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

Supplementary information

Self-Assembled and Perfusable Microvasculature-on-chip for Modelling Leukocyte Trafficking

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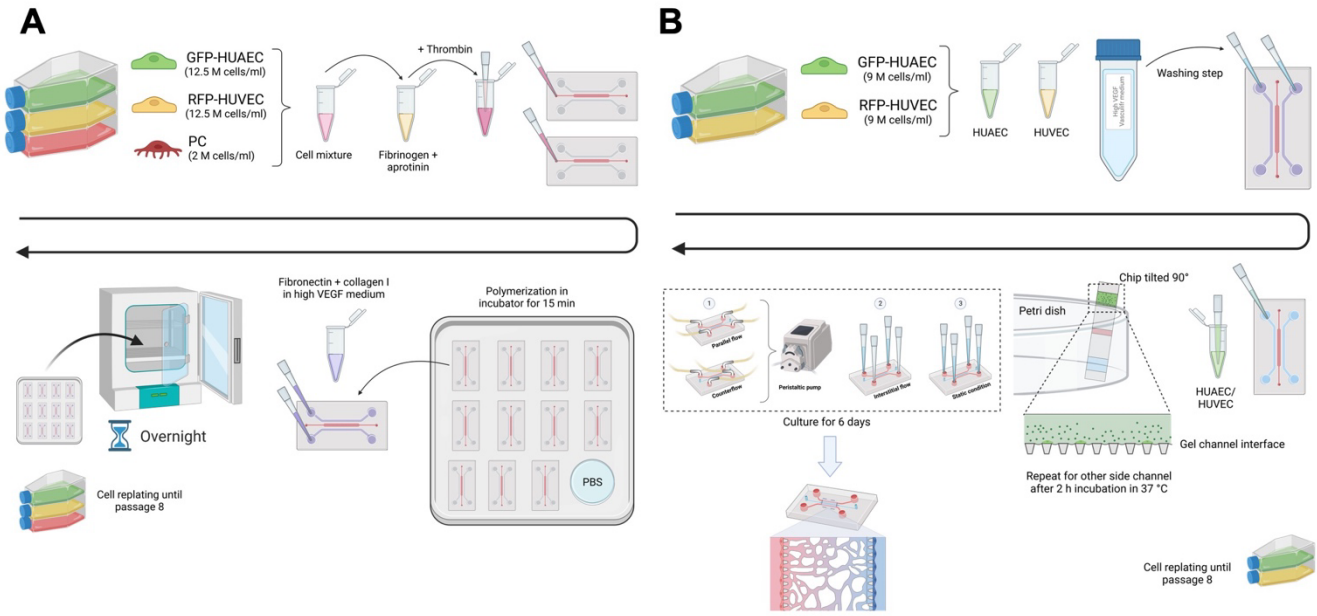

Figure SI1. Schematic workflow of the device seeding and flow conditions. **(A)** Seeding of the cell-laden fibrin mixture into the central compartment. **(B)** Seeding of side channels to create connected arteriole- and venule-like vessels. Figures made with BioRender.com.

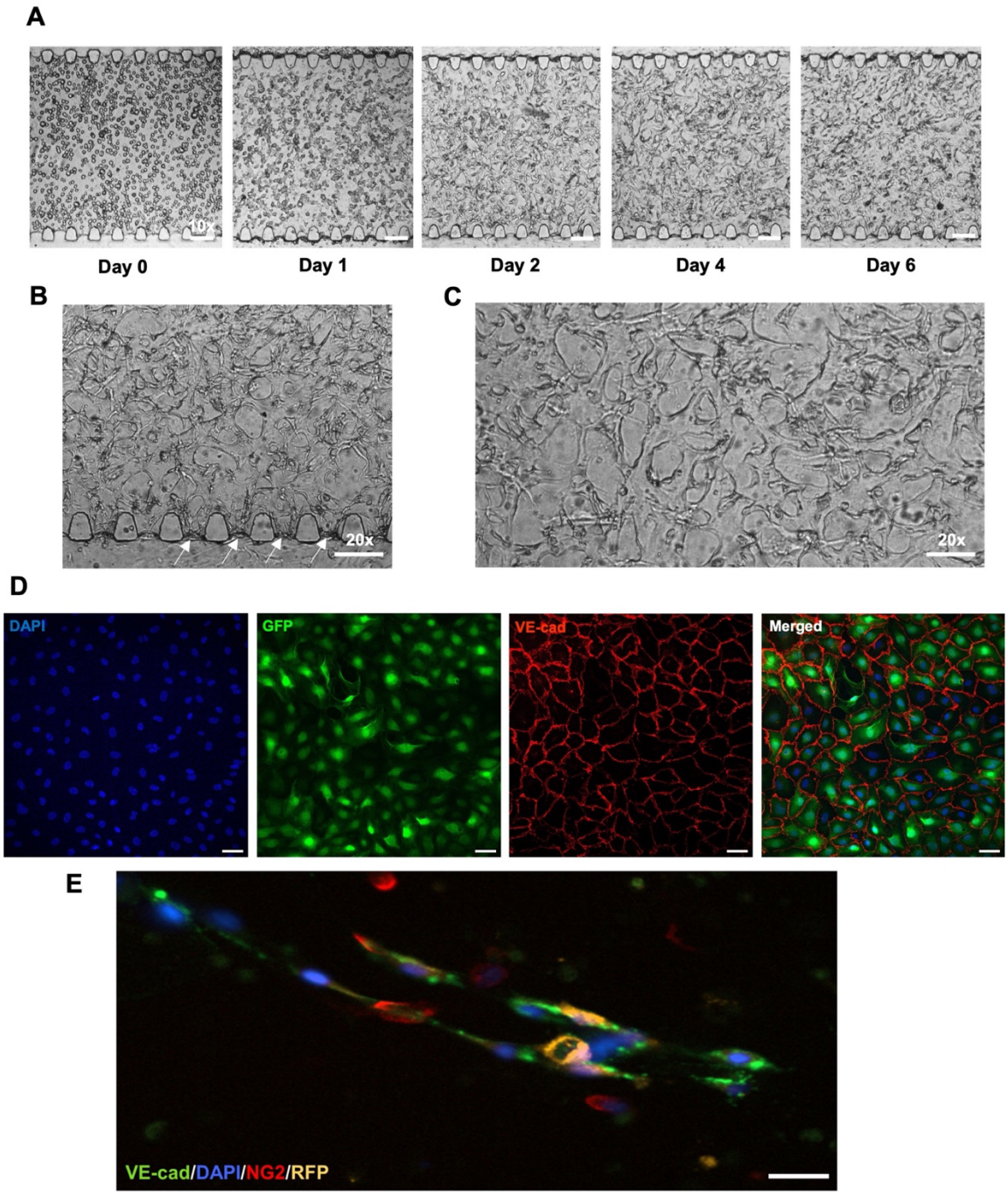

Figure SI2. Network formation, supplementary images. **(A)** Brightfield images depicting the formation of a microvascular network over time under peristaltic flow. Images were acquired on day 0, immediately after seeding, and at subsequent time points until a continuous network was formed on day 6. **(B)** Vessel sprouts from the gel channel were connected across the entire gel region to the parallel arteriole- and venule-like side channels. Arrows display the formation of perfusable vessels in the gel interface. **(C)** Brightfield image of perfusable network formed within the gel channel highlighting the ability of the device to support the formation of a functional microvasculature. **(D)** Confocal micrographs of VE-cadherin (red) expression control in GFP-expressing HUAECs. **(E)** Immunofluorescence staining for HUVEC (yellow), and VE-cadherin (VE-cad, green) proves vascular barrier functionality *in vitro*. NG2 staining shows PCs residing on the microvessels (red). Scale bars: (A-C) 50 µm, (D) 20 µm, and (E) 10 µm.

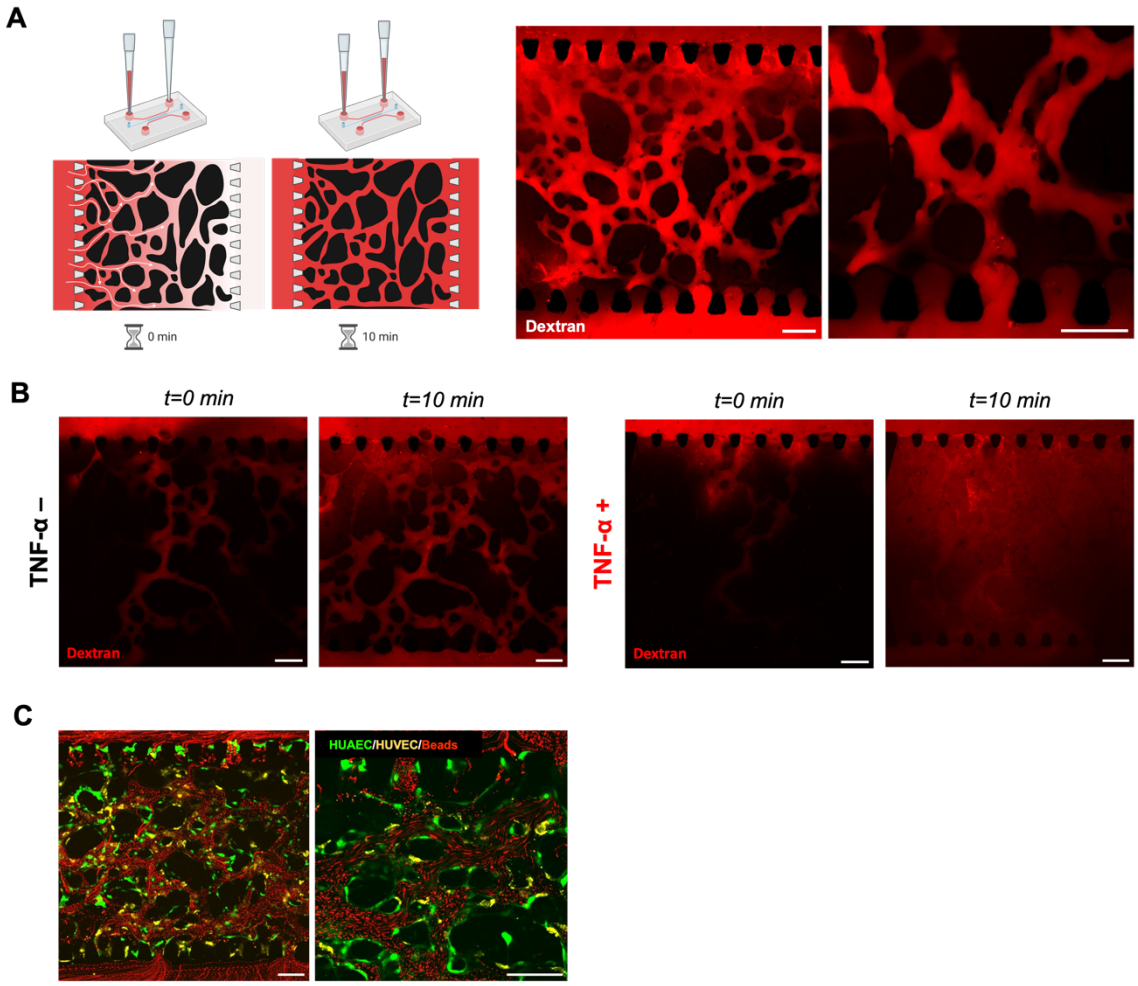

Figure S3: (A) Dextran-based permeability assay to assess vessel permeability across the microvascular network. 70 kDa CF®633-labeled dextran was perfused from one side channel to the other of the microfluidic device to evaluate endothelial barrier integrity. Scale bar: 50 µm. (B) 70kDa CF®633-labeled dextran was perfused after 6 days of culture on treated (TNF-α +) and untreated (TNF-α -) vessels to study endothelial permeability in response to inflammation. Vessels treated with TNF-α weakened the endothelial barrier and increased vascular permeability compared to untreated controls. Total acquisition time was 600 s. Scale bar: 50 µm. (C) Time-lapse images of 1 µm fluorescent beads introduced via one side channel to evaluate vessel perfusability of microvascular networks containing HUAECs (green), HUVECs (yellow) and PCs (not shown). Traversing beads are indicated by red lines. Scale bar: 50 µm.

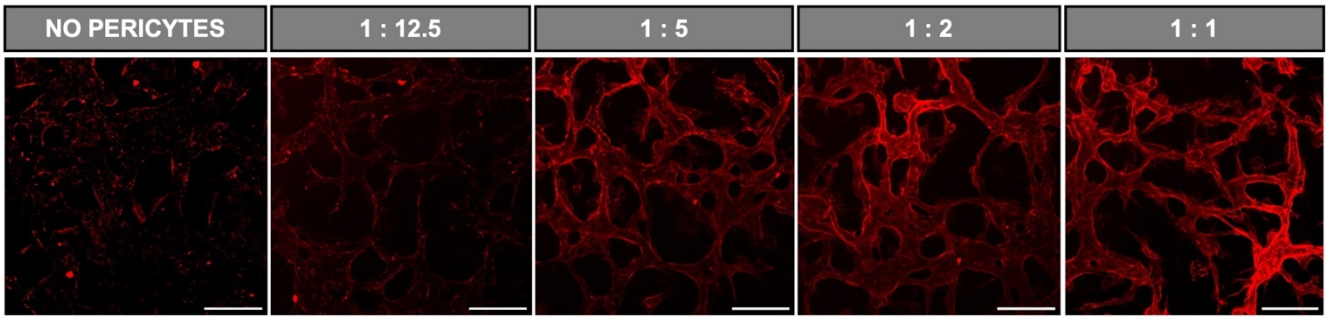

Figure SI4. Increased PC-to-EC ratio leads to increased collagen IV deposition in the perivascular space compared to cultures of HUAECs and HUVECs without pericytes. Scale bar: 50 µm.

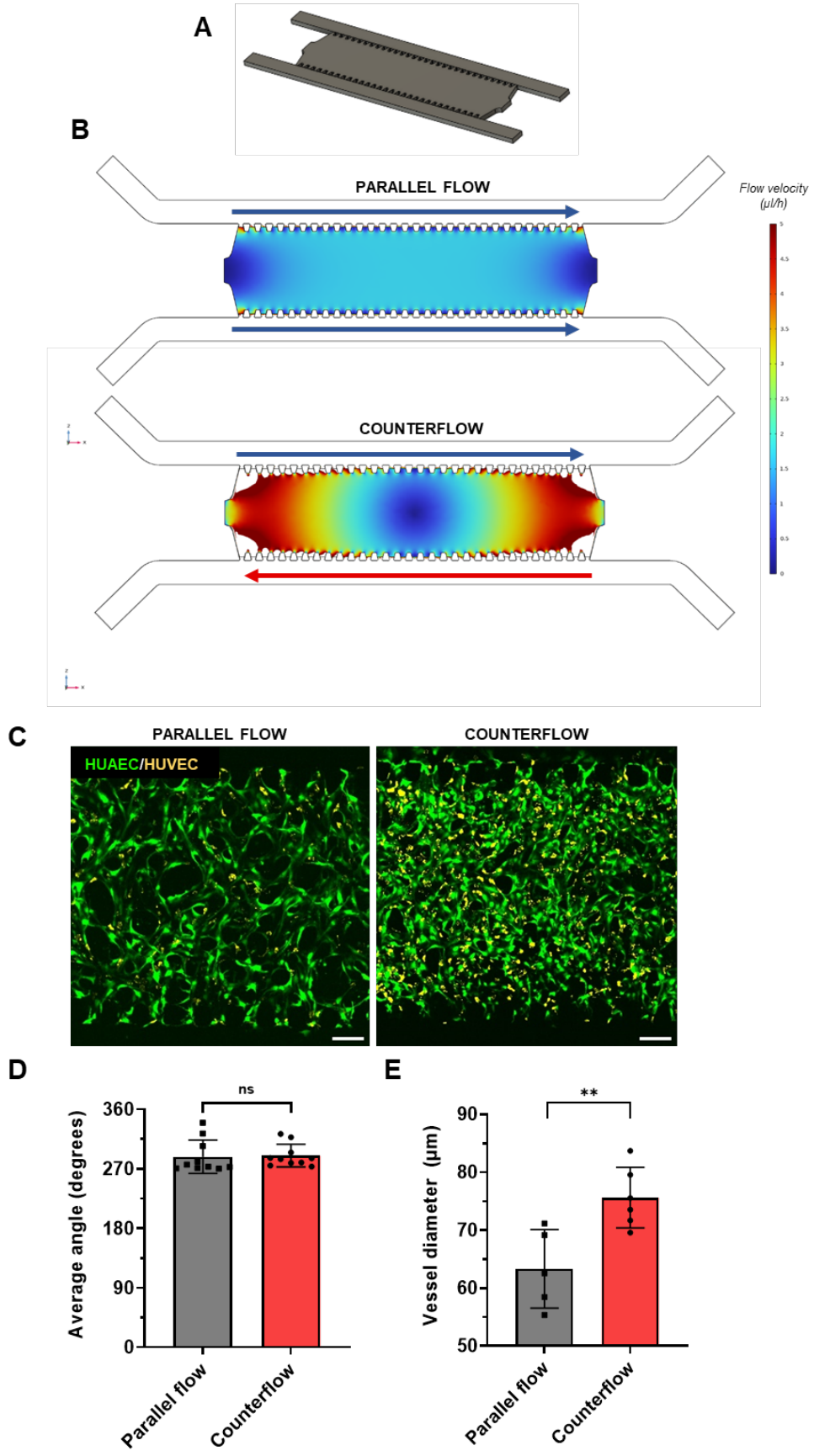

Figure SI5. COMSOL Multiphysics® software simulation depicting the fluid velocity under different flow directions. (A) Autodesk Fusion 360 model (100 µm channel height) representing the microfluidic chip design

and channel dimensions prior to being exported to COMSOL Multiphysics® software. **(B)** COMSOL Multiphysics® software models depicting the fluid velocity in the gel channel simulating the flow behavior during different flow directions (counterflow and parallel flow) under peristaltic flow (60 µl/h). The 3D simulation was generated by importing the device geometry into the COMSOL laminar flow module (addition of 100 µm channel height). The gel interface was created by choosing the 'Free Porous Media Flow' physics setting and solved by analysis of the time-independent Darcy-Brinkman equation applied over the gel region. Matrix permeability was set based on already established measurements for acellular fibrin gels.[1] All boundaries were set as no-slip. **(C)** Representable maximum intensity projection images containing HUAECs (green), HUVECs (green), and PCs (not shown) acquired from confocal microscopy comparing the microvascular network morphology during parallel flow and counterflow. Scale bar: 50 µm. **(D)** Average angle of vessel orientation compared between devices cultured under parallel and counterflow. Significance was calculated with a non-parametric Mann Whitney U test (N=10 devices for each condition). Data are presented as median ± SD. ns = not significant. **(E)** Average diameter of vessels in devices cultured under parallel or counterflow. Each data point is calculated by averaging the diameter of 10 randomly sampled vessels within one maximum intensity projection image ROI from one device. Significance was calculated with a non-parametric Mann Whitney U test. Data are presented as median ± SD (**$p \leq 0.01$).

References:

1 C. L. Chiu, V. Hecht, H. Duong, B. Wu and B. Tawil, *BioResearch Open Access*, 2012, **1**, 34–40.

Video SI1: Representative time-lapse video capturing the flow of 1 µm fluorescent beads through microvascular networks containing HUAECs (green) and HUVECs (yellow) cultured under peristaltic flow. Red lines highlight the trajectory of traversing beads, providing insights into vessel perfusability. Similar results were observed for networks cultured under interstitial flow. Images from this video are displayed in Figure SI3D.

Video SI2: Same as before, zoom into the network.

Video SI3: The video shows the trace (blue) of a T cell labelled with CellTracker™ Deep Red as it rolls along a vessel consisting of HUAECS (green) and HUVECS (yellow) and its final extravasation across the endothelial barrier. Images from this video are shown in Figure 6B (main manuscript).