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

Supplemental table 1. Primer sequences used for qPCR

Target gene	Sense primer sequence	Antisense primer sequence
Beta actin	TCCCTGGAGAAGAGCTACGA	AGCACTGTGTTGGCGTACAG
VEGFR2	TCTCTGCCTACCTCACCTGT	GCTCTTTCGCTTACTGTTCTGC
PECAM	GATGTCAGCACCACCTCTCAG	GAAGTGTATTGGGGCCTTTTC
PDGFRB	GAGGAATCCCTCACCCTCTC	GGGTATATGGCCTTGCTTCA
NG2	CTGTCCTTCCCAGTGACCAT	GGAGCCTGAACCACCTCATA
VE-cadherin	TTGGAACCAGATGCACATTAT	TCTTGCGACTCACGCTTGAC
ZO-1	ATTTTGTCCGCTCAGCCTGTT	GCCAGCTTTTCTCTGGCAAC
CX43	TGGATTCAGCTTGAGTGCTG	GGTCGCTCTTTCCCTTAACC

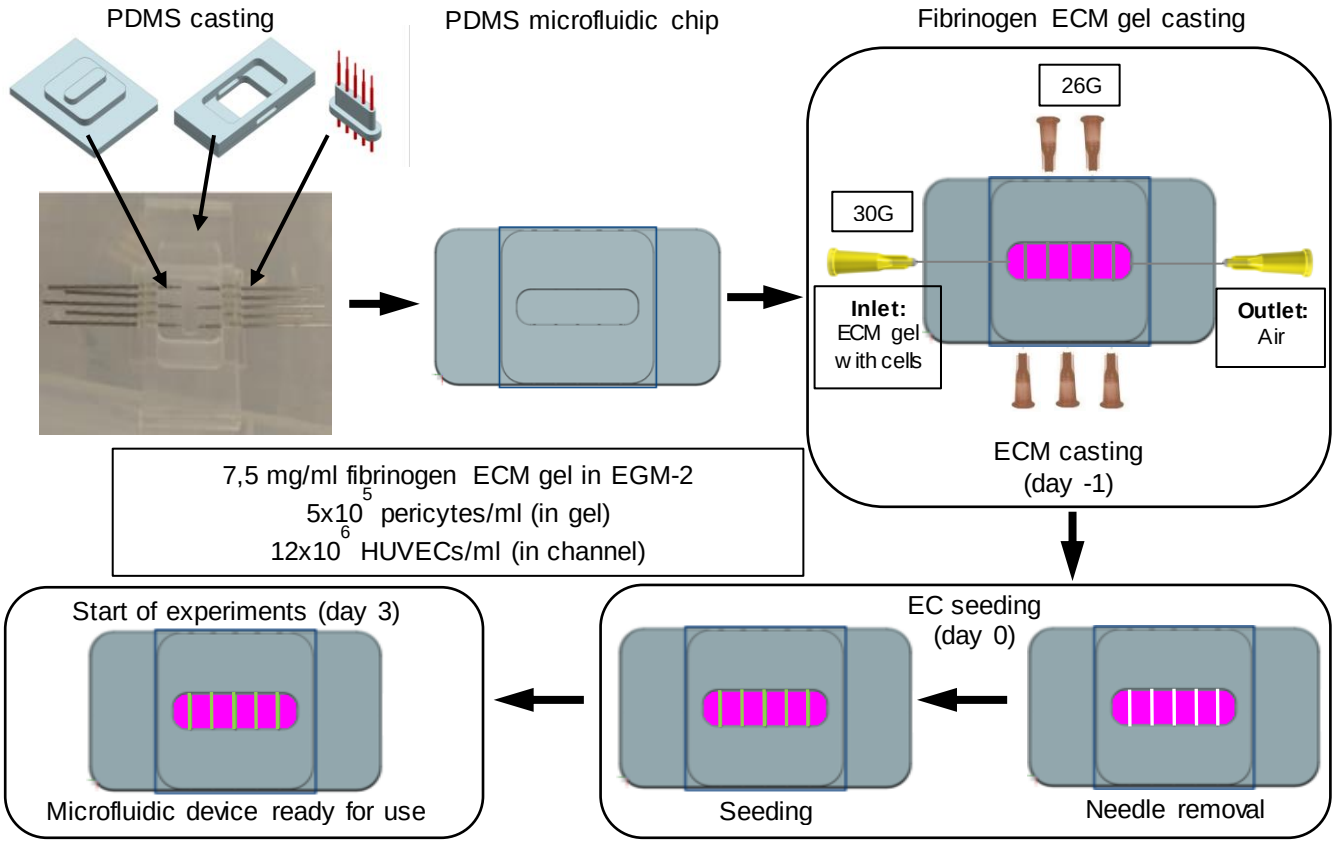

Fig. S1. Schematic overview of the work flow. Shown are step by step illustrations of the process to create a biological functional microfluidic device. PDMS microfluidic device are produced, sealed with a coverslip and sterilized with UV prior to use. Five 26G needles are placed in the pre-made channels of the PDMS microfluidic device and act as mold for the channels. Fibrinogen gel is dissolved in EGM-2 medium (7.5 mg/ml) and put under a vacuum to remove air bubbles. A pellet of pericytes ($5x10^5$/ml) is resuspended in the fibrinogen solution. The ECM/cell mix is injected in the reservoir using a syringe and 30G needle. A 30G needle on the opposite site of the reservoir acts as outlet for air. The casted microfluidic device is turned over every 30 min for 2 hours to ensure a homogenous distribution of the pericytes. After overnight polymerization of the ECM, 26G needles are removed, leaving a hollow, tube shaped channel (indicated as day 0). A suspension of HUVECs ($12x10^6$/ml) is injected in the channels and the microfluidic device is turned over every 30 min for 2 hours to ensure homogenous seeding of the HUVECs at the surface of the channel. The microfluidic device is placed in a dish with medium (EGM-2) for further maturation of the vessel-on-a-chip. At day 3, experiments as described in this study are started (indicated as day 3 in the results).

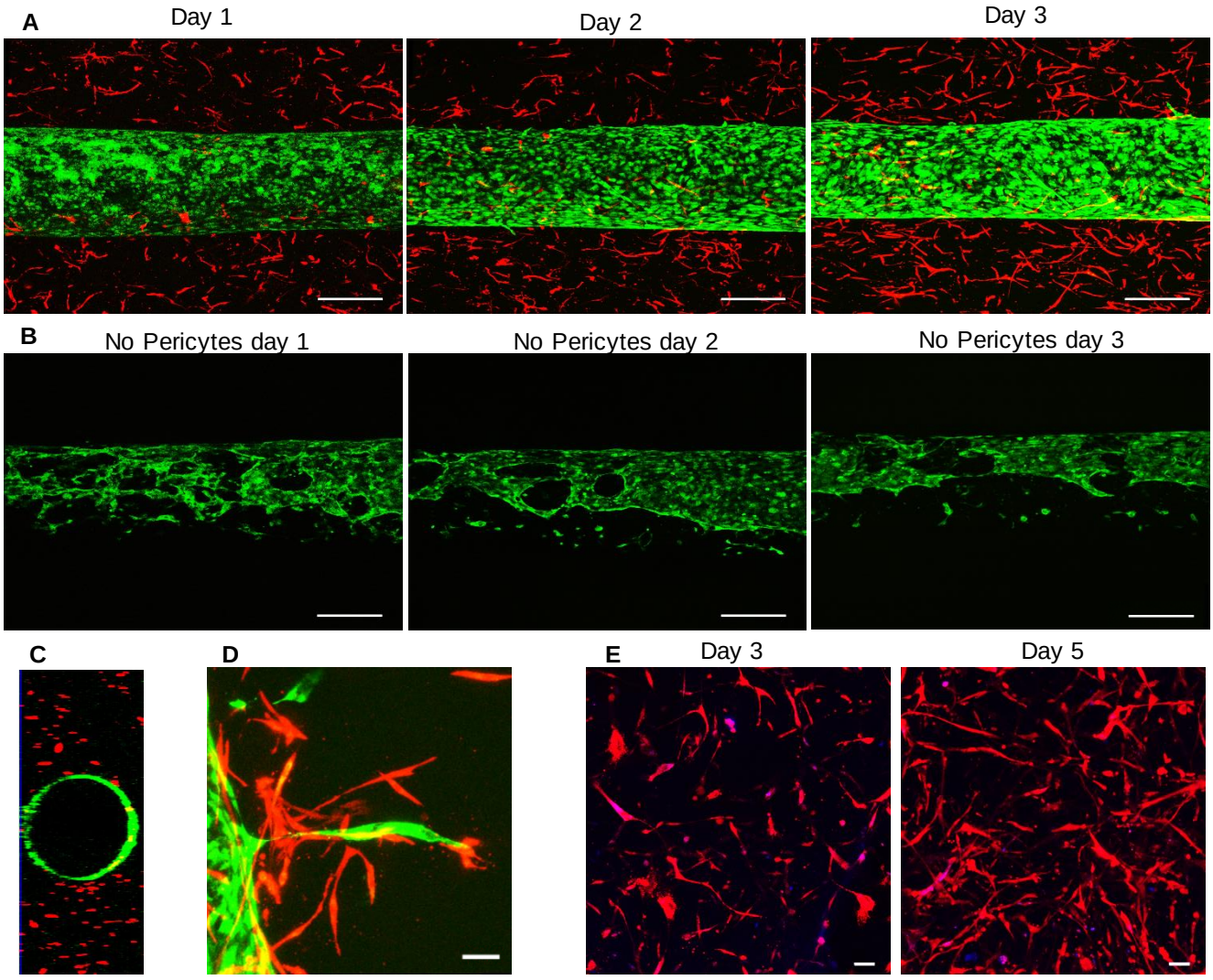

Fig. S2. Endothelial channel development. (A) Merged images of an endothelial channel during development. HUVEC (green) do not form a full monolayer and pericytes (red) do not cover and stabilize the neovessel until day 3. Scale bar = 250 µm. (B) Endothelial channel development over time without pericytes in the ECM gel. Scale bar = 250 µm. (C) Cross section of the microfluidic fluidic device. The ECs (green) form a round, open lumen with pericytes (red) in the surrounding ECM gel. (D) High magnification image of an endothelial sprout (green) supported by pericytes (red). Pilot experiments shows the sprouting capacity of ECs towards a gradient with pro-angiogenic factors. Pericytes are attracted and support the newly formed vessel. Scale bar = 50 µm. (E) Morphology of pericytes do not change in time. To assess if dsRED area (red) is a valid method to quantify pericyte proliferation, pericytes are stained with DAPI (blue, overlap magenta). No changes in pericytes morphology was observed in time. Scale bar = 50 µm.

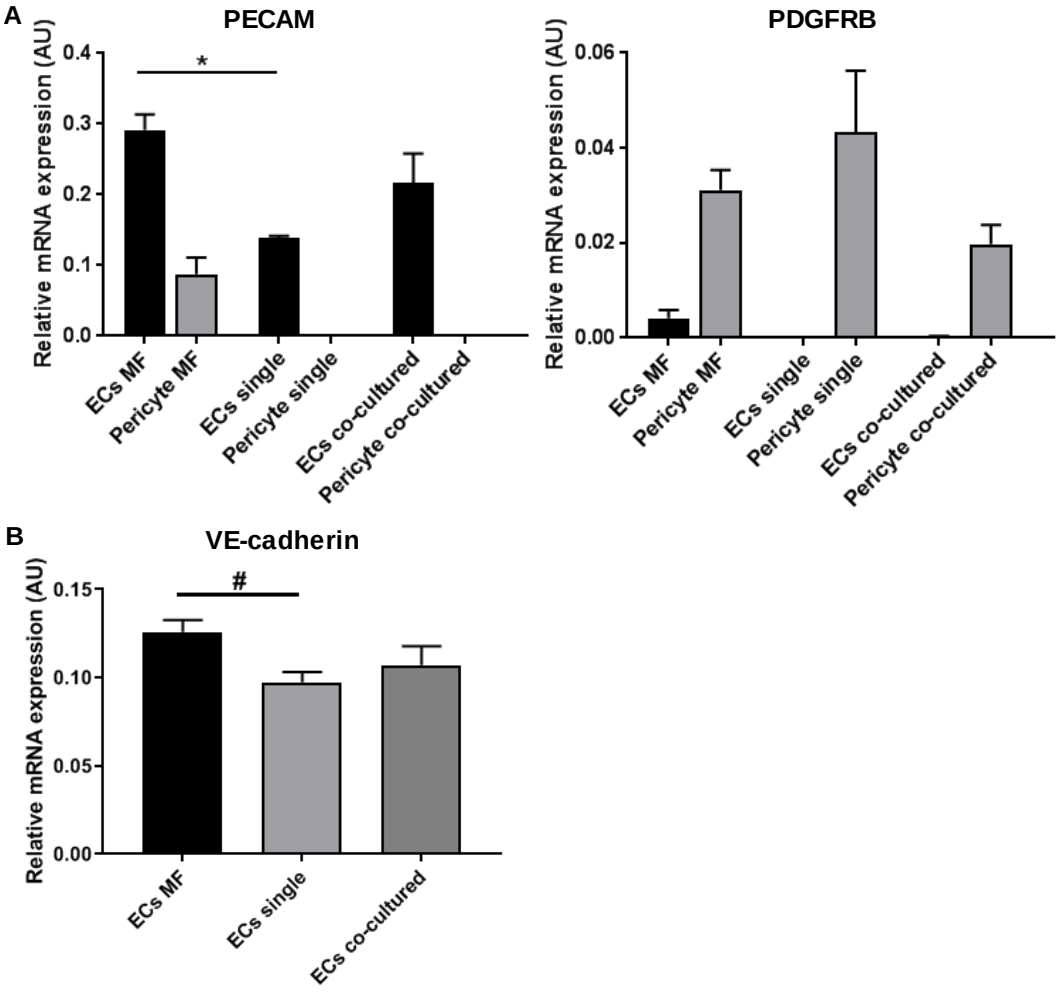

Fig. S3. Specific cell fractions can be isolated for mRNA isolation. (A) mRNA expression levels of endothelial specific PECAM and pericyte specific PDGFRB. Cells lysed in the ECs fraction show more PECAM expression compared to single and co-cultured ECs. Cells lysed in the pericyte fraction show similar PDGFRB expression compared to single and co-cultured pericytes. N=3, *P<0.05. (B) mRNA expression levels of VE-cadherin in ECs cultured in the microfluidic channel (ECs MF), single cultured ECs (ECs single) and ECs co-cultured with pericytes in a transwell system (ECs co-cultured). N3, # P=0.0985. mRNA levels are normalized to beta actin. MF: microfluidic device.

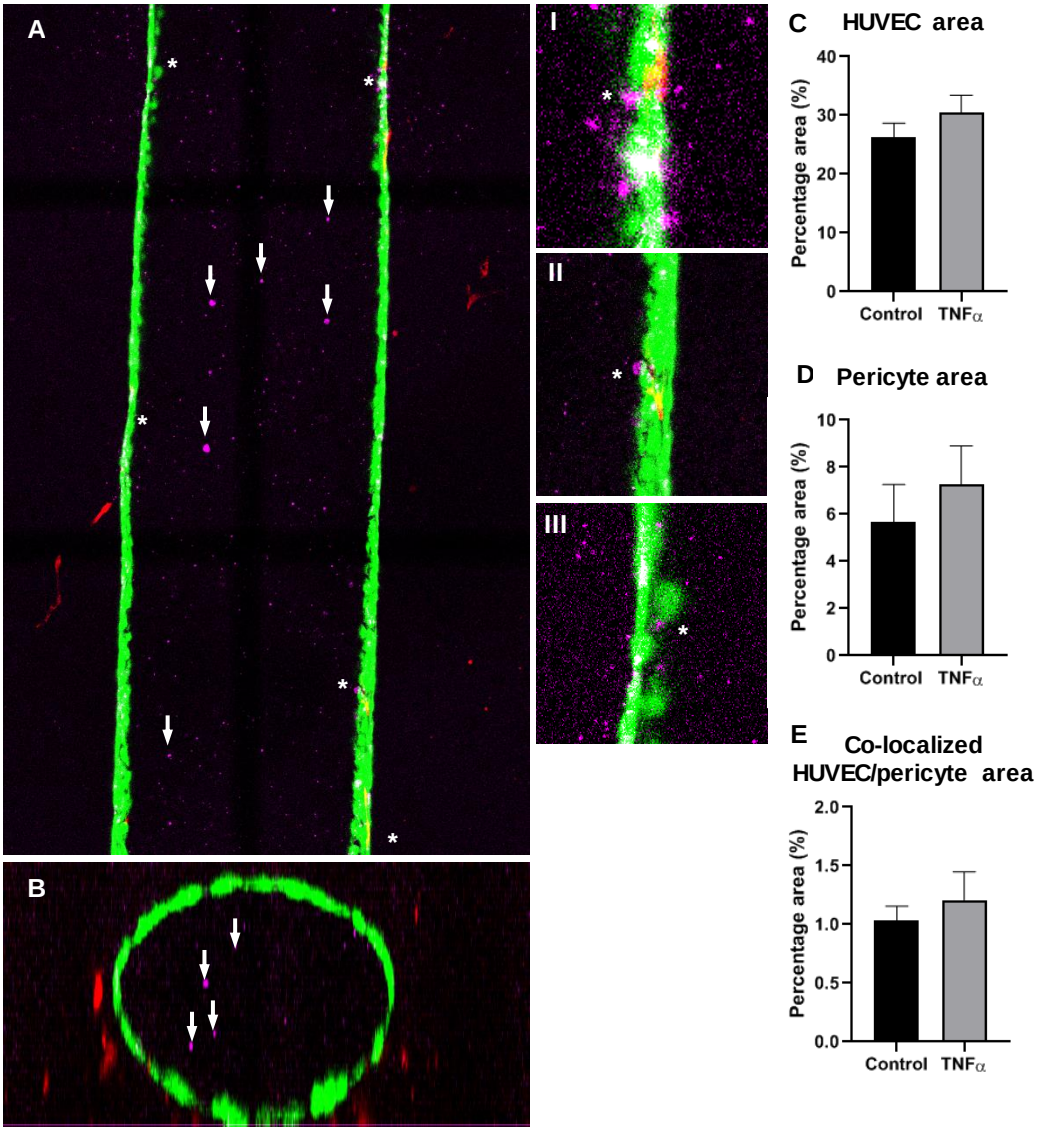

Fig. S4. Flowing the tissue-engineered channels with circulatory THP-1 cells. (A) Projection of the mid-section of the neovessel shows attachment of THP-1 cells to the vessel wall (asterisks) and suspended THP-1 (magenta) in the lumen (indicated with arrows). High magnifications images of THP-1 attachment (asterisks) are shown in I, II and III. (B) Projection of multiple cross sectional Z stacks shows suspended THP-1 in the lumen during flowing (arrows). There is no influence of TNFα on (C) total HUVEC nor (D) pericyte area, as shown in the bar graphs in percentage of co-localized area of total image area. (E) Percentage co-localization area of HUVECs with pericyte is not changed in the presence of TNFα. N=6.

Supplemental movie 1. GIF movie of a longitudinal, composite view of the endothelial (green) channel supported by the ECM containing pericytes (red). The channel shows an open lumen.

Supplemental movie 2. GIF movie of endothelial sprout (green), starting from the tissue-engineered blood vessel wall into the ECM supported by pericytes (red).

Supplemental movie 3. Small movie of circulating THP-1 (blue) in the endothelial channel (green borders) embedded in the pericytes (magenta) supported ECM. Movie is made at a specific z-position almost halfway the endothelial channel.