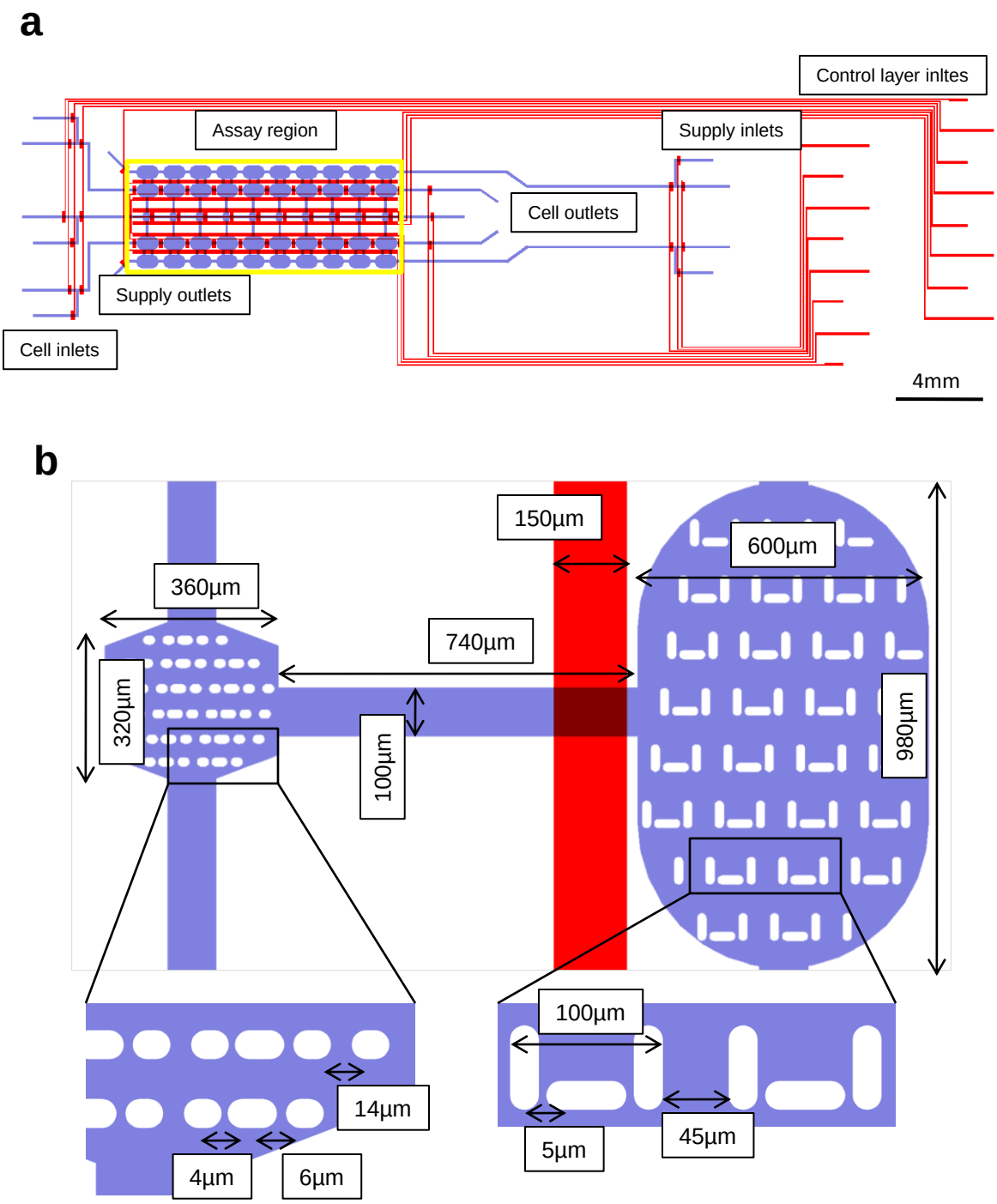
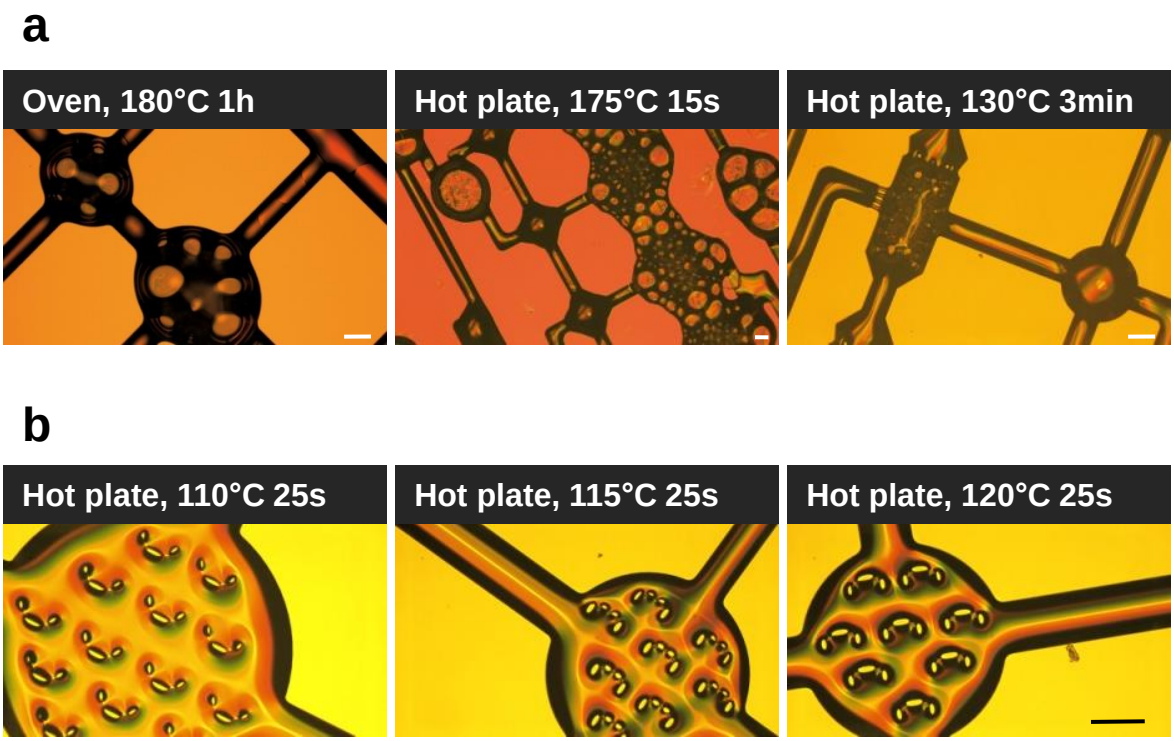
Electronic Supplementary Material (ESI) for Lab on a Chip.



Supplementary Figure 1

Design of the chemotaxis co-culture device.

Full design for co-culture chemotaxis analysis (**a**) with control layers in red and flow layers in blue. Cell inlets are on one side and supply inlets on the other side of the assay region (marked by a yellow box). Zoomed views (**b**) of the responder (left) and producer chamber (right) connected to the migration channel with details about their geometry. The traps in the responder chambers are made of pillars of 20 x 26 µm or 20 x 34 µm. The rows of traps are spaced at 32 µm distance from each other and offset from the previous row by 4 - 12 µm (randomized). The traps in the producer chambers are made of pillars of 20 x 58 µm with a row spacing of 58 µm. Each row of traps is offset from the previous by 15 – 25 µm (randomized). We used asymmetric offset rows of traps because they were previously described to be better at capturing cells throughout the chamber when compared to symmetrically offset rows. [17]

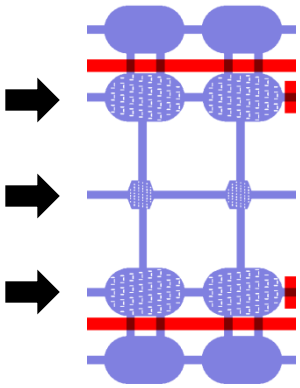


Supplementary Figure 2

Optimization of master mold production for the flow layer .

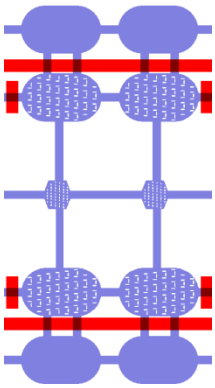
The efficient closure of flow channels in the assembled device requires rounded channel profiles. Twenty one baking conditions for the wafer with the flow layer design were tested to optimize reflow of the photoresist after photolithography. Images diplay examples of (**a**) sub-optimal and (**b**) optimal baking conditions for preserving the pillar structures which are necessary for highly efficient trapping of mammalian cells. Scale bars, 100µm.

a



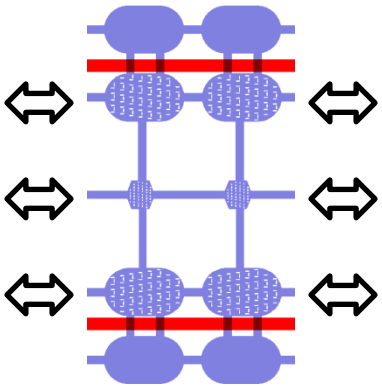
Collagen purge
45 min, RT

b



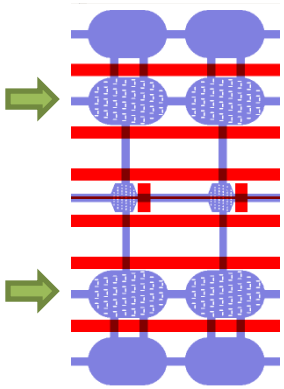
Incubation
45 min, 37°C

c



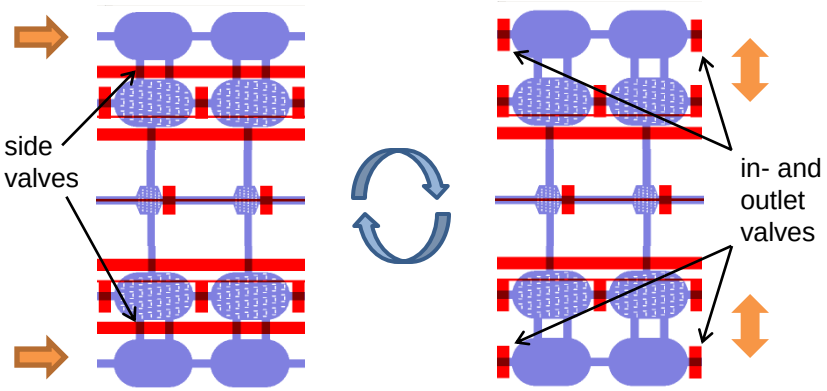
Chip rinsing
with 1xPBS

d



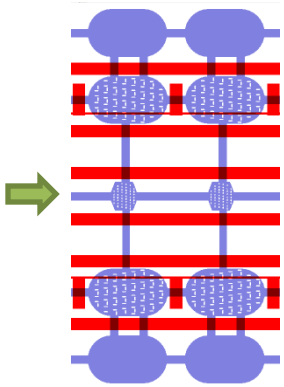
producer loading

e



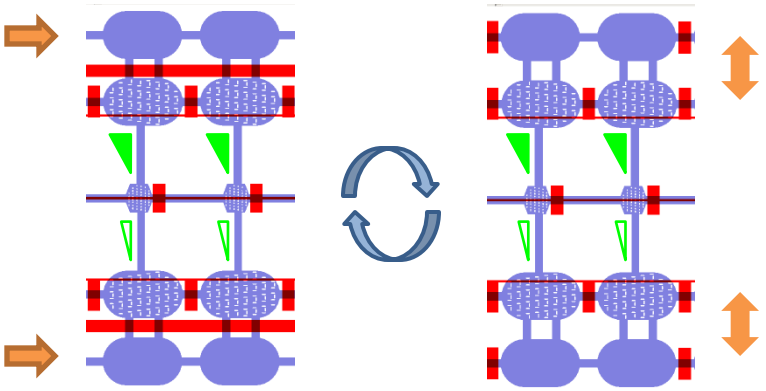
pulsed supply with 60/30 cycling for 12h

f



responder loading

g

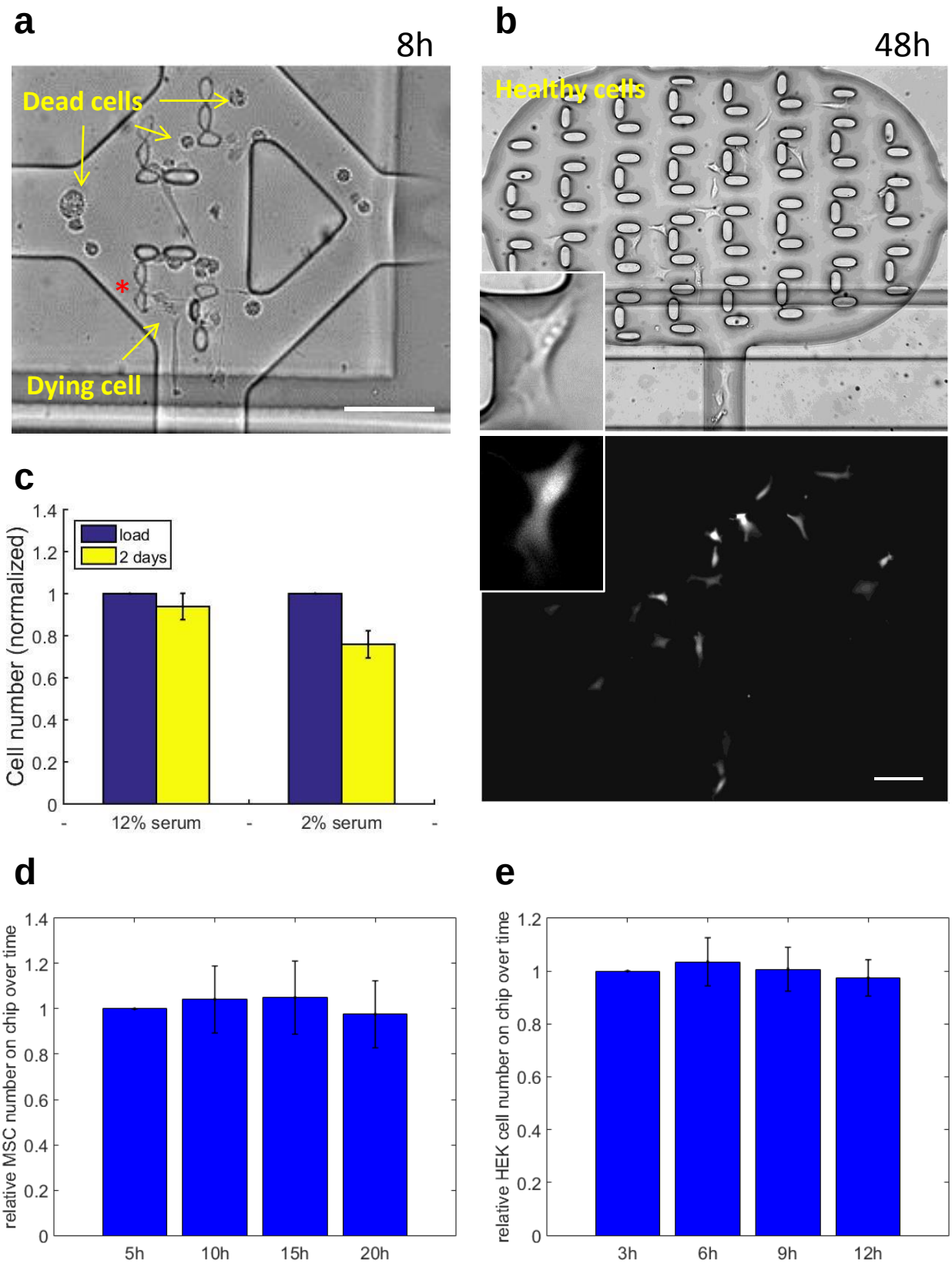


Co-culture and pulsed supply with 35/5
cycling for 12h to 20h

Supplementary Figure 3

Schematic overview of the protocol for chip preparation, cell loading and pulsed supply.

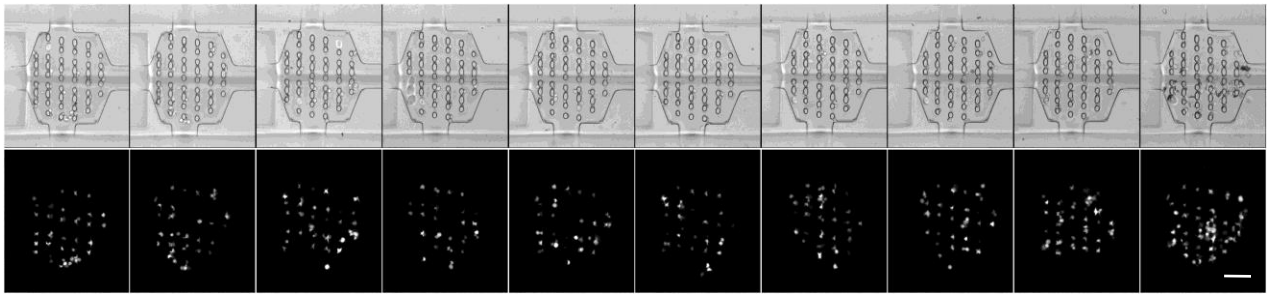
Two basic units are displayed to exemplify the procedures used for the chemotaxis assay. On-chip valves (red) were used during collagen coating of the chip (**a, b** and **c**), cell loading (**d** and **f**), cell feeding (**e** and **g**) and protein gradient generation (**g**). The empty chip was first purged (**a**) with a collagen solution (black arrows) through the cell inlets while the cell outlets were closed. Then the inlets were closed (**b**), the flow was stopped and the collagen-filled chip was incubated at 37°C. The chip was rinsed twice with PBS (**c**; white arrows), alternating from both sides, for 10 min. Next, producer cells (green arrows) were loaded (**d**) into the producer chambers. For this, valves on both sides of the chambers were closed while the interconnecting valves were opened. The chip is symmetrically designed and thus the loading of both control and experimental cells is simultaneous. After the producer cells adhere they are fed by "pulsed supply" (**e**) through the side channels: first i) fresh culture medium (orange arrows) was flown through the side channels while the side valves were closed, then ii) the flow was stopped by closing valves at the in- and outlets, the side valves were released and the medium was allowed to diffuse between side channels and chambers. This cycle of medium flow/medium exchange by diffusion was automatically repeated in defined intervals (e.g. for L-cells, 60 min flow, 30 min diffusion, abbreviated as 60/30). Nutrient deprivation in regular cultures served as an estimation to determine the pulsed supply frequency, e.g. in regular cultures 293T cells require frequent medium change while the L-cells do not show decreased viability even after 3-4 days without medium change. After 12h, responder cells were loaded (**f**) to the central chambers and the co-culture (**g**) was started after 1 h by releasing the valves between the chambers and the migration channels. Using again pulsed supply (now 35/5 cycles) during the co-culture period resulted in formation of concentration gradients (green triangles) in the experimental (top) and control migration channel (bottom). The chemotaxis assay was stopped when the first responder cells were reaching the end of the migration channel and/or when they started mixing with the producer cells. Thus the length of the assay was set to 12h for HEK and to 20h for MSC.


a
8h
Dead cells
Dying cell
b
48h
Healthy cells
c
Cell number (normalized)
load
2 days
12% serum
2% serum
d
relative MSC number on chip over time
5h
10h
15h
20h
e
relative HEK cell number on chip over time
3h
6h
9h
12h

Supplementary Figure 4

Viability of primary stem cells in microfluidic chambers of nanoliter scale

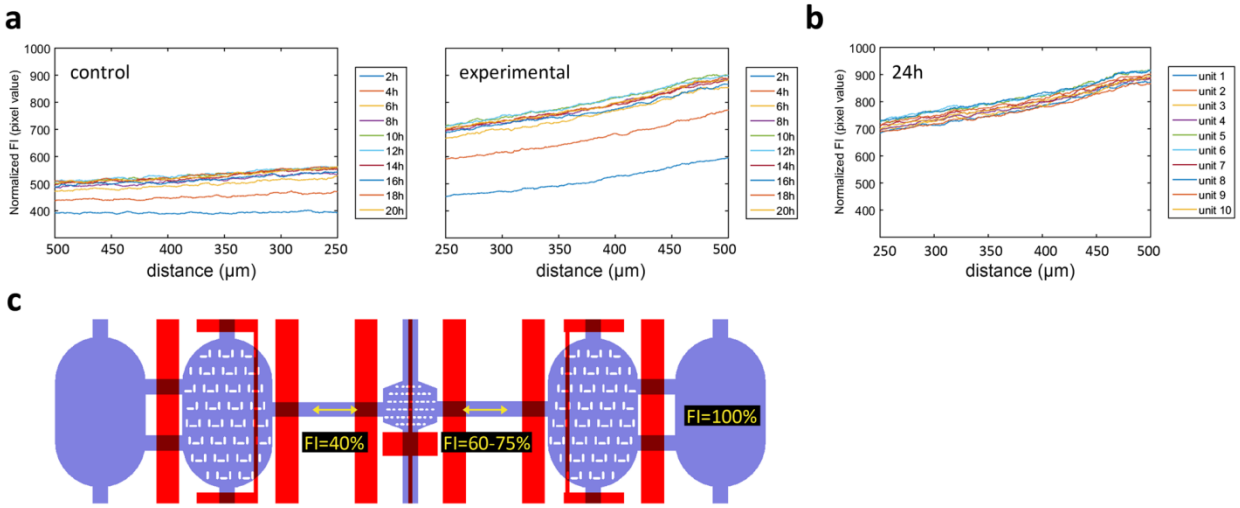
Primary MSC were isolated from the bone marrow of Actin-GFP mice, enriched by MACS, maintained in regular cultures for three days and then loaded into the micro-fluidic device. (**a**) Without using the full set of optimizations (specified in Supplementary Table 1), cells did not survive the overnight culture in the micro-fluidic device (culturing in 10% serum). (**b**) Applying the optimizations, led to increased viability of primary MSC for up to 5 days (longer experiments were not performed). Images shows bright field (top) and fluorescent views of healthy MSC that moved from the responder chamber, through the migration channel to the empty producer chamber after 2 days of on-chip culturing. Zoomed image of one of the cells is displayed on the left. (**c**) Viability of cells will depend on the serum concentration in the supply medium. Using only 2% serum in the medium decreased viability from 95% to 75% (means and standard errors are presented, n=5 and 3 for 12% and 2% serum, respectively). In co-culture setting the cells were supplied with 10% serum in the medium. The viability of (**d**) day 6 CXCR3 MSC and (**e**) CXCR3 HEK responder cells was not affected during the assay time (means and standard errors are presented, n=10 units). Scale bar, 100μm. Note that too thin trapping structures in the initial design are destroyed during multilayer soft-lithography fabrication (**a**, red star).



Supplementary Figure 5

Equal loading of primary mesenchymal stem cells into 10 responder chambers of one device.

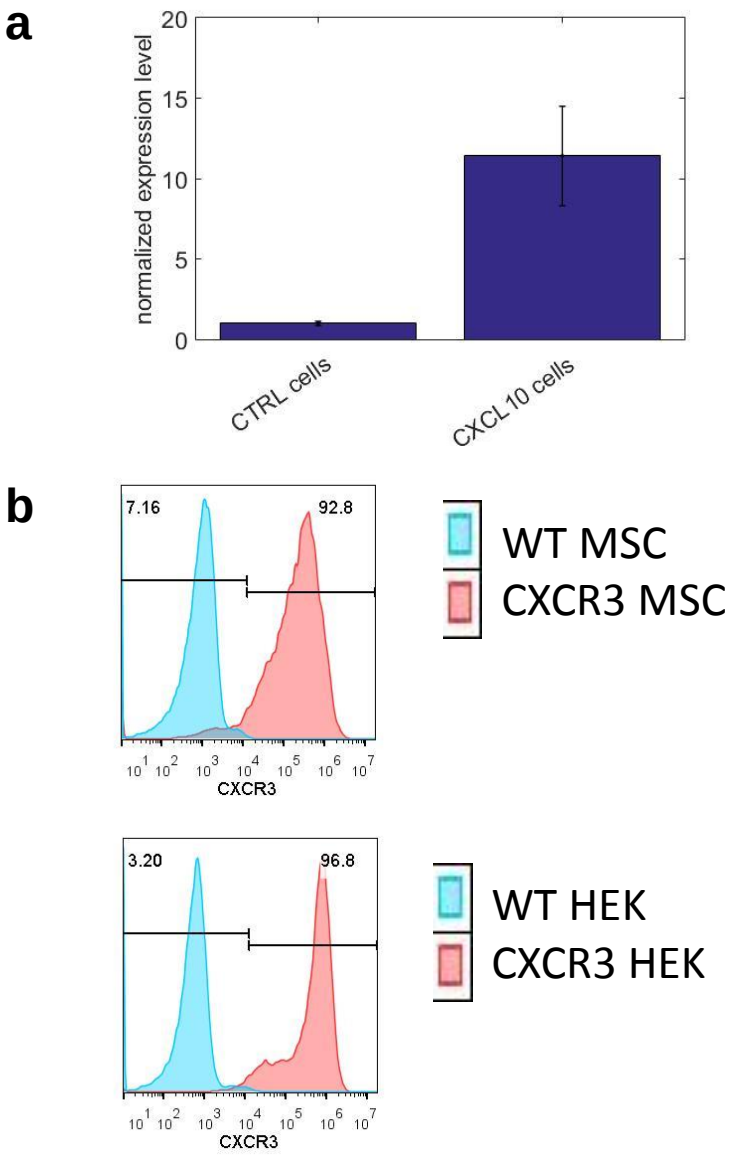
Primary MSC were isolated and enriched by MACS from Actin-GFP mice, maintained in regular cultures for three days and then loaded into the microfluidic device. Bright field and corresponding fluorescent images are shown. Cell were loaded from right to left. Except for the first chamber (right) trapping is very homogeneous throughout the whole chip. Scale bar, 100µm.



Supplementary Figure 6

Stable gradient formation from external factor using pulsed supply.

The design was also tested for gradient generation using conditioned medium from secNeonGreen^{+} HEK cells as an added, external factor vs. control conditioned medium from parental HEK cells and the pulsed supply method at 4/4 cycling (4 min flow / 4 min diffusion). (**a**) The protein gradient of secNeonGreen was stabilizing within the first 4-6 hours after which it was stable until the end of the assay. (**b**) At 24h, the fluorescent intensity (FI) profile was measured in all 10 basic units showing that the gradient was very similar between each unit. The measured area is depicted by yellow arrows in (c). Noise was removed by applying moving average filter with 10µm window size. (**c**) Based on the fluorescent intensity of the conditioned medium in the side channel, ~40% of the protein is present in the control while the experimental migration channel shows a stable gradient between 60% and 75%.

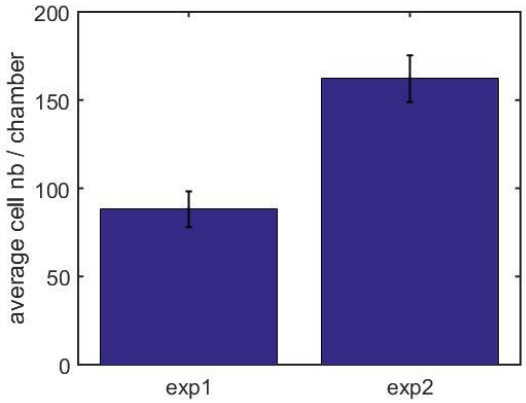


Supplementary Figure 7

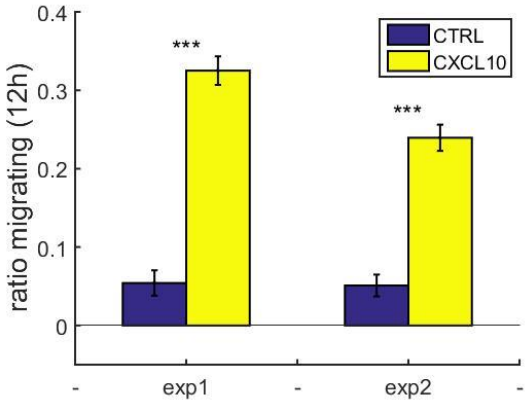
Expression of CXCL10 and CXCR3.

(**a**) Expression level of CXCL10 was measured by RT-PCR in control and stable transfected L-cells. Expression level was normalized to GAPDH (**b**) Expression level of CXCR3 was measured by cytometry in control and lentiviral transduced, primary mesenchymal stem cells (MSC) (top) or in control and stable transfected HEK293T cells (bottom).

a

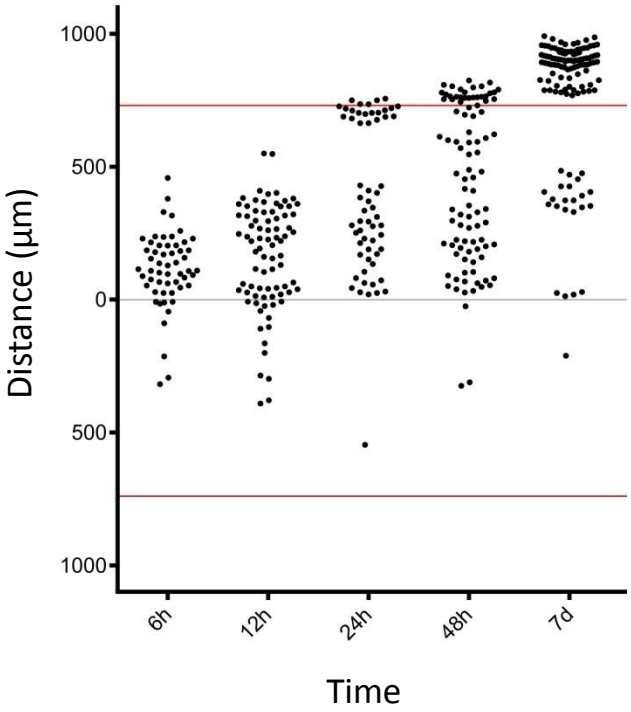


b

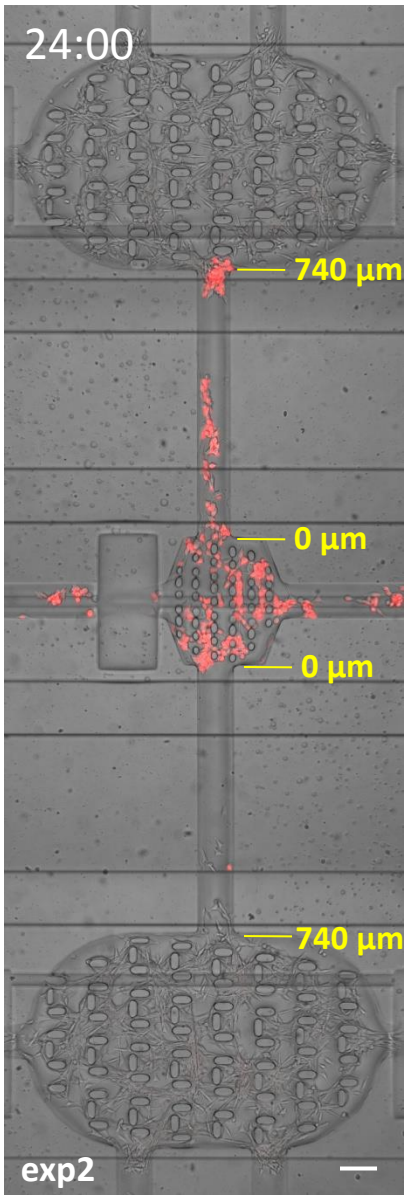


exp1: CXCL10 on bottom, 20% R3 HEK at loading
exp2: CXCL10 on top, 40% R3 HEK at loading

c



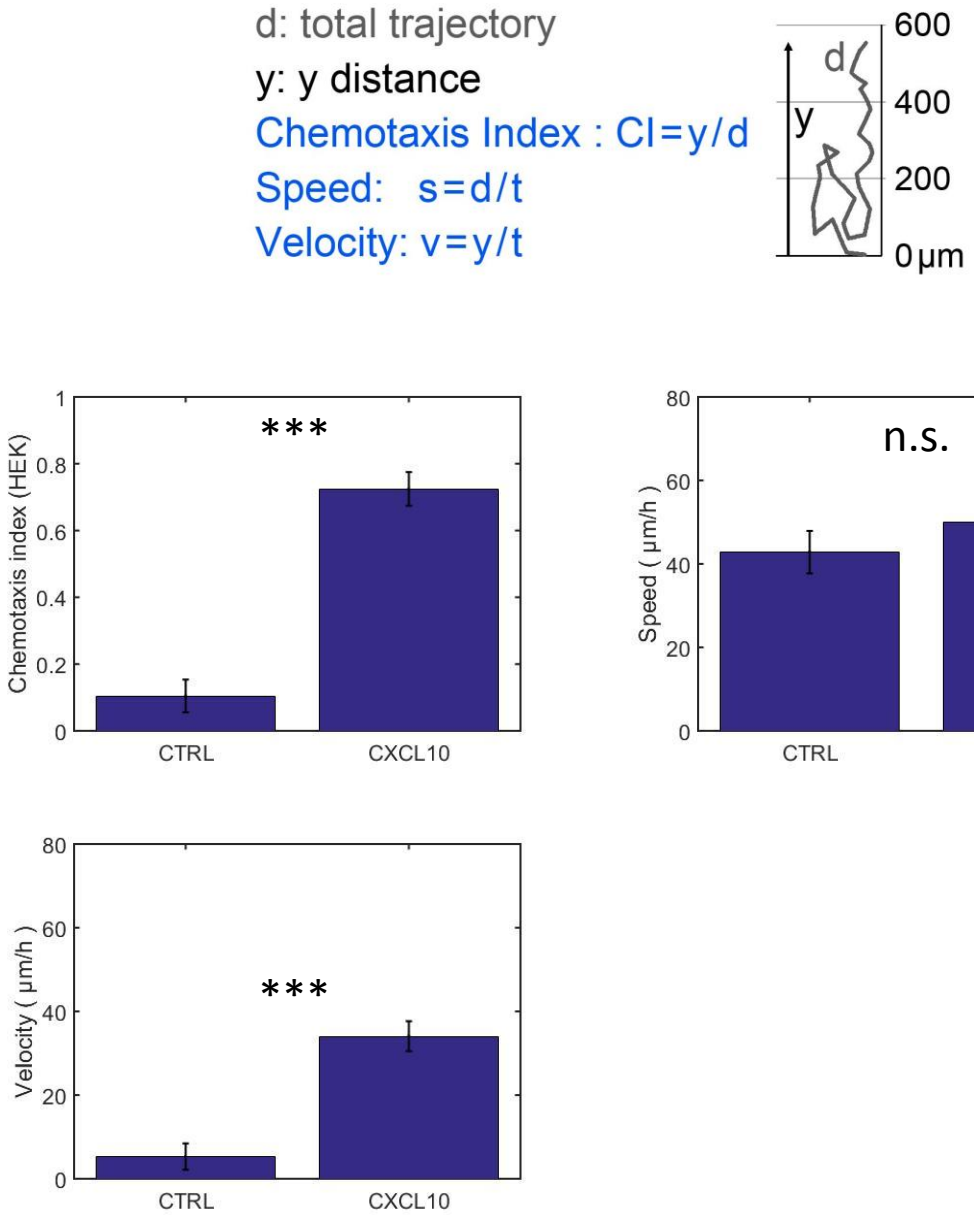
d



Supplementary Figure 8

CXCR3^{+} mCherry^{+} HEK293T (R3 HEK) chemotaxis in the co-culture microfluidic device.

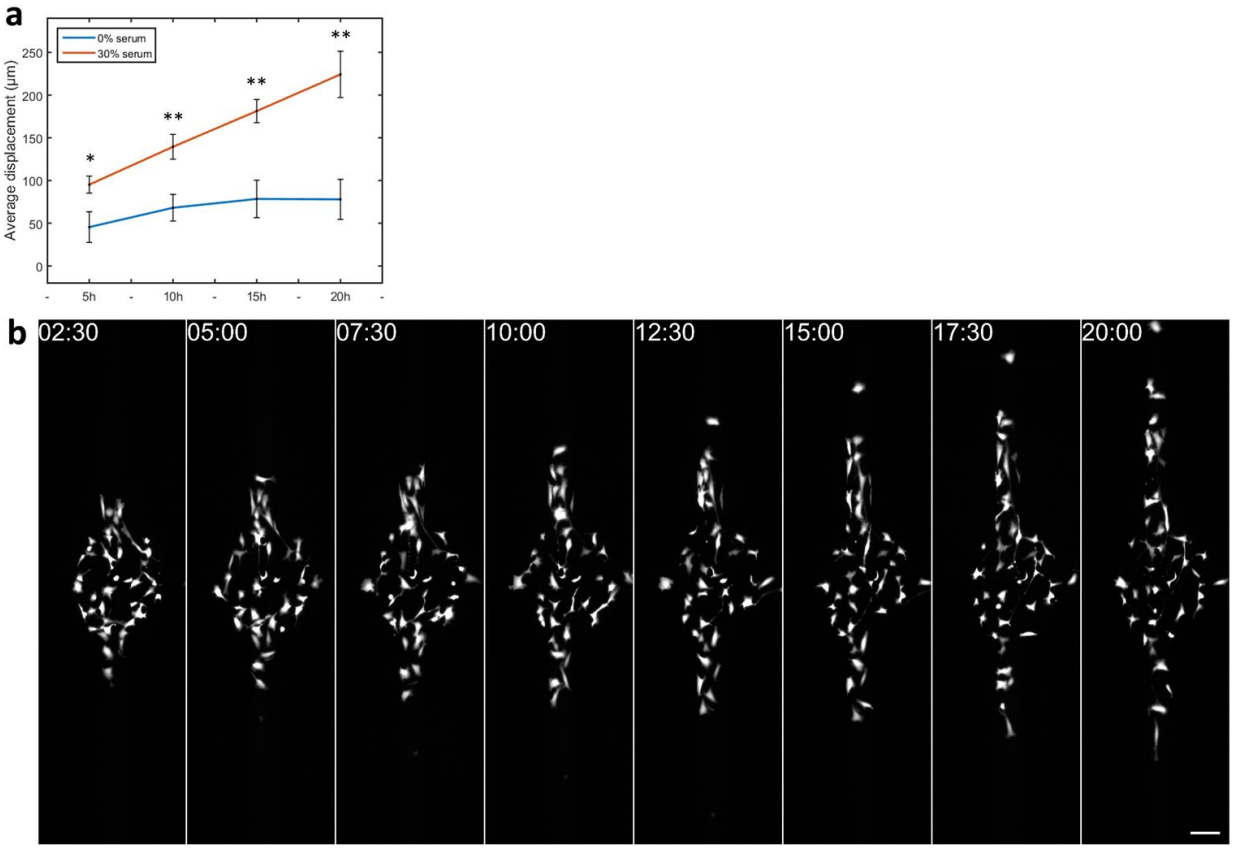
Two experiments were performed to investigate the chemotaxis properties of R3 HEK cells in co-culture with CXCL10 ligand secreting L-cells seeded at different densities either to the bottom (exp1) or the top (exp2) producer chambers. Control L-cells were loaded on the opposite side of the chip in each experiment. (**a**, **b**) Significant preferential migration (b) of R3 HEK towards CXCL10 was observed independently of the seeding density (a) of responder cells or the localization of the CXCL10 producer cells (n=10 units, mean and s.e.m are presented). (**c**, **d**) Even after 7 days (c), cells were still migrating towards the source of CXCL10. This confirms the stability of the system over a long period of time. The bee swarm plot displays the Y position of each R3 HEK cell in two opposing migration channels of one unit over time. Red lines depict the border of the producer chambers. Cells were cultured on the microfluidic device with the 'pulsed supply' method using 35/5 cycles. (d) Composite of bright field images of the basic unit quantified in (c) overlayed with the red fluorescent channel to reveal the position of the mCherry^{+} R3 HEK responder cells at 24h. (Scale bars, 100µm)



Supplementary Figure 9

Description of chemotactic properties of R3 HEK based on real-time single cell tracking.

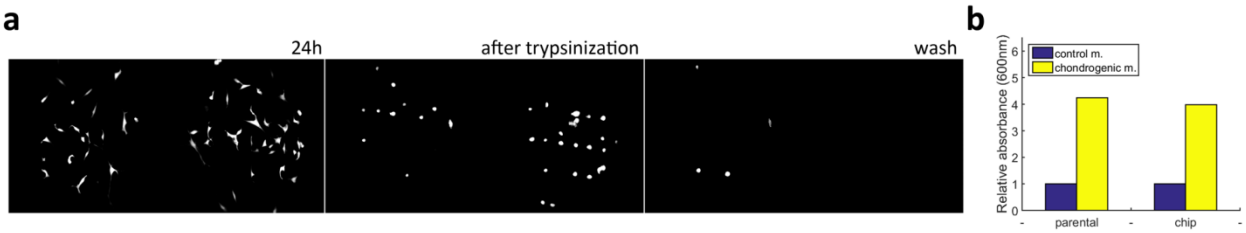
Chemotactic properties of R3 HEK cells in co-culture with CXCL10 ligand secreting L-cells were measured over 12h and manual object tracking was performed by ImageJ (http://rsb.info.nih.gov/ij/plugins/track/track.html) to retrieve spatial coordinates of cells every 15 mins over 12h and the resulting data were analyzed in MATLAB (MathWorks). The chemotaxis index was calculated based on the net distance the cells moved in the direction of either producer chamber (termed *y* distance) which was divided by the total trajectory *d* the cells moved in any direction. Speed was calculated by dividing the total trajectory *d* by the time the cells spent in the migration channel. Similarly, velocity was calculated by considering the *y* distance instead of the total trajectory. Graphs show the comparison of cells moving in the control (CTRL) vs. experimental (CXCL10) channel. The data was acquired by tracking a total of 65 cells (54 in the experimental and 11 in the corresponding control channel. Only the cells that entered the migration channel were analyzed. *** $p<0.001$.



Supplementary Figure 10

Chemotaxis towards external provided factor using pulsed supply.

Primary MSC were isolated and enriched by MACS from Actin-GFP mice, maintained in regular cultures for three days and then loaded into the microfluidic device The design was tested for chemotaxis of these cells using high (30%) vs low (0%) serum containing medium and the pulsed supply method at 4/4 cycling (4 min flow / 4 min diffusion). The cells moved preferentially towards higher serum concentration with (a) statistically significant results already after 5 h (combined results of 10 basic units, mean and s.d. shown). (**b**) Fluorescent imaging of Actin-GFP^{+} MSC at different time points document the preferential migration towards the high serum concentration (top).



Supplementary Figure 11

Retrieval of cells after the chemotaxis assay for further downstream applications.

The design permits to isolate cells after the chemotaxis assay. In this example, MSC were isolated from the bone marrow of Actin-GFP mice and enriched by MACS. Cells were cultured for 3 days in regular culture before loading into our chemotaxis device. We re-isolated cells from the device after 24h of chemotaxis with a serum gradient (Suppl. Fig. 10): side valves were closed and trypsin was loaded (**a**, left). After 20mins the cells started to detach (**a**, middle) and were washed out from the chip by flowing new, complete medium from the inlets while a collecting tube was plugged to the outlet (**a**, right). The recovery rate was around 90% (comparing the number of cells that stayed on-chip after trypsinization/washing with the initial number in the responder chamber). Isolated cells were plated onto collagen coated dishes and expanded for 2 weeks in regular culture. Afterwards, cells were differentiated in a chondrogenic medium and chondrocyte lineage contribution was measured by Alcian blue staining. (**b**). The rate of differentiation was similar to parental cells that were not cultured on-chip (chondrogenic differentiation used equal cell number of parental and on-chip MSC). This experiment shows that the on-chip culture does not alter the properties of MSC.

Supplementary Video

Chemotaxis of R3 HEK cells

Time-lapse video of R3 HEK cells migrating towards L10 cells (loaded on the bottom) over 12h. Producer chambers with cells are not shown.

Supplemetary Table

Factors influencing the viability of mammalian cells in a microfluidic device

Action	Function for high cell viability	Additional advantage
Microfluidic device fixed in the incubation holder	Thermometer incorporated in the holder enables measuring the temperature in close proximity to the device	The incubation holder fixes firmly the tygon tubes and the moving stage does not perturb the microfluidic system while acquiring images at multiple positions
Microfluidic device submerged with bicarbonate buffer	Maintains osmolarity by preventing evaporation	-
Stabilized CO_2 gas atmosphere in the incubation holder	Controls pH of the medium in the device (which is also based on a bicarbonate buffer) since PDMS is permeable for gases	Gas atmosphere can be adjusted according to the cell type (e.g. hypoxia-dependent cells)
Precise pressure regulator (0-2psi) for loading	Decreases flow speed and shear stress on cells while loading (especially for chambers with traps)	Easier manipulation of rare cells in small volume
Avoid usage of new tygon tubes, sterilization by 70% EtOH only	Slight toxicity was observed when new or autoclaved tygon tubes were used for cell loading and medium supply	Reusing the tygon tubes even up to 50 experiments is more economical
Avoid molding PDMS chip from freshly TMCS treated wafer	Decreases the toxic effect of the new wafer	-
Loading sensitive cells in 20% serum	MSCs and L-cells are more sensitive to on-chip culturing conditions. Loading them in 20% serum increased the cell viability. After the cells are fully adherent complete medium with 10% serum is used for supply	-
Using 1% Pen/Strep	Antibiotics is all solutions getting in contact with the device decreases the risk of bacterial contamination and increases cell viability	Whole setup is clear from contamination for at least 10 days allowing also other, longer functional assays for future need (cell cycle control, reporter assay, differentiation, etc.)