Electronic Supplementary Material (ESI) for Lab on a Chip.
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Adding the 'heart' to hanging drop networks for microphysiological multi-tissue experiments

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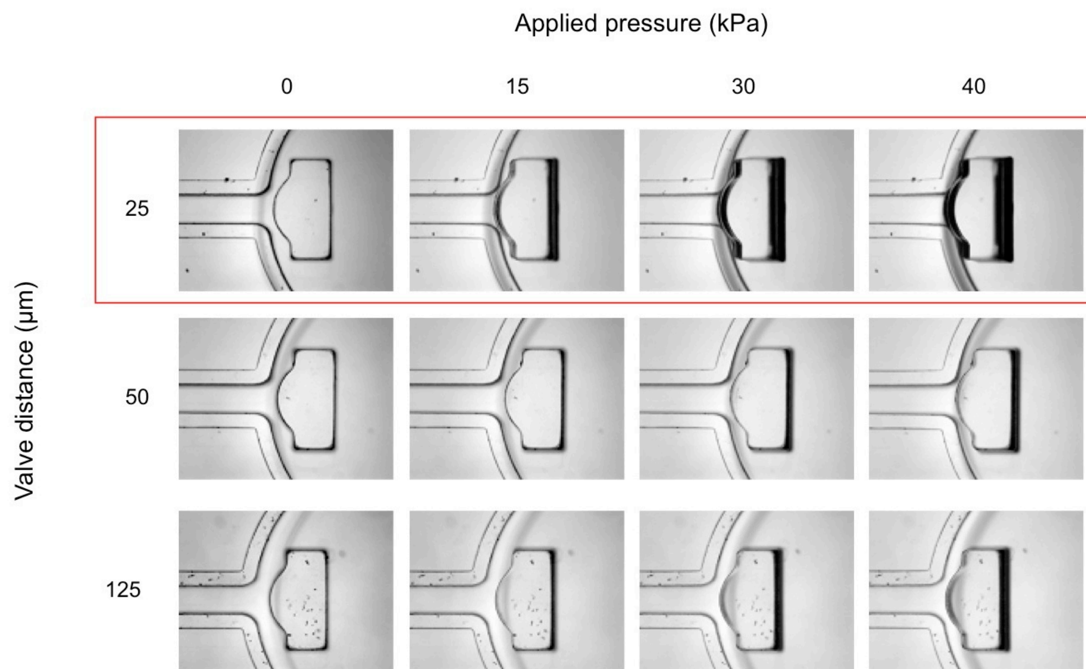
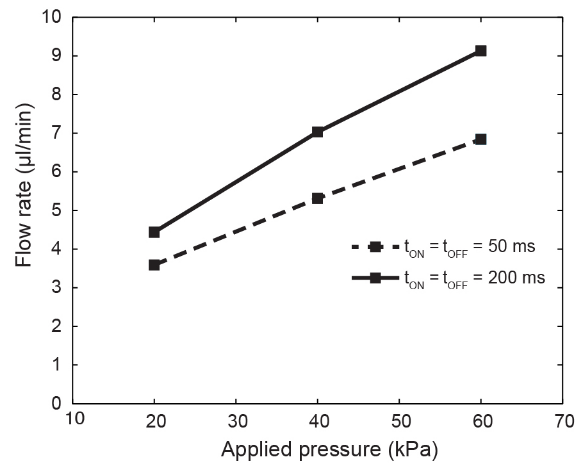
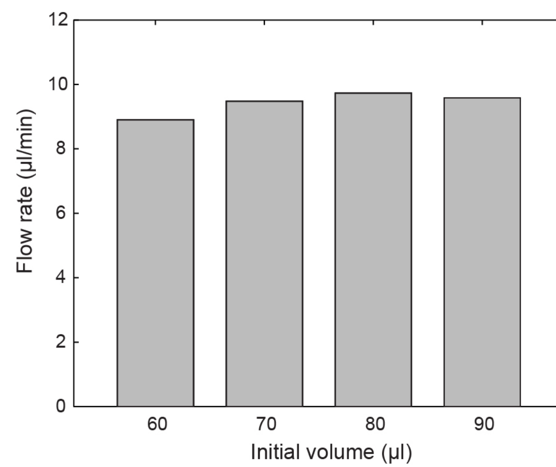
Supplementary Information



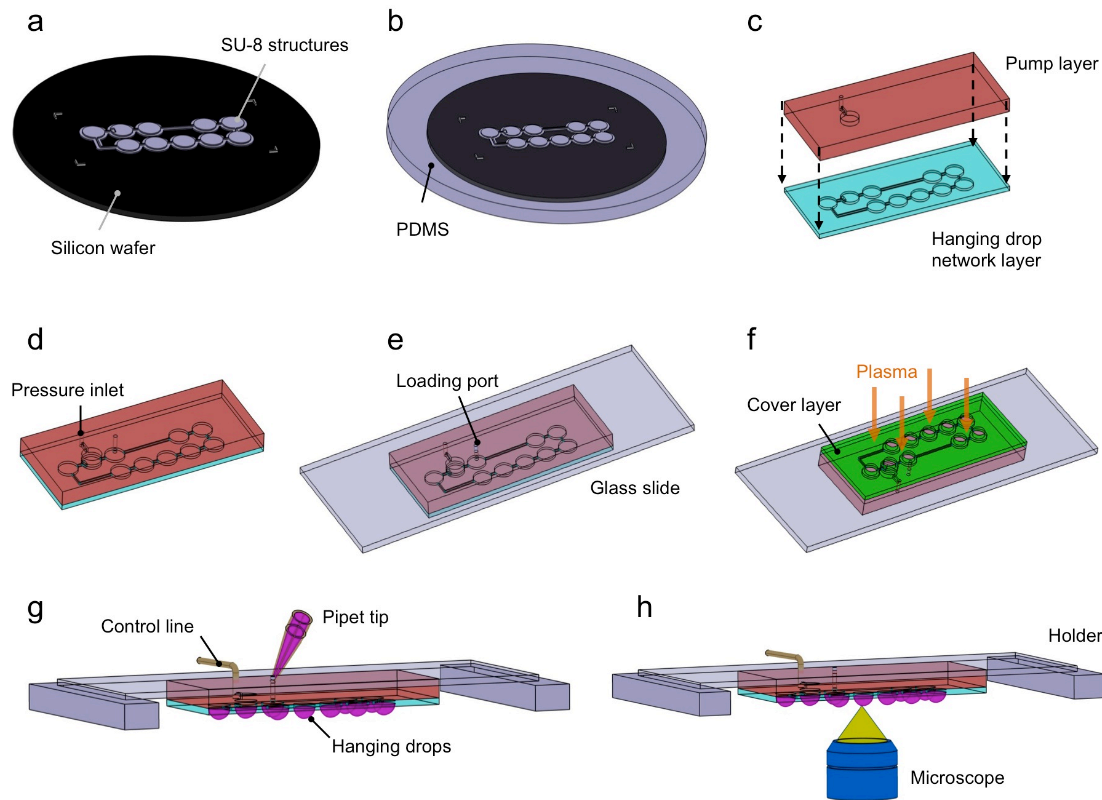
Supplementary Figure 1. Micrographs of different valve designs, in which the PDMS block has been positioned at different distances from the inlet (25, 50 and 125 µm). The closing characteristics is shown using different applied pressures (0, 15, 30 and 40 kPa) in the pressure chamber above. The final version used is framed in red.



Supplementary Figure 2. Variation of the flow rate in dependence of the pressure applied to the pneumatic chamber above the pump drop.



Supplementary Figure 3. Effect of the loaded liquid volume into the chip on the flow rate. A total chip volume between 60, 70, 80 and 90 µl results in drop heights of 540, 700, 820 and 920 µm, respectively ($t_{ON} = t_{OFF}$ = 200 ms; applied pressure of 60 kPa).



Supplementary Figure 4. Fabrication process of the microfluidic devices. (a) A 4-inch silicon wafer is patterned with two layers of SU-8 using standard photolithography to create a casting mold. (b) Freshly mixed PDMS is poured onto the SU-8 mold and cured to from the hanging drop network layer. (c) Using the same process, a second SU-8 mold is fabricated (single SU-8 layer), from which the pump layer is obtained. Both PDMS layers are aligned and bonded to each after oxygen plasma activation. (d) An inlet for pressurized air is bunched through the pump layer before bonding. (e) The two bonded layers are bonded to a glass slide after oxygen plasma activation for mechanical stability. The glass layer has drilled holes for liquid loading and connecting tubing. (f) Before liquid loading, a cover layer is placed onto the structured PDMS, covering the rims and only exposing the drop structures to oxygen plasma thereby rendering them hydrophilic. (g) Liquid is loaded using a conventional pipet forming the hanging drop network and the control line is connected. (h) The hanging drop network device is placed into a holder on an inverted microscope for characterization and cell culturing.

Supplementary Movie 1. Real-time video of 5-µm beads flowing in the 200-µm-wide bottom-open channel. Continuous flow using actuation protocol: t_{ON} = 50 ms, t_{OFF} = 50 ms; applied pressure of 40 kPa.

Supplementary Movie 2. Real-time video of 5-µm beads flowing in the 200-µm-wide bottom-open channel. Pulsatile flow using actuation protocol: t_{ON} = 200 ms, t_{OFF} = 200 ms; applied pressure of 40 kPa.

Supplementary Movie 3. Time-lapse movie of ink circulating through two 4 x 6 arrays in parallel. Actuation protocol: t_{ON} = t_{OFF} = 200 ms, applied pressure of 40 kPa, initial liquid volume of 250 µl. Time indicated are MIN:SEC.

Supplementary Movie 4. Circulation of beads through the complete network in real-time. Microscope stage is adjusted manually while the pump was running. Actuation protocol: t_{ON} = t_{OFF} = t_{DELAY} = 200 ms, applied pressure of 40 kPa.

Supplementary Movie 5. Real-time video of a beating cardiac microtissue (hCdMT) during pump actuation. Temperature was 37 ^{o}C, resulting beating rate was 91 min^{-1}.