Supplementary Material (ESI) for Lab on a Chip
This journal is © The Royal Society of Chemistry 2009

Electronic Supplementary Information (ESI)

Continuously Perfused, Non-Cross-Contaminating Microfluidic Chamber Array for Studying Cellular Responses to Orthogonal Combinations of Matrix and Soluble Signals

Edward S. Park[a], Ashley Carson Brown[b], Michael A. DiFeo[a], Thomas H. Barker[b,c,d] and Hang Lu[a,c,d]*

[a] *School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA, USA.*
[b] *Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, USA.*
[c] *The Parker H. Petit Institute of Bioengineering and Biosciences, Georgia Institute of Technology and Emory University, Atlanta, GA, USA.*
[d] *Interdisciplinary Program of Bioengineering, Georgia Institute of Technology and Emory University, Atlanta, GA, USA.*
*Fax: 404 894 4200; Tel: 404 894 8473; Email: hang.lu@chbe.gatech.edu

Other considerations in device design

On-chip valves are extensively integrated into the design to provide fluidic isolation and execute different modes of operation. The valves are described as elastomeric "push-up" valves.[1] In order to close, fluid flows through a channel in the top layer, while a control channel in the bottom layer is pressurized. This causes the elastomeric membrane between the two layers to deflect into the upper channel, which stops flow. The push-up valve is chosen for its ease of integration, small dead volume, and robustness of its design (specifically with respect to membrane thickness) when contrasted with its counterpart, the "push-down" valve.[2]

Another important feature incorporated into the design is the seeding and attachment of cells onto the surface of the underlying (supporting) substrate of the device. Life scientists are well-familiarized with cell behavior on glass surfaces. Thus, glass is assumed to be a preferred substrate upon which cells are grown and assayed. However, a consequence of using push-up valves is that cells most conveniently approach each chamber from the top flow layer. For cells to make contact with glass in the chamber, they must travel by way of through-holes from the top layer to the bottom layer. Otherwise, cells would seed in the top layer on a surface made of the bulk material of the device.

The device in this work is designed with the default option to seed cells on glass or other rigid substrate (e.g. polystyrene). This is necessary if high-magnification microscope objectives with small working distances are utilized (requiring cover slips as the substrate). Nevertheless, if one desires for cells to grow on PDMS or another soft material, it can be coated to the appropriate thickness on the substrate prior to attaching the device.

Fabrication: further notes

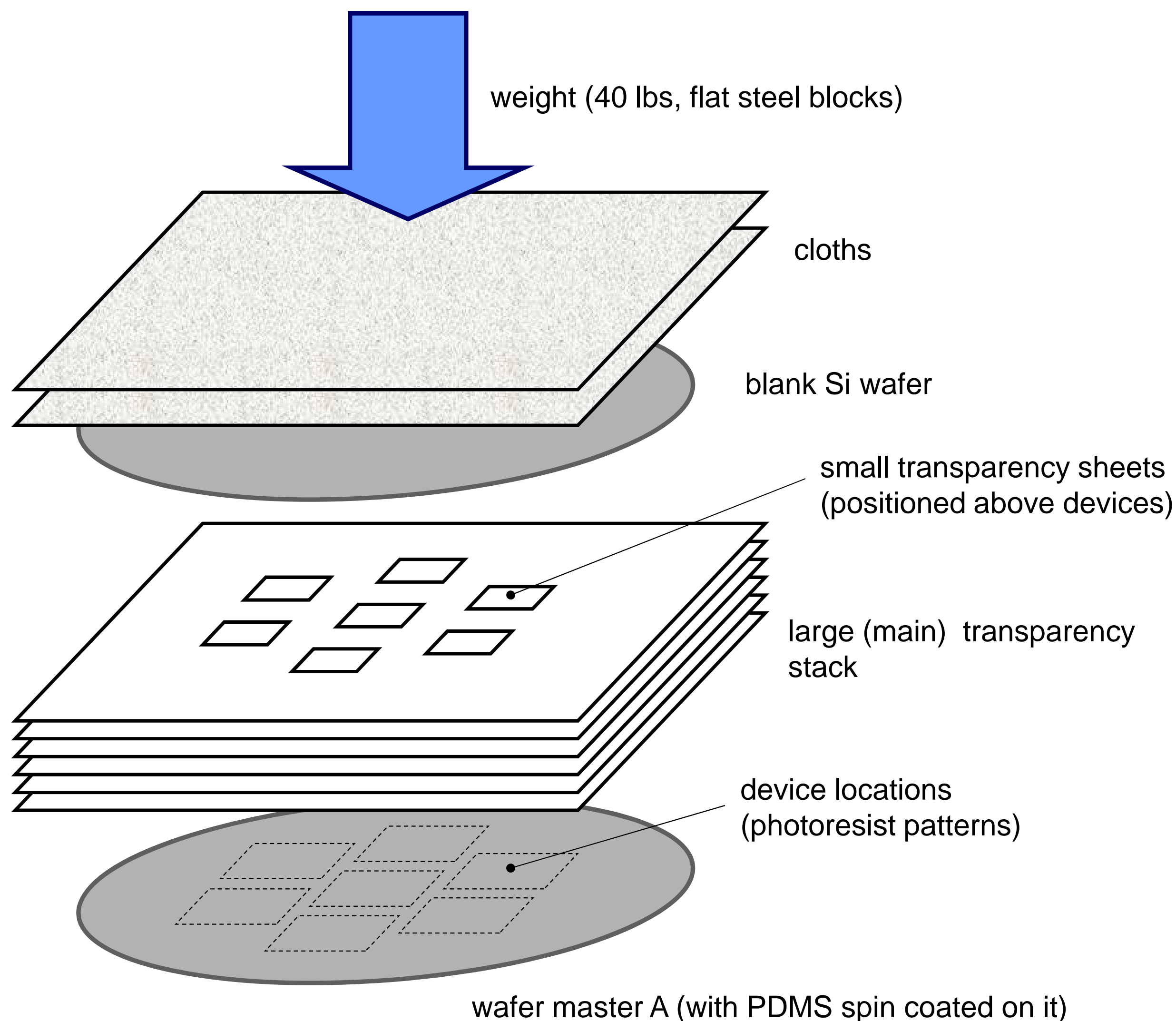
Wafer master A: Unlike most soft lithography processes, no silane is deposited on the surface of master A. In previous iterations, we observed that using a master with a silane layer causes the PDMS to stick to the transparency during the compression-molding process. Therefore, when the transparency is peeled off the master, the PDMS is peeled off with it. We found that an unsilanized master causes the PDMS to remain on the master; at the same time, there were no problems releasing the PDMS from the master after the full cure had taken place.

Transparencies: To further prevent the PDMS from sticking to the transparency, a silane layer is vapor-deposited onto the transparency. A few drops of silane (T2492, United Chemical Technologies, Bristol, PA, USA) are placed in a glass vial in a desiccator with the transparency. The desiccator is evacuated and silane vapor allowed to deposit for 30 min.

Wafer master B: Master B is first patterned with SU-8 photoresist. A layer of SU-8 10 µm-thick is spin coated onto the wafer and blanket exposed, forming an adhesion layer. A second layer of SU-8 is spin coated to a thickness of 7 µm and patterned to form the conduits that link through-holes from the diverted flow channel to through-holes in the protected area. The wafer is then well rinsed in isopropanol and blow dried with nitrogen. Hexamethyldisilazane (MicroPrime HP Primer, Shin-Etsu Chemical Co., Tokyo, Japan) is spin coated onto the wafer at 3000 rpm, and the wafer is placed on a hotplate at 110 C for 45 sec to evaporate residuals. AZ P4620 is then spin coated and processed per manufacturer's specifications.

1. M. A. Unger, H. P. Chou, T. Thorsen, A. Scherer and S. R. Quake, *Science*, 2000, **288**, 113-116.
2. V. Studer, G. Hang, A. Pandolfi, M. Ortiz, W. F. Anderson and S. R. Quake, *J. Appl. Phys.*, 2004, **95**, 393-398.

This journal is © The Royal Society of Chemistry 2009

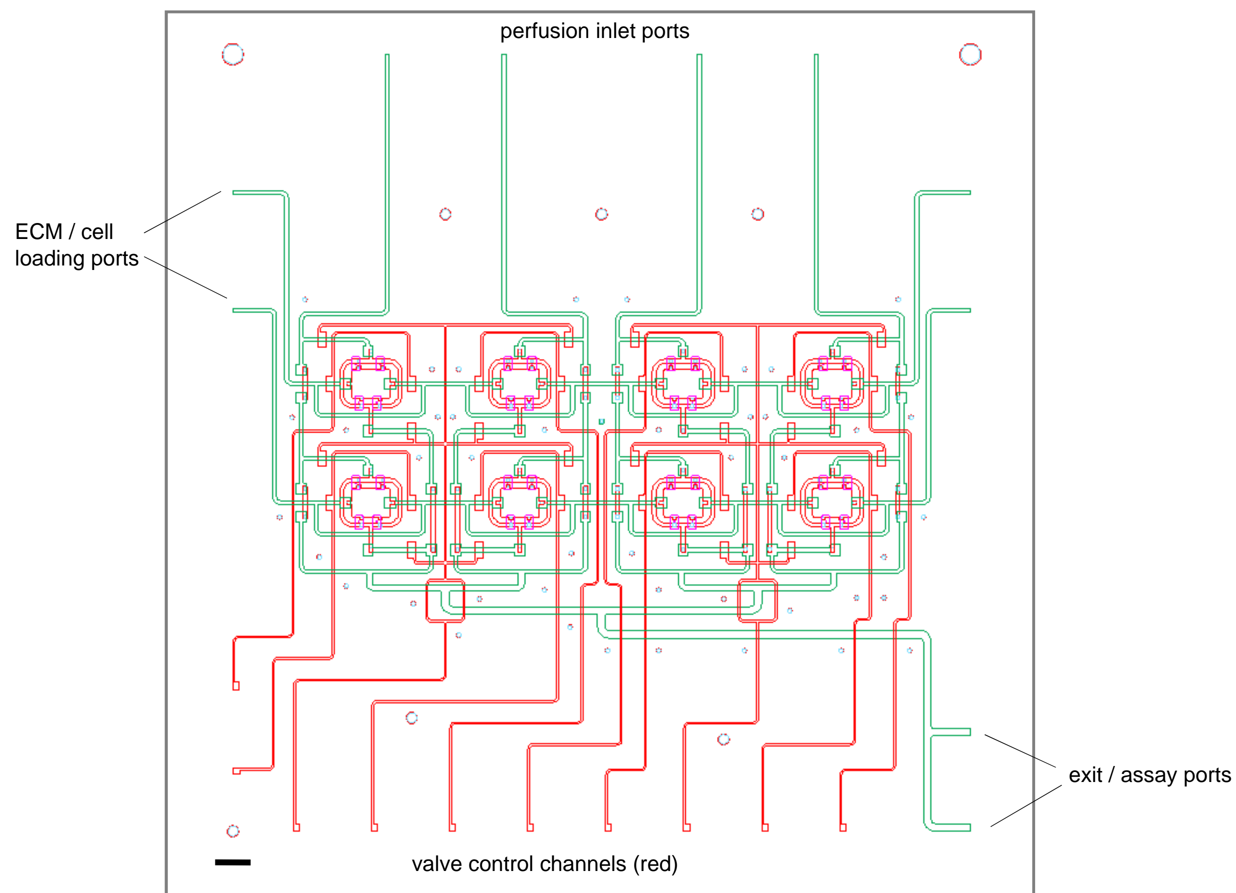


ESI Figure 1. Stack for compression-molding fabrication process. Small transparency sheets cut to the size of each device are placed on the main transparency stack. Small sheets are aligned to device locations. The main stack and small sheets are Scotch®-taped together to maintain correct relative positioning. When weight is applied, pressure is focused above each device, and PDMS is laterally displaced from the tops of photoresist features. The use of small sheets improves the reliability of defining through-holes (completely removing residual PDMS that could block the flow path) in the device described in this work.

This journal is © The Royal Society of Chemistry 2009

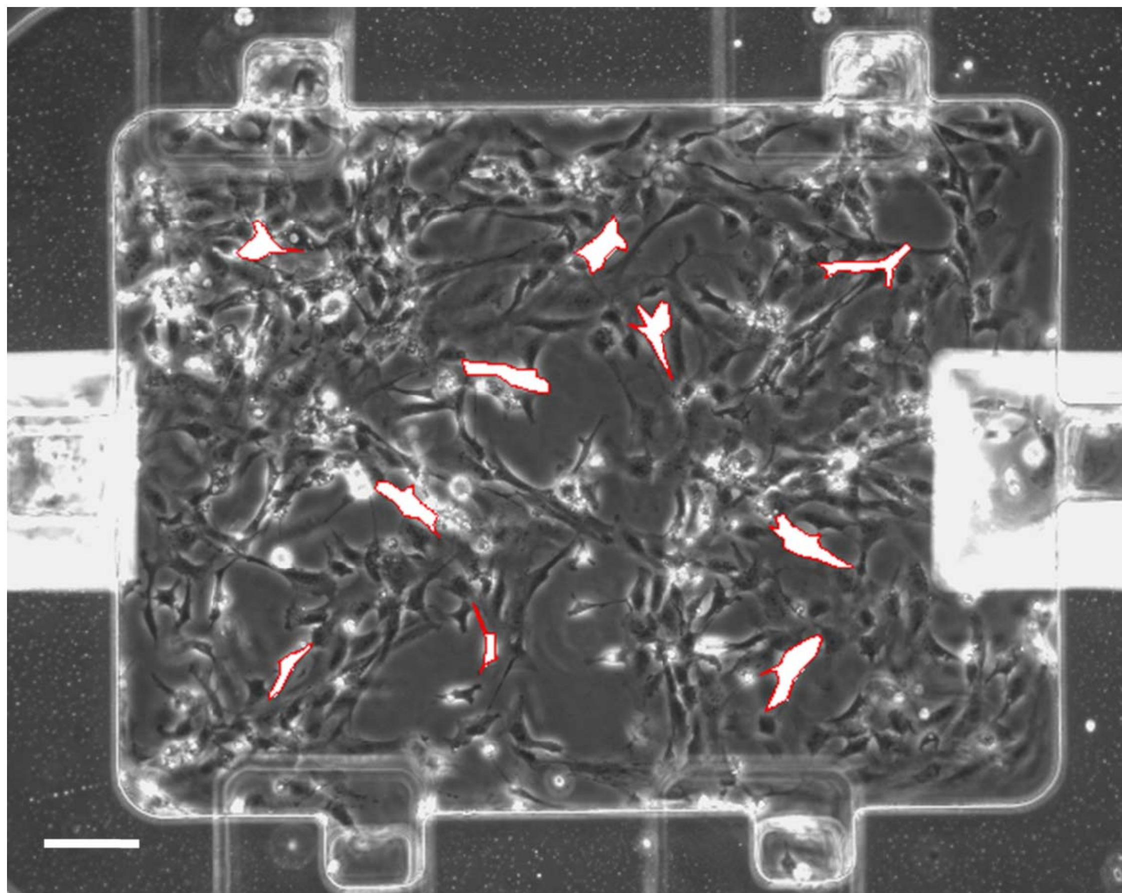
ESI Figure 2. Array device mounted to microscope stage. Line of 10 L-shaped steel pins are inserted into ports for on-chip valve control channels. Other pins are inserted into flow ports. Dimensions of PDMS device are 25 x 25 x 4 mm. Underlying substrate is a 25 x 75 x 1 mm glass slide. For high magnification observation (40X+), a glass cover slip can be used as the substrate.

This journal is © The Royal Society of Chemistry 2009



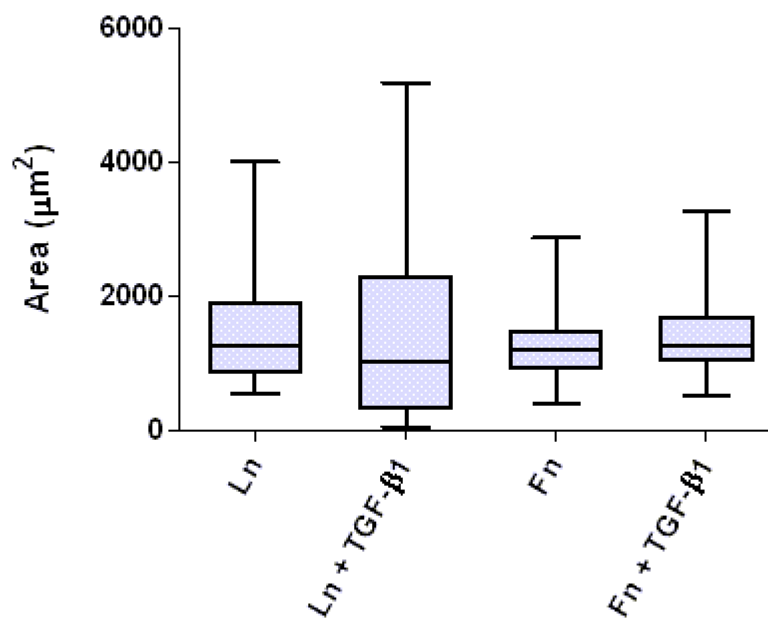
ESI Figure 3. Diagram of array device. Scale bar 1 mm.

This journal is © The Royal Society of Chemistry 2009



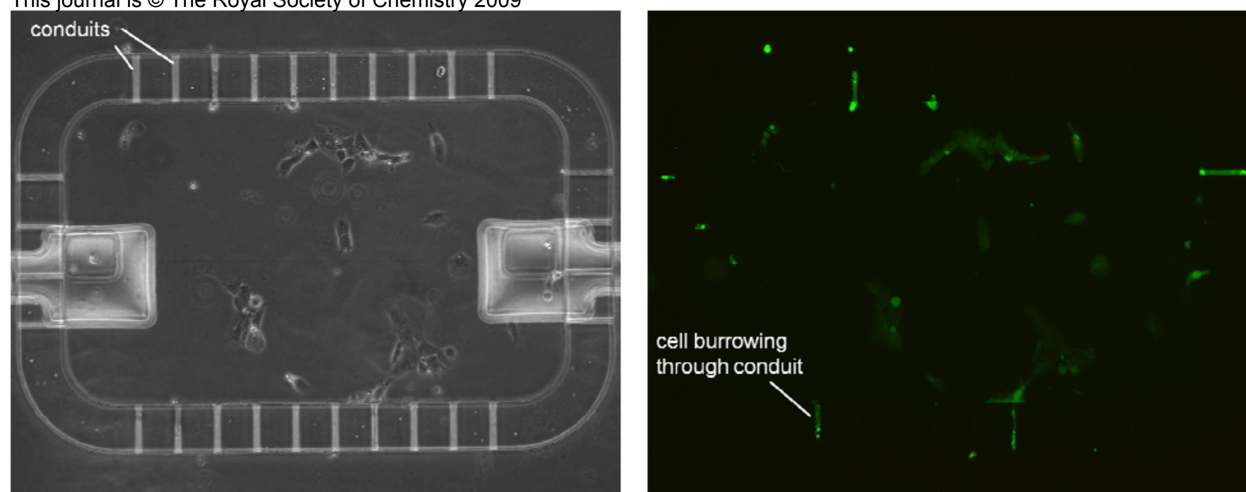
ESI Figure 4. Sample images of cell traces (Fn only condition). Cells in phase contrast images are traced by polylines and closed into polygons. Polygons are color-filled for easy identification, after which the image is thresholded to remove all features except polygons. Morphological characteristics (circularity and area) are then calculated. Cell tracing and calculations performed using Image Pro 6.1 software. Scale bar 100 μm.

This journal is © The Royal Society of Chemistry 2009



ESI Figure 5. Cell areas calculated from traces. Slight differences observed; no statistical significance ($p > 0.05$). Box plot shows 25th and 75th quartiles (median line inside). Whiskers denote max and min values. Data from 30 cells (3 trials, 10 per trial) for each condition.

This journal is © The Royal Society of Chemistry 2009



ESI Figure 6. In the first generation device design, high-resistance conduits were located on the floor of the chambers. Cells tended to clog high-resistance conduits on the floor, as shown by the phase contrast and fluorescent LIVE/DEAD images of the same field of view. Cells in conduits evident from green fluorescence. Migration/proliferation of cells into the conduits disrupted the flow field and mass transport into the protected area (cell growth area). Conduits in the first generation device were 50×10×2 μm (L×W×H). To avoid clogging, most recent designs incorporated conduits on the ceiling of chambers, as described in the main article. We observed that cells rarely migrated/proliferated into conduits on the ceiling.

ESI Movie 1. Cells are loaded into the array. Cells begin flowing into a row of chambers, and each chamber is scanned for proper cell density. The stage briefly pauses at each chamber as valves are re-opened and closed to sample a new set of cells; once satisified, the stage is translated to the next chamber. This is followed by an overall scan of the device. Movie is 2 fps, running at 4X real time.