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


# Supplementary Information for:

# Flow shear stress controls the initiation of neovascularization via heparan sulfate proteoglycans within biomimic microfluidic model

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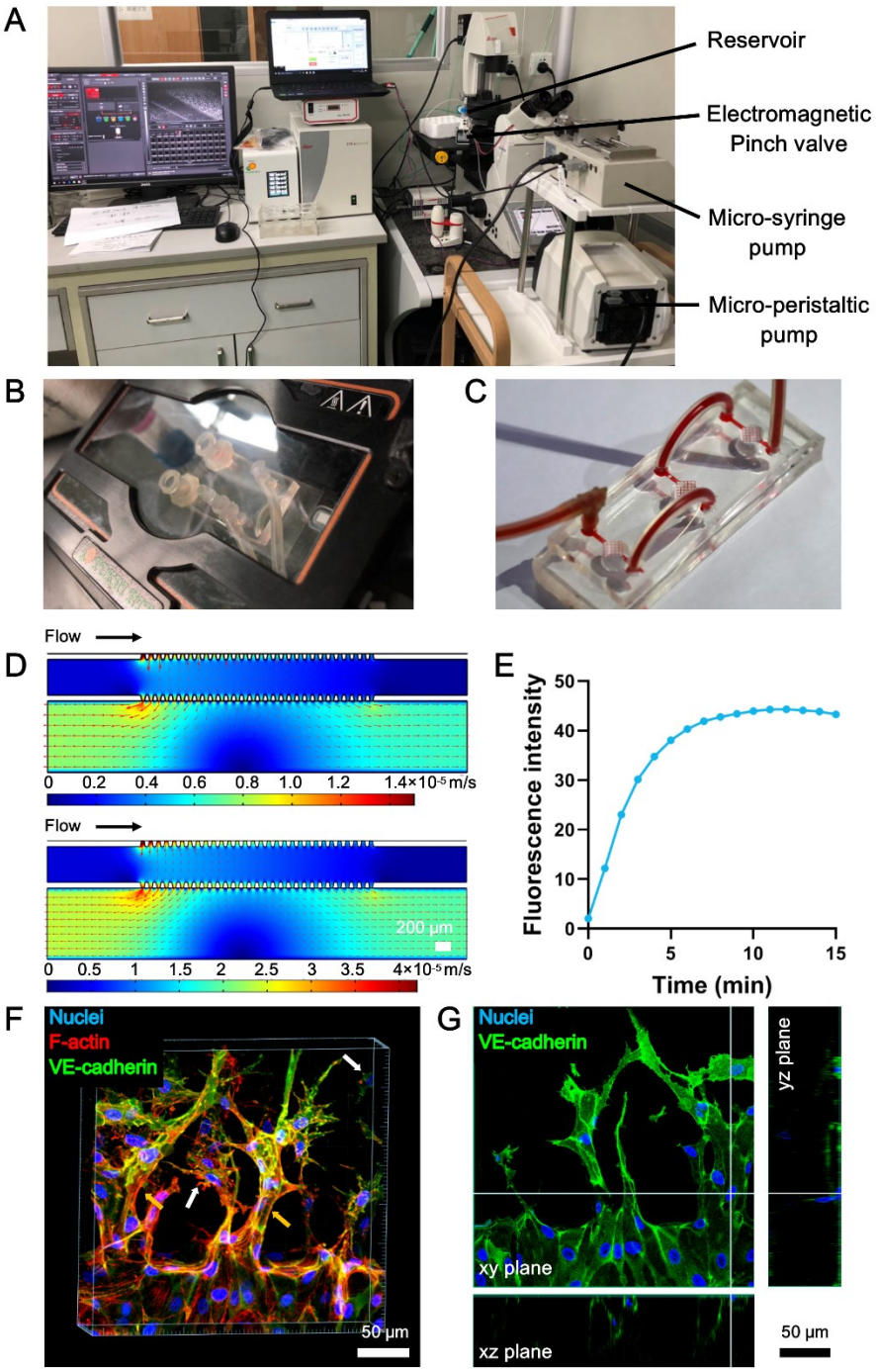


Fig. S1 Structure and function of the microfluidic control system. (A) Picture of working microfluidic control system. The process of sprouting can be observed in real time. A custom LabVIEW program is used to control the micro-syringe pump and electromagnetic pinch valve. (B) Microfluidic sprouting chip in stage top incubator. (C) Picture of bubble trap chip. We connect three chips in series to achieve better bubble removal. (D) The velocity of transendothelial flow from EC channel to central hydrogel channel in the microfluidic sprouting chip without cell lining under 5 dyn/cm$^2$ (top) or 15 dyn/cm$^2$ (down) luminal shear stress. Without cell lining, the velocity of transendothelial flow increases rapidly but still maintains a low level. (E) Averaged fluorescence intensity in hydrogel channel as a function of perfusion time. Data are from images taken every 1 min for 15 minutes. (F) 3D confocal image of sprouting, from which both migration (white arrows) and invasion (yellow arrows) of HUVECs could be observed. (G) Cross-section of sprouts showing the lumen of stalk cells.

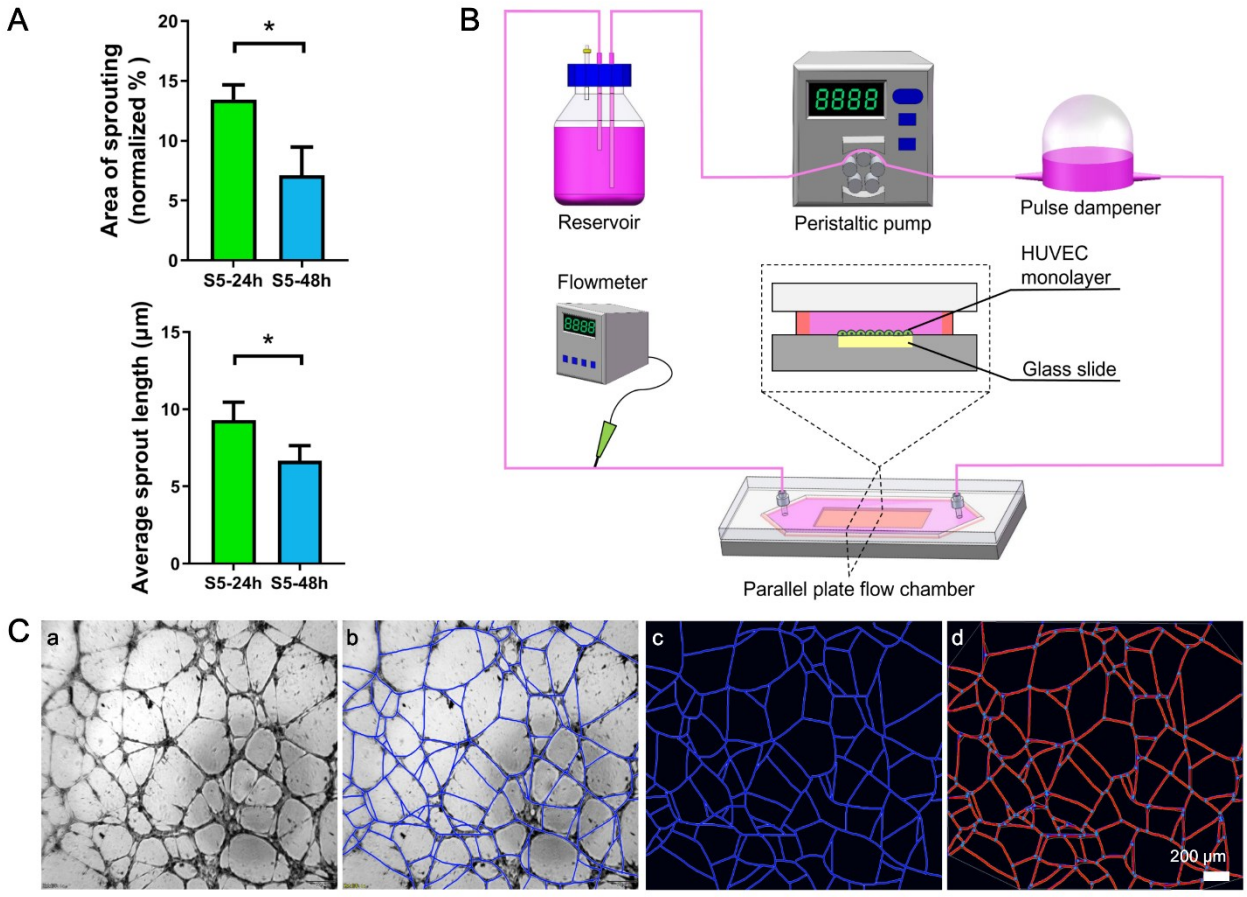


Fig. S2 Traditional tubule formation assay. (A) Quantified area of sprouting and average sprout length for ECs exposing to 5 dyn/cm$^2$ for 24 h or 48 h. (B) Schematic of macroscale parallel-plate flow chamber flow system. The system is consisted of a parallel-plate flow chamber made by sandwiching a silicone gasket between a plexiglas plate and a concave aluminium plate, a flowmeter, an in-house made glass pulse dampener to take out most of the pulsation caused by the peristaltic pump and remove the small bubbles in the flow system, a peristaltic pump and a medium reservoir. (C) The image processing steps for quantifying endothelial tubule formation. a: an image gets from phase contrast microscope at 4× magnification; b: endothelial tubules are manually identified and traced onto a new layer in Photoshop; c: the image imported into AngioTool; d: the final image processing result get from AngioTool, including total tubule length and total number of branch points.

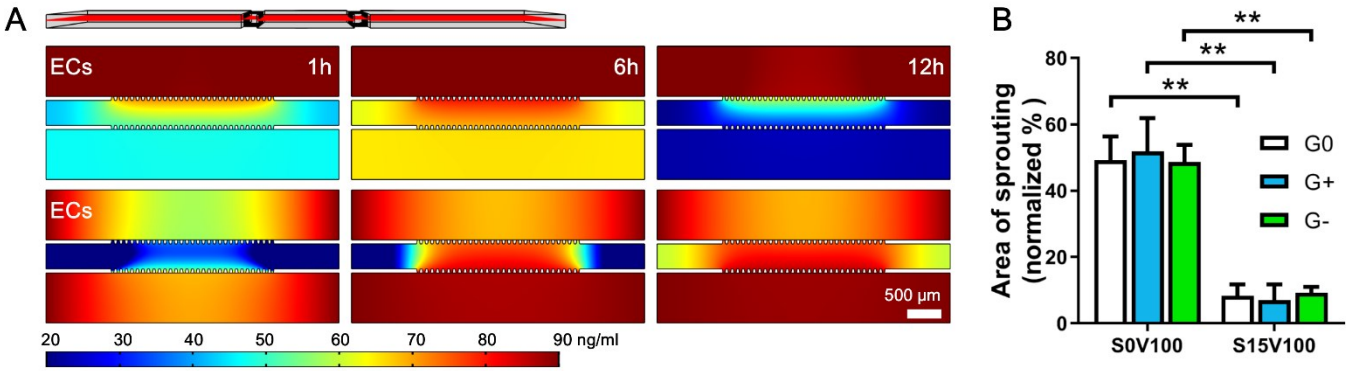


Fig. S3 VEGF gradient in the microfluidic sprouting chip. (A) Simulated results of VEGF negative gradient change over time in static condition (up) and simulated results of VEGF gradient change over time in static condition when VEGF is added to both EC channel and fluid channel (down). (B) Quantified area of sprouting under different VEGF gradients (G0, G+, and G-) and different shear conditions (S0, S15). Neither positive nor negative VEGF gradient affects neovascularization. **, $p < 0.01$.

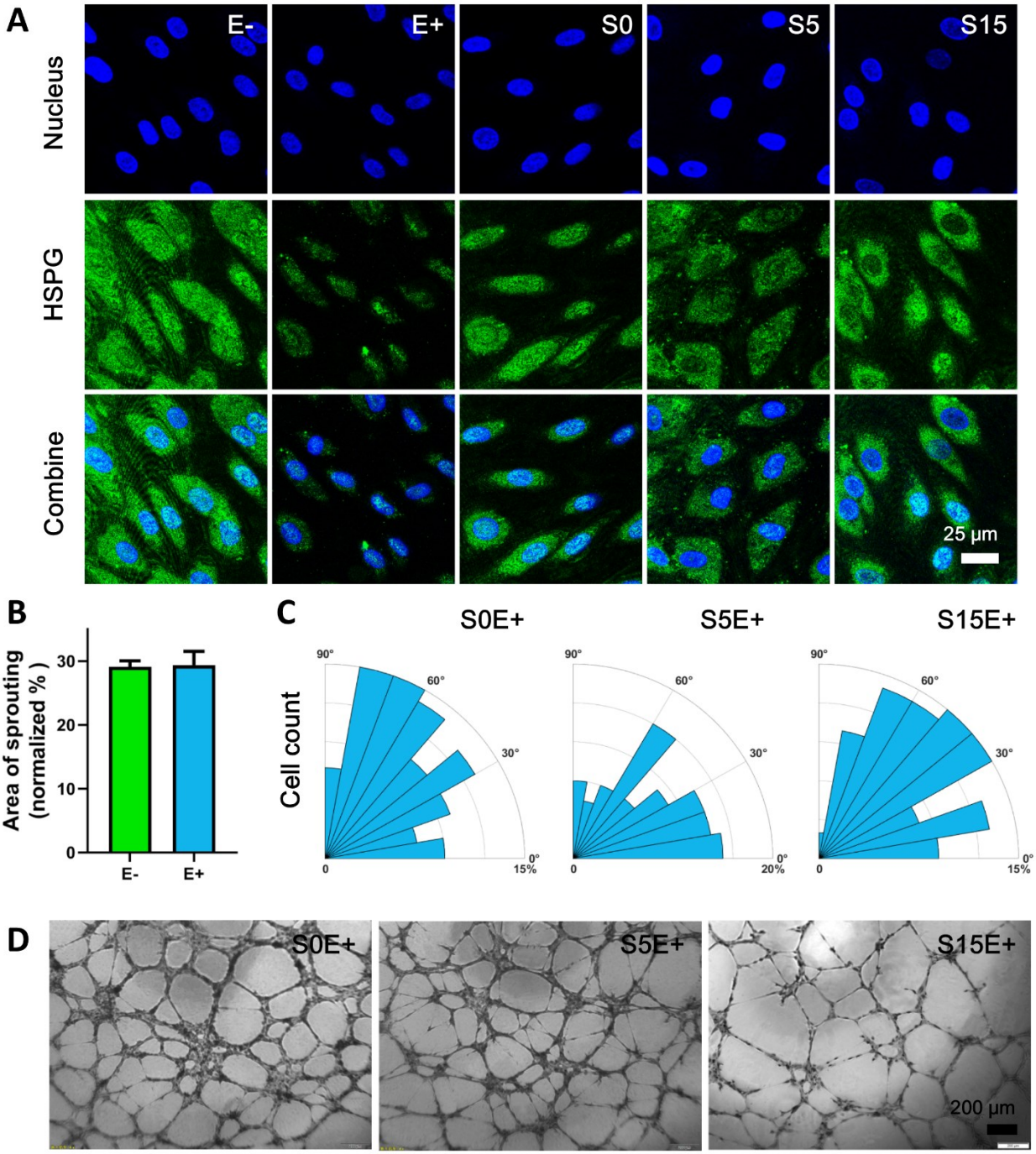


Fig. S4 The effect of Hep.II itself on neovascularization under static condition was not obvious. (A) The distribution of HS on HUVECs with nucleus in blue and heparan sulfate proteoglycan (HSPG) in green. After treated with 250 mU/ml Hep.II for 1 h (E-), the distribution of HS significantly decreased and it regrew at different degree after 24 h culturing or shearing (S0, S5, S15) in the presence of 30 mM/ml NaClO3. (B) Quantified area of sprouting for ECs with (E+) or without (E-) Hep.II treatment in static condition. Without VEGF and shear stress, HSPG disruption would not affect endothelial sprouting. (C) Quantification of Hep.II treated ECs angle orientation distribution. Instead of aligned along the flow direction, treated cells were elongated randomly in all possible angles from 0° to 90°. (D) Representative images of Hep.II treated endothelial tubule formation under different shear conditions.

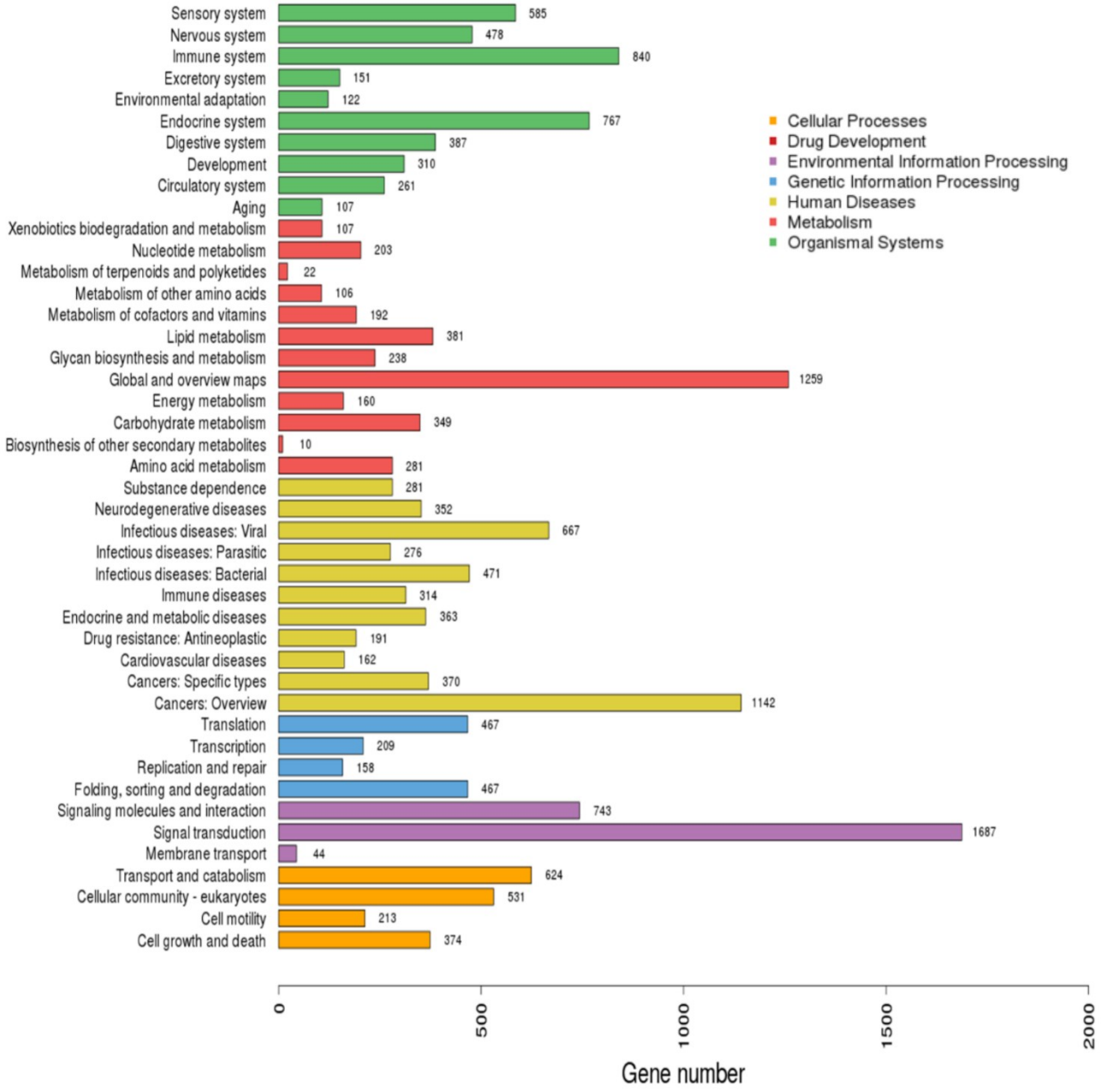


Fig. S5 Second class of KEGG classification.

Video 1. Bubble trap chip

Video 2. Sprouting in 48 h under static culture