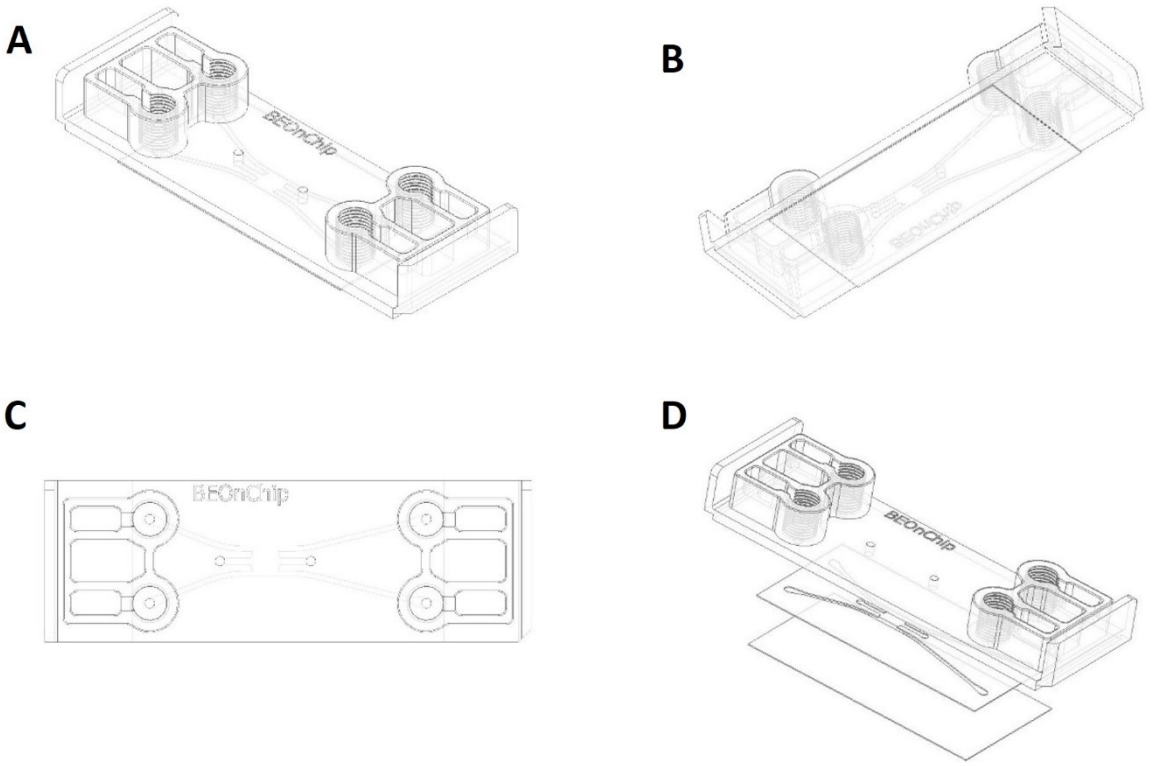
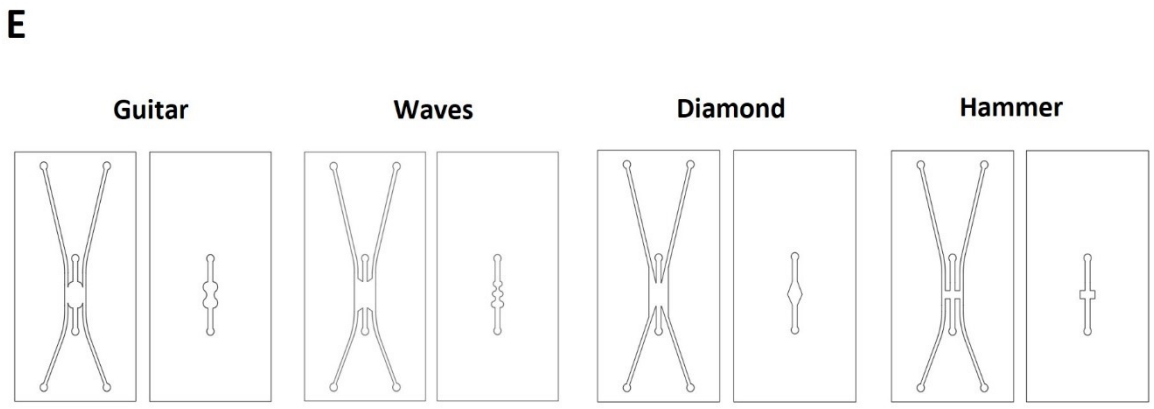
Electronic Supplementary Material (ESI) for Lab on a Chip.

TUNEABLE HYDROGEL PATTERNS IN PILLARLESS MICROFLUIDIC DEVICES

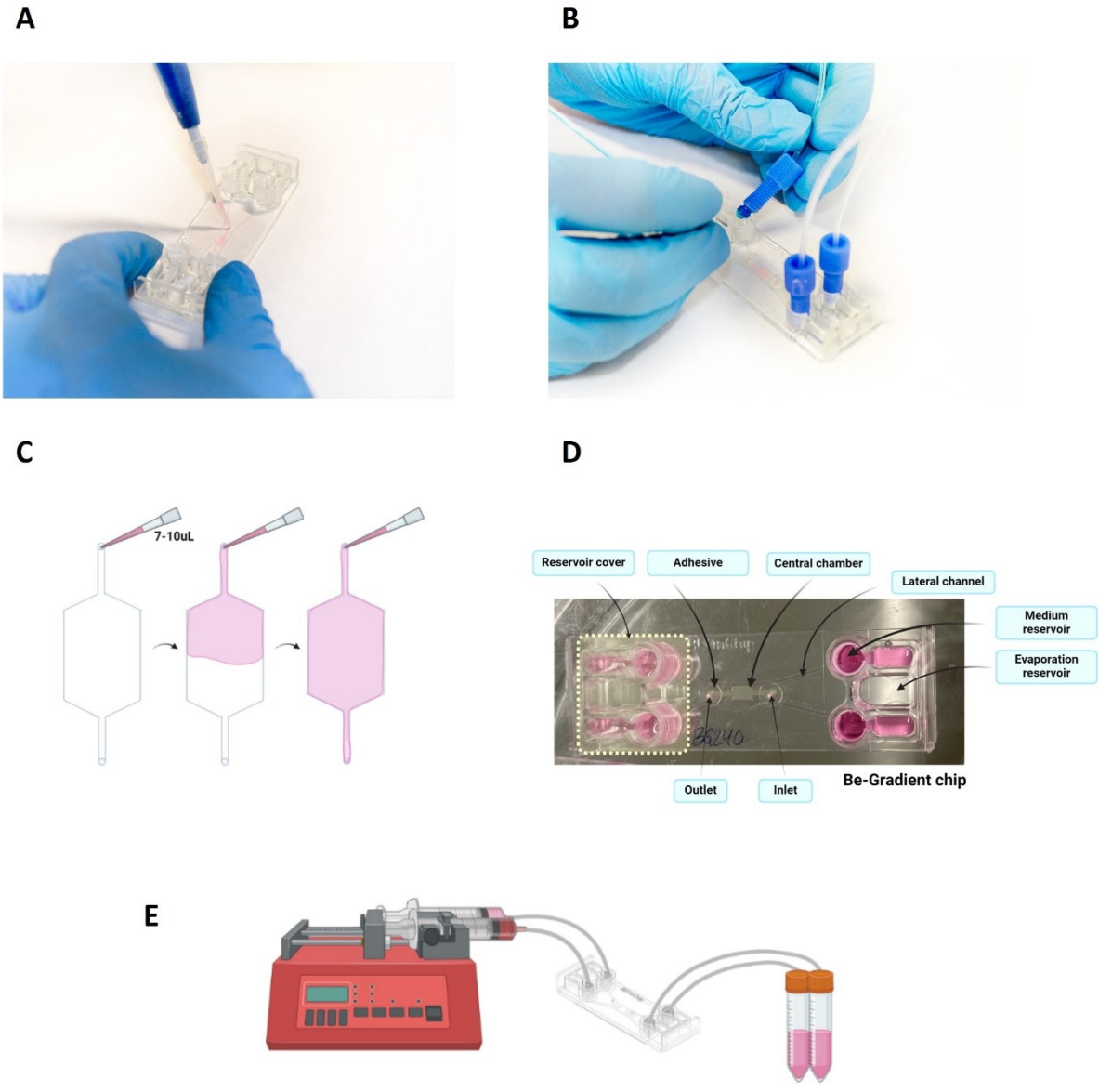
Supplementary material

Supplementary Figure 1: Views of the Be-Gradient without pillars. *A) General view, B) bottom view, C) top view and D) decomposed piece. E) Channels and mask (central channel) designs for the corresponding different geometries.*

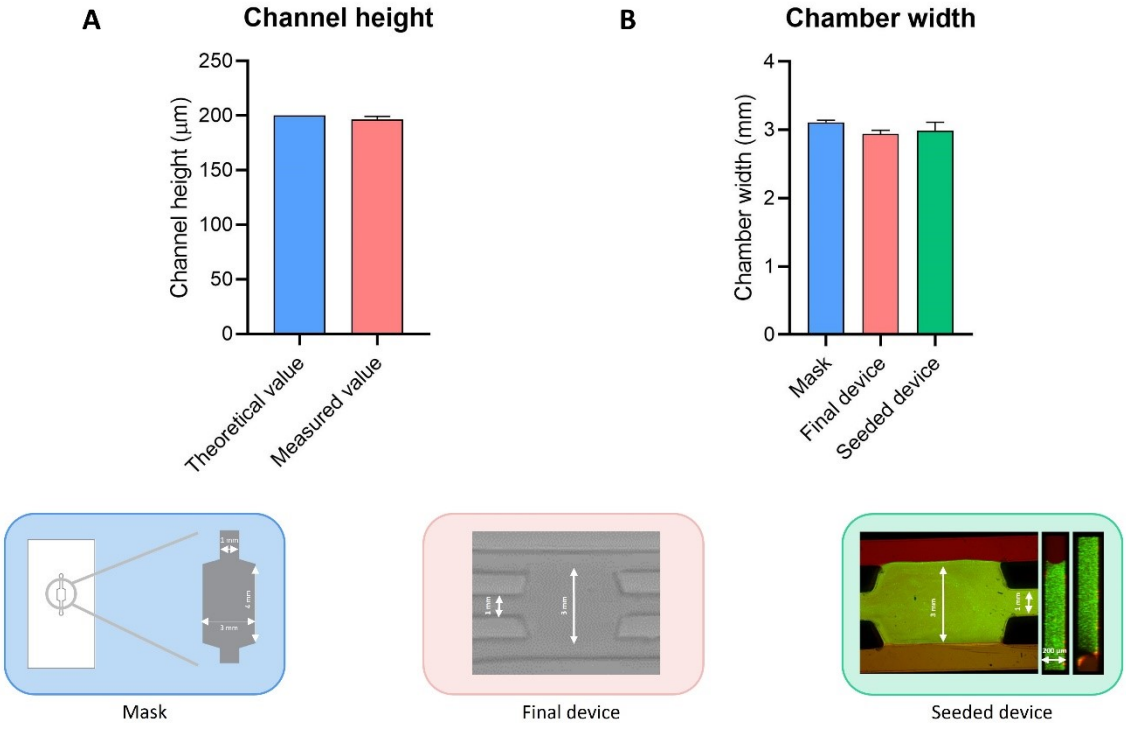




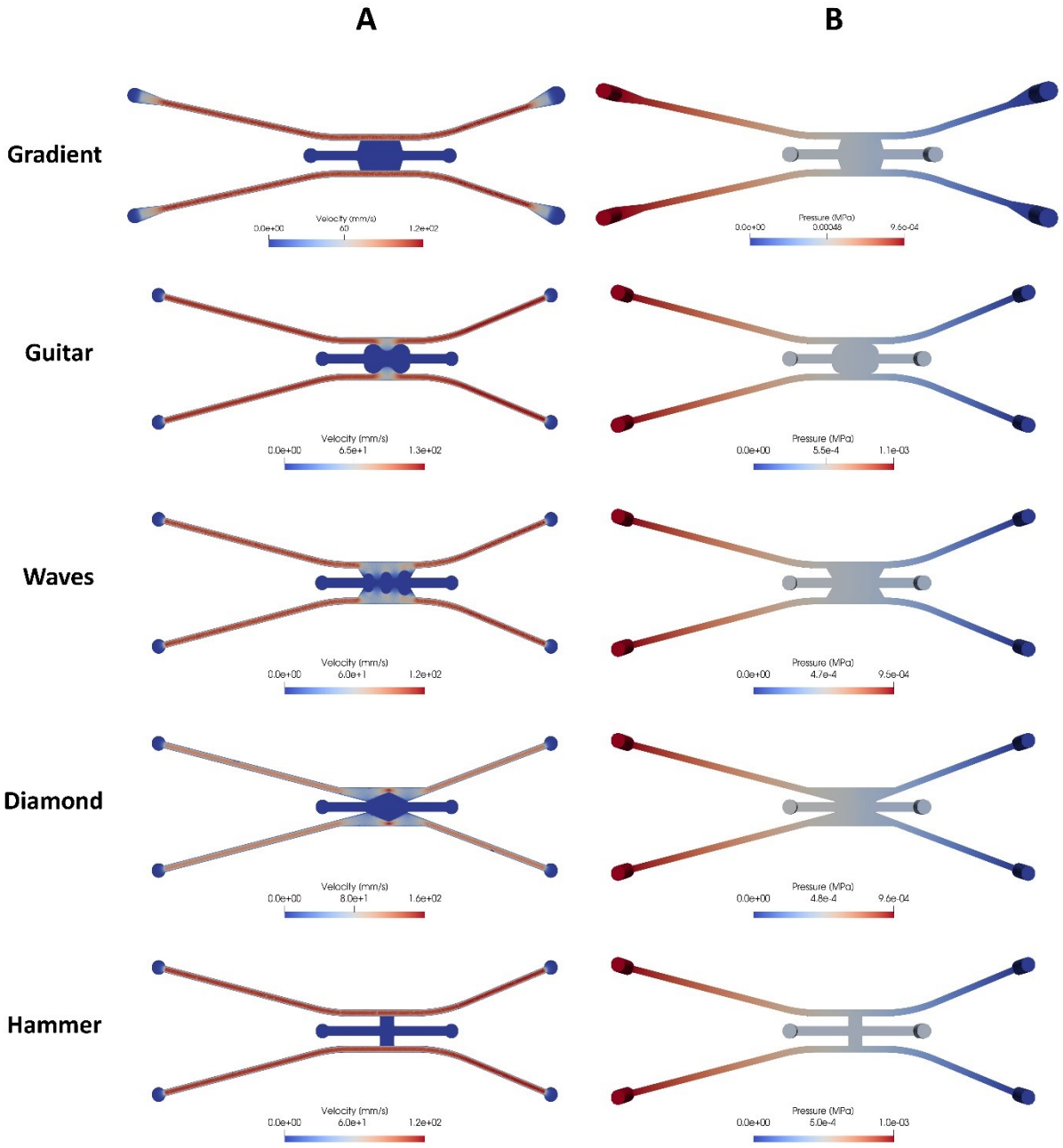
Supplementary Figure 2: Seeding the Be-Gradient without pillars and flow system setup. *A) Seeding the Be-Gradient device, B) flow connecting process, C) seeding process, D) parts of the chip and E) flow system with the syringe pump.*



Supplementary Figure 3: Characterization of the Gradient device. *(A) This graph shows measurements of the height of the channels. (B) The graph shows measurements of the central chamber (mask, final device and seeded device). Seeded devices are the devices that are filled with the hydrogel. The side views shown in the image "Seeded device" are made along the central channel (channels and camera), thus measuring the height of the channels of the device. Mask and final device are measured by the Nikon SMZ745 magnifying lens and seeded devices by the Nikon Eclipse Ti confocal microscope.*



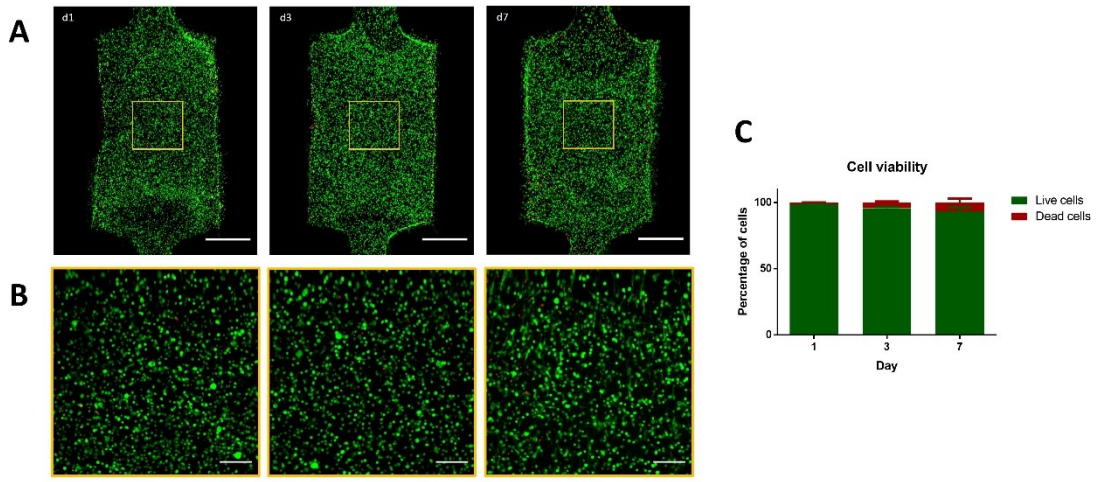
Supplementary Figure 4: *A) Velocity and B) pressure profiles for different designs.*



Supplementary Figure 5: Biocompatibility test.

To test the biocompatibility of the device, a week-long test was conducted. U251-MG cells (Sigma, 09063001) were stably transduced with a green fluorescent protein (GFP)-expressing lentiviral vector [1]*, kindly provided by Dr. Prats, University Paul Sabatier, Toulouse, France. Cells were cultured in high glucose Dulbecco's modified Eagle's medium (DMEM: Lonza, BE12-614F), supplemented with 10% fetal bovine serum (FBS: Sigma, F7524), 2 mM L-glutamine (Lonza, 17-605C) and penicillin/streptomycin (Lonza, 17-602E). A collagen hydrogel (4 mg mL^{-1}) was prepared following the same process as in section 2.3 with a final volume of 100 µL. The cell suspension (50 µL) was mixed with the final collagen hydrogel, and the mixture was seeded in the central chamber (cell seeding density: $4x10^6$ cells mL^{-1}* [2]*). The device was turned upside down every 30 seconds for 5 minutes, and placed in the incubator for 15 minutes, turning it every 5 minutes to achieve a homogeneous distribution of cells over the entire height of the device. Once the gel was polymerized, the medium was added in lateral channels, and the chip was maintained in the incubator at 37°C and 5% CO_2.*

Biocompatibility of the device was determined by propidium iodide (PI) staining. Stock solution of 2 mg mL^{-1} PI (Sigma Aldrich, P4170) was dissolved in distilled water, and further diluted to 6 µg mL^{-1} in phosphate-buffered saline (PBS; Lonza BE17-516F). PI solution was perfused through lateral channels of the device on days 1, 3 and 7, and incubated for 15 min. Confocal images



(A) Confocal microscope images of a hydrogel containing U251-GFPcells at a concentration of 4 × 106 cells ml-1 within the central chamber at days 1, 3 and 7. Viable cells were stained green with calcein AM and dead cells were labelled red with propidium iodide. Scale bar 1000 µm. (B) Detail of the area selected to obtain values for cell viability and (C) graph showing cell viability. Scale bar 200 µm. The graph summarizes the data showing the percentage of live/dead cells over the days.

were acquired using a Nikon Eclipse Ti-E C1 confocal microscope.

It was observed that on day 1 the percentage of live cells was 98.58 ± 0.18 while the percentage of dead cells was 1.42 ± 0.18. By day 3, the percentage of dead cells increased until 4.32 ± 0.90; and by day 7, the percentage of live cells was 93.38 ± 2.92 and dead cells was 6.62 ± 2.92. The results showed that no significant differences over the days were detected in the number of dead cells.

References:

[1] F. Morfoisse et al., "Hypoxia induces VEGF-C expression in metastatic tumor cells via a HIF-1α-independent translation-mediated mechanism," Cell Rep., vol. 6, no. 1, pp. 155–167, 2014, doi: 10.1016/j.celrep.2013.12.011.

[2] J. M. Ayuso et al., "SU-8 based microdevices to study self-induced chemotaxis in 3D microenvironments," Front. Mater., vol. 2, May 2015, doi: 10.3389/fmats.2015.00037.