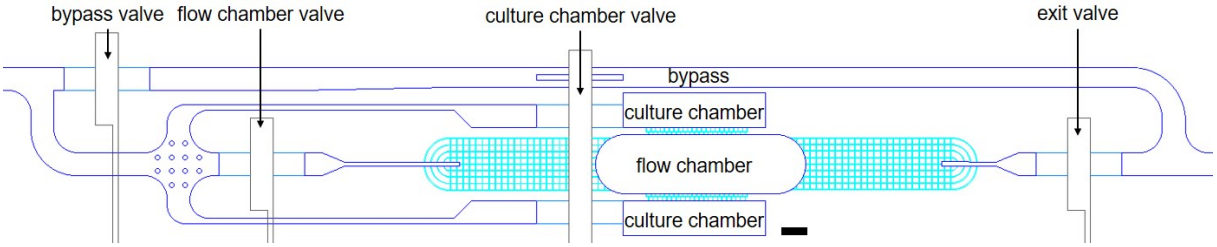
Electronic Supplementary Material (ESI) for Lab on a Chip.

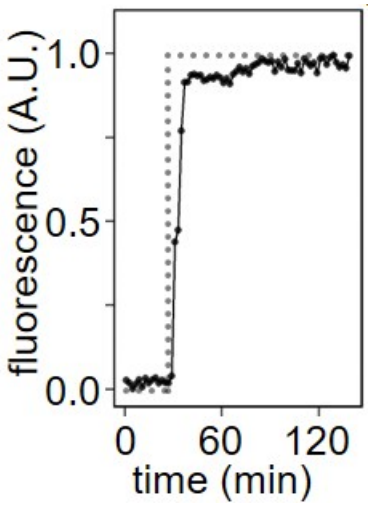
Supplementary Material

Supplementary Figures



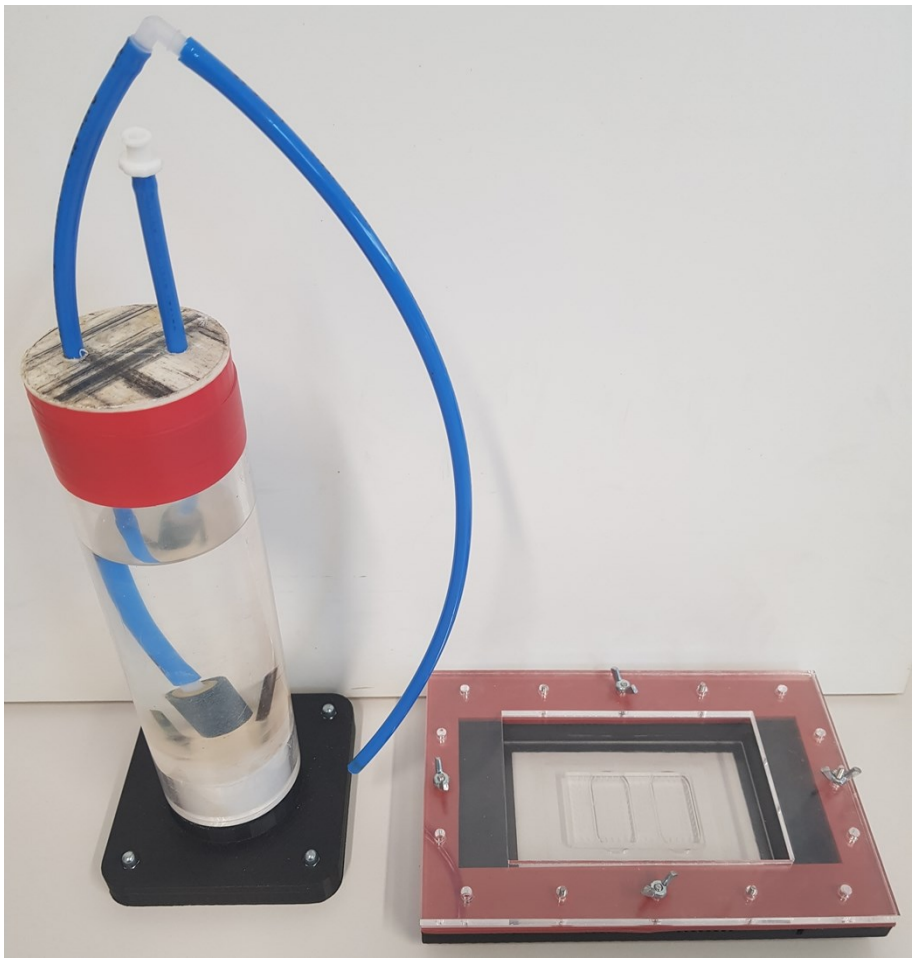
Supplementary Figure 1: Chamber unit design.

Two culture chambers flank a shared flow chamber. PDMS membrane valves control flow into bypass channels, culture chambers, and flow chambers. Diffusion between flow chamber and culture chambers via capillaries establishes equilibrium. A 3 µm wide 4 µm tall capillary network before and after flow chambers prevents bulk fluid flow during valve opening and closing. Scale bar = 100 µm.



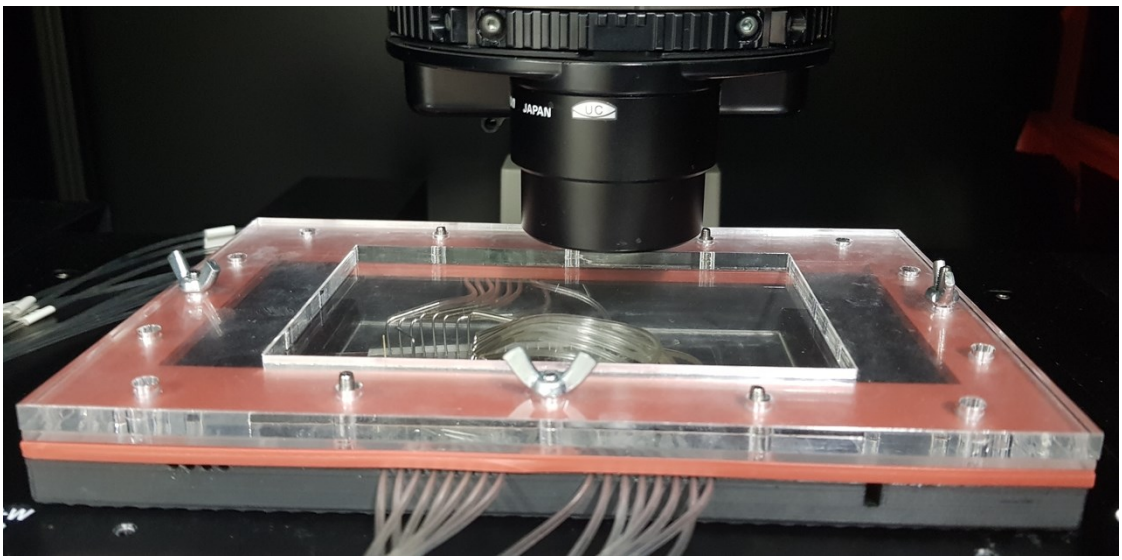
Supplementary Figure 2: Large molecules can be applied to chambers on short timescales

Molecules with the typical size of standard cell culture cytokines rapidly diffuse into cell culture chambers. 40 kDa FITC-Dextran (300 µM) is imaged every 90 s as it is applied on a 2 min interval, 2 min with flow followed by 2 min without, and reaches 90% saturation in culture chambers within 6 minutes of diffusion from the flow chamber.



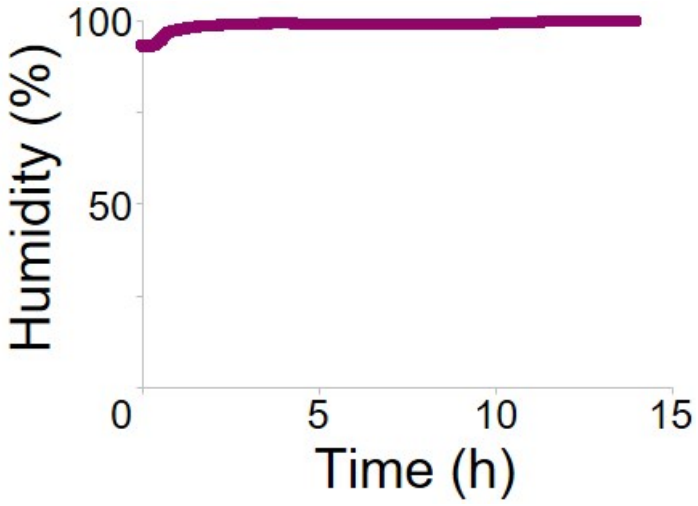
Supplementary Figure 3: 3D printed incubation chamber and humidifier produce near saturation humidity levels.

Our 3D printed gas humidifier and chip enclosure allow fast setup of chip experiments and provide maximum humidification of PDMS devices for effective cell culture experiments. Note that the lid is hard to see due to its transparency in this picture. See Suppl. Fig. 2 to see its location.



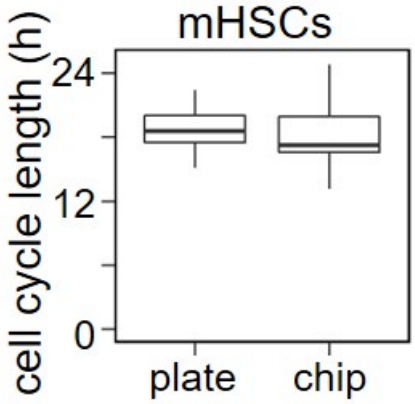
Supplementary Figure 4: 3D printed incubation chamber provides easy tube management and robust chamber sealing.

Our custom-made enclosure provides slots for tubing organization that enable proper function of all chip components and fluids while minimizing gas consumption during atmospheric conditioning. Note that unused tubing access slots are plugged to seal the incubator during experiments (not done here for improved recognition).



Supplementary Figure 5: 3D printed incubation chamber and humidifier produce near saturation humidity levels.

Relative humidity measured within our incubation chamber stabilized at or above 99% for at least 15 h.



Supplementary Figure 6: Murine HSCs cultured in the chip behave normally.

Murine HSCs cultured in our chip and a 384 well plate showed comparable mean cell cycle lengths (calculated from the slope of $\log_2$ transformed hourly cell counts over 72 hours). 3 replicates with mean cell cycle lengths 20.7 (20.1, 21.3, and 19.3) and 19.1 (20.0, 19.5, and 18.7) hours, respectively ($p = .228$, Welch two sample t-test, 3 experiments).

Supplementary Movies

Supplementary Movie 1: Capillary networks effectively capture 10 µm polystyrene beads.
10 µm Polystyrene beads in PBS flow into a set of culture chamber units and cannot pass through the capillary network. Bright field images were inverted for improved identification of beads.

Supplementary Movie 2: Murine HSCs are concentrated within cell culture chambers.
mHSCs flow into a cell culture chamber and are efficiently captured by the capillary network by size exclusion.

Supplementary Movie 3: Rapid signal oscillations can be sustained via diffusion.
40 kDa FITC-Dextran in a PBS solution oscillates between 300 µM and 0 µM within cell culture chambers over 15 min during a simulated oscillating signal pattern.

Supplementary Movie 4: Flow-free stimulation limits motion of non-adherent particles.
Single 10 µm polystyrene beads in cell culture chambers remain stationary during 60s of media flow through the flow chamber at 0.14 µl min^{-1}. Images were taken every 1 s in order to observe rapid bead translocations that may occur during flow. No major bead translocations could be detected. Time code = mm:ss.

Supplementary Movie 5: Flow-free stimulation enables tracking of hHSCs.
Single hHSCs are reliably tracked during a two-hour flow-free stimulation experiment.

Supplementary Movie 6: Murine HSCs can be cultured for 72 hours.
mHSCs cultured in serum free and phenol red free expansion media survive for multiple generations during a time-lapse imaging experiment conducted in the chip over 72 h.