Supplementary Information for “3D-Printed Microfluidic Automation”
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Supplementary Notes:

Modularity in SL

Modular design is commonplace in microelectronics, aeronautics, and car design, among many fields. In SL, by “modular design” we refer to a design paradigm where designs can be constructed by digitally joining and editing other design portions (“modules”) before printing. Very large CAD libraries of 3D modules are already available online for free (e.g. http://grabcad.com, http://www.partcloud.net). This terminology has been extended to physical modules, i.e. designs that are assembled from several pieces after printing: complex modular 3D microfluidic circuits have been built with SL (see, e.g. 4-6).

Importance of SL in the commercialization of devices

SL, a form of 3D-printing invented in the 1980s, facilitates the commercialization path by simplifying the fabrication process10. SL-printed “plug-and-play” (industry-standard) connectors substantially enhance user-friendliness compared to PDMS devices. There are many mail-order SL services that offer quasi-automated fabrication and quotes via the web (which helps project costs). Inexpensive SL desktop printers are now available (SL has seen a rapid decline in cost due to the expiration of key patents in 2014). SL can deliver from a single part to medium-volume throughputs (hundreds of parts), which facilitates the launching phase of a product (the 3D-Printing news website http://www.3ders.org offers daily news on new SL products).

Cost of devices

In SL commercial services, cost is computed as a function of the time spent printing by the machine, which tends to be a function of the dimensions of the device, and a one-time service cost is added to account for the engineer-operator’s time in setting up the SL machine. The devices described in this paper cost between $20 and $36 per unit, not counting initial setup costs. For example, the first print of the single-valve device (the least expensive) cost $158, but a second (and third, fourth, etc.) copy cost only $20; similarly, the four-valve device (the most expensive) cost $159, but a second (and third, fourth, etc.) copy cost only $36. These costs are much smaller than the costs of any PDMS-molded device once salaries and materials are computed10. Injection-molded devices can be produced at much smaller cost per unit (<$1), but only if very large number of units are produced and after a substantial investment due to the cost of the metal molds.

Biocompatibility of WaterShed resin

WaterShed XC 1112 meets biocompatibility standards ISO 10993-5 Cytotoxicity, ISO 10993-10 Sensitization, ISO 10993-10 Irritation, and USP Class VI13. C2C12 cells cultured for 24 h on planar WaterShed surfaces were morphologically indistinguishable from cells cultured on tissue culture polystyrene (Fig. S1). In some cases, the solvents used to rinse uncurd resin from complex microchannels can result in decreased cell viability on those devices. A set of processing standards for maximizing biocompatibility, requiring the use of fresh isopropanol as the rinse solvent, allows devices built in WaterShed to be designated as “BioClear”.

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Fig. S1. Cell viability on WaterShed resin. (A) C2C12 cells cultured on tissue culture polystyrene (TCPS) control surfaces coated with Matrigel; the cells have been stained for viability with Live/Dead cell stain after 1 day in culture. Green cells are viable and red cells are dead. (B) C2C12 cells cultured on WaterShed surfaces coated with Matrigel using the same seeding and coating protocols as in (A); cell viability on WaterShed surfaces is indistinguishable from that on tissue culture polystyrene surfaces.
Fig. S2. The six-phase actuation sequence used to control the pump. (A) Schematic illustration of the valve states in the six-phase actuation sequence. (B) Waveforms of the open and closed states, with valves V₁ and V₃ open for time T.
Fig. S3. Analysis of individual CHO cell responses to multiple ATP applications. A mosaic of the 947 ATP-responsive cells identified by increase in the fluorescence of Fluo-4 calcium indicator is shown for before (A) and after (B) perfusion with ATP at the times indicated in Fig. 8b,c. Cells were exposed to 4 applications of 3 concentrations of ATP.
Fig. S4. Implementation of drainage holes to simplify device architecture. (A) Each of the control channels are fabricated with a 635 µm x 635 µm outlet hole (indicated by arrows) so that the resin can be easily drained to the exterior (arrows). (B) The drainage holes are sealed with adhesive tape.
Movie Captions for “3D-Printed Microfluidic Automation”

Movie S1
**Actuation of a single valve unit.** A single-valve device with blue dye in the fluid channels is repeatedly opened and closed. The valve is closed by applying 5 psi of pneumatic pressure to the control channel of the device, which is sufficient to close the valve against 0.5 psi fluid driving pressure. The valve is opened by returning the control channel to atmospheric pressure.

Movie S2
**Switching between two dyes.** A two-valve switching device, with blue dye in the left valve and red dye in the right valve, is cycled between various states to allow only blue dye to reach the outlet, to allow only red dye to reach the outlet, or to allow a combination of blue and red dye to reach the outlet. A small air bubble trapped in the right valve moves when that valve is opened.

Movie S3
**Operation of a peristaltic pump.** A peristaltic pump device filled with blue dye is actuated in the six-phase sequence (Fig. S2) with a minimum valve opening time of 200 ms. A control pressure of +5 psi is applied to close the valves, while -2.5 psi control pressure (vacuum) is applied to open the valves. The fluid flow resulting from this peristaltic sequence can be seen in the tubing placed next to the device.

Movie S4
**Fluid switching in the four-valve device.** A four-valve switch is connected to a cell chamber by a length of tubing, and the dye which is being perfused into the cell chamber is controlled by sequentially adjusting the states of the four valves, such that only one of the valves is open at a given time. At the end of the movie, the dye is replaced with clear buffer.

Movie S5
**CHO cell responses to ATP.** This movie shows the calcium response of Fluo-4-loaded CHO cells in response to perfusion with solutions of ATP. This excerpt depicts only the second series of three concentrations of ATP over the time frame indicated in Fig. 8c. The timing of ATP applications is indicated on the bottom. The field of view corresponds to the same full image (4x) shown in Fig. 8a. The movie plays at 20x speed.