-8 2025 were purchased from Microchem Corp. AZ 50XT was purchased from AZ Electronic Materials. All biological reagents were purchased from Invitrogen and Sigma-Aldrich and were used according to manufacturer's protocol. Sterility of experimental setting was achieved as follows. Peripheral components, including metal pins, tubing and handling tools were autoclaved at 120^{o}C for 30 min or cleaned with 70% ethanol. Before use, the Cell-μChips were exposed for 15 min to bactericidal UV light[1]. Cell-μChip was inside a biosafety cabinet (Thermo Electron Corp., USA) during preparation and monitoring. For cell culture experiments, the Cell-μChips were maintained at 37^{o}C and 5% CO_2 in a conditioned incubator (HERAcell 150). A microscope (Nikon, TS100) equipped with a CCD camera (Hitachi, KP-D20BU) was utilized for routine monitoring during the Cell-μChip operation. Fluorescence imaging was performed on a Nikon TE2000-S equipped with a fluorescence lamp house (Photonic Solutions Inc., X-Cite 120) and a CCD camera (Photometrics, Cascade-II 512). The imaging software MetaMorph (Molecular Devices Corp.) was used to process the acquired images.

Cell culture

NIH 3T3 mouse fibroblast, HeLa human cervical and B16 mouse melanoma cells were obtained from the American Type Culture Collection. They were cultured using cell culture dishes in a humidified incubator (5% CO_2, 37^{o}C). Cells were grown in Dulbecco's Modified Eagle Medium (DMEM), containing 10% heat-inactivated fetal bovine serum (FBS), 0.2 mM glutamine, 100 units mL^{-1} penicillin and 100 μg mL^{-1} streptomycin (P/S). The cell lines were passaged at 80–90% confluence. Following trypsinization, cells at a concentration of 2 x 10^6 cells mL^{-1} in 10% FBS DMEM were introduced into the Cell-μChips.

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Fabrication of the Cell-μChip

The Cell-μChips were fabricated using multilayer soft lithography method.[2, 3] Two different molds were first produced by photolithographic processes to create the fluidic channels (channel width ranging from 100 to 500 μm, channel height: 40 and 100 μm) and the control channels (channel width: 25 μm, channel height: 35 μm).

Prior to the Cell-μChip fabrication, both the fluidic and control molds were exposed to trimethylchlorosilane (TMSCl) vapor for 5 min. A well-mixed PDMS pre-polymer (Sylgard 184, A and B in 10 to 1 ratio) was poured onto the fluidic mold located in a Petri dish to give a 6 mm-thick fluidic layer. Another portion of PDMS pre-polymer (A and B in 10 to 1 ratio) was spin-coated onto the control mold (2000 rpm, 30 s, ramp 5 s) to obtain a 60 μm-thick control layer. The thick fluidic layer and thin control layer were cured in an 80^{o}C oven for 20 min. After incubation, the thick fluidic layer was peeled off from the mold, and holes were introduced into the fluidic layer for access of reagent solutions. The fluidic layer was then trimmed, cleaned and aligned onto the thin control layer. After baking at 80^{o}C for 20 min, the assembled layers were peeled off from the control mold, and another set of holes were punched for access of control channels and for constructing tall medium reservoirs. These assembled layers were then placed on the top of a glass slide that was coated (2000 rpm, 20 s, ramp 5 s) with the PDMS pre-polymer (A and B in 10 to 1 ratio) that had been cured for 20 min in the 80^{o}C oven. The Cell-μChips were ready for use after baking at 80^{o}C for 48 h.

The control interface

The pneumatic control setup consists of one set of eight-channel manifolds (Fluidigm) controlled through a NI-DAQPad-6507 controller board (National Instruments) connected to a computer through the USB port. Compressed air provided pressure (20 psi) to the manifolds. Twelve control channels in the Cell-μChip were first filled with DI-water and connected to the corresponding channels on the manifolds with metal pins (23 Gauge, New England Small Pin Corp) using Tygon microbore tubing

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(Cole-Parmer East, Bunker Court). When a regulator on either the electronic or the manual manifold was activated, compressed air entered the respective control line connected with the regulator, thus providing pressure to close valves in the microfluidic device. The control interface for electronic manifold was created using LabVIEW program (Version 8.0, National Instrument Inc.), which allowed for the automation of the pumping processes. The manual control of individual valves was handled by toggle valves (MSC Industrial Direct Inc.) mounted on manifold to provide quick and free access.

Notes and references

1. A. Paguirigan, D.J. Beebe. Lab Chip **6**, 407–413 (2006) Medline doi:10.1039/b517524k
2. Y.N. Xia, G.M. Whitesides. Annu. Rev. Mater. Sci. **28**, 153–184 (1998) doi:10.1146/annurev.matsci.28.1.153
3. M.A. Unger, H.P. Chou, T. Thorsen, A. Scherer, S.R. Quake. Science **288**, 113–116 (2000) Medline doi:10.1126/science.288.5463.113

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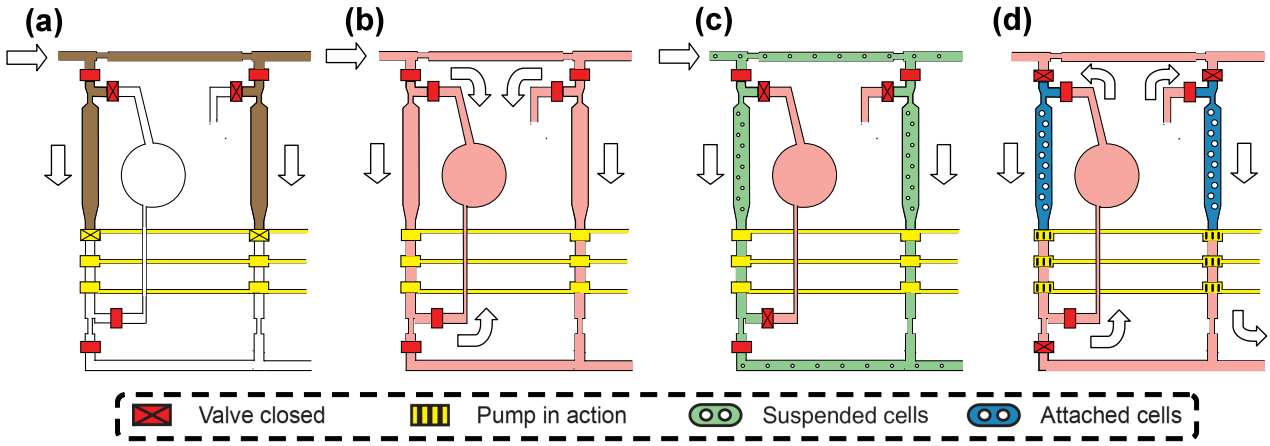

Fig. S1 Schematic diagrams that illustrate the four sequential processes for performing the on-chip cell culture experiments via the cooperation of valves and pumps. (a) Fibronectin coating: A fibronectin solution (1 mg mL^{-1}) is introduced to fill the cell culture chambers by a dead end filling approach in order to enhance the biocompatibility of the microenvironment. (b) Culture medium loading: A cell culture medium is loaded to replace the fibronectin solution. Sequentially, the medium reservoir is filled with the culture medium at an external pressure (10-15 psi). (c) Cell loading and immobilization: A cell suspension solution (2×10^6 cells mL^{-1}) is loaded into the chambers by gravitation, and the microchip is maintained at 37°C for cell adhesion. (d) Medium circulation or feeding: The conjugated peristaltic pumps are turned on to circulate medium in the cell culture chamber on left and to directly feed medium through the one on right. The circulating/feeding flow rates (0.1-4 nL sec^{-1}) are synchronized by the operating frequency of the pumps.

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