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

Direct current electric field regulates endothelial permeability under physiologically relevant fluid forces in a microfluidic vessel bifurcation model

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Supplementary Information

Electrical current measurements in the microfluidic devices

Significant and relevant past work with electrical stimulation of biological cells reports on the applied potentials and the equivalent electric fields generated is discussed in the main manuscript. However, whenever an electrode-based system with a conducting fluid (i.e., saline or media) is used, there is also an accompanying current flow. Table S1 presents a summary of the recorded current flow for the microfluidic devices with both collagen and cells seeded. Furthermore, Table S1 also summarizes the electric current recorded for various device states of media only (no collagen and no cells) and media with the collagen chamber filled. As various species are added to the microfluidic system, the device resistance increases and the measured current drops for the same applied potential as noted in Table S1. All the current data were acquired using NI-DAQ 9365 and the data was recorded in LabVIEW.

Table S1. Measured current values in the devices for different collagen and cell seeding cases.

Case	Current Measured (μA)
No Collagen + No Cells	0.37 ± 0.07
Collagen + No Cells	0.22 ± 0.02
Collagen + Cells	0.15 ± 0.02

Ionic composition of endothelial cell growth media

Table S2. Composition of the major electrolytes in the endothelial cell growth media (ECGM) media and their respective concentrations as provided by Promocell.

Electrolyte	Concentration (M)
$MgSO_4$	0.01
NaCl	0.11
$NaHCO_3$	0.014
$CaCl_2$	0.0016

Consolidated endothelial hydraulic conductivity (L_P) values at the bifurcation point (BP) and branched vessel (BV) for all stimulatory conditions.

Table S3. Quantitative effects of 1 μL/min perfusion (generating 3.8 dyn/cm^2 BFF at the BP and 0.3 dyn/cm^2 LSS at the BV) or 10 μL/min perfusion (generating 38 dyn/cm^2 BFF at the BP and 3 dyn/cm^2 LSS at the BV) both in the absence and presence of 70 V/m DC-EF on L_P after 1 hour and 6 hour treatments, compared to static control condition (control case).

Time (h)	Inlet Potential (V)	BFF (dyn/cm^2)	LSS (dyn/cm^2)	L_P at BP (x 10^{-4} cm.s^{-1}.cmH_2O^{-1})	L_P at BV (x 10^{-4} cm.s^{-1}.cmH_2O^{-1})
1	0	3.8	0.3	0.24 ± 0.04	0.19 ± 0.03
		38	3.0	0.64 ± 0.09	0.57 ± 0.08
		50	8.8	0.55 ± 0.09	0.41 ± 0.08
		78	11.5	0.35 ± 0.05	0.32 ± 0.05
	1	3.8	0.3	2.21 ± 0.39	2.13 ± 0.23
		38	3.0	1.39 ± 0.24	1.11 ± 0.17
6	0	3.8	0.3	0.28 ± 0.05	0.27 ± 0.06
		38	3.0	0.33 ± 0.05	0.32 ± 0.04
	1	3.8	0.3	0.31 ± 0.04	0.39 ± 0.03
		38	3.0	0.27 ± 0.08	0.32 ± 0.07
Control Case	0	0.0	0.0	0.32 ± 0.02	0.32 ± 0.06

Table S4. Quantitative effect of 70 V/m DC-EF co-applied with 1 μL/min perfusion (generating 3.8 dyn/cm^2 BFF at the BP and 0.3 dyn/cm^2 LSS at the BV) and 10 μL/min perfusion (generating 38 dyn/cm^2 BFF at the BP and 3 dyn/cm^2 LSS at the BV), in the presence of Akt inhibitor MK – 2206 on L_P after 1 hour of treatment, compared against the static control condition (control case).

Time (h)	Inlet Potential (V)	BFF (dyn/cm^2)	LSS (dyn/cm^2)	L_P at BP (x 10^{-4} cm.s^{-1}.cmH_2O^{-1})	L_P at BV (x 10^{-4} cm.s^{-1}.cmH_2O^{-1})
1	0	3.8	0.3	0.27 ± 0.01	0.24 ± 0.04
		38	3.0	0.25 ± 0.07	0.21 ± 0.06
	1	3.8	0.3	0.21 ± 0.04	0.21 ± 0.04
		38	3.0	0.43 ± 0.06	0.43 ± 0.1
Control Case	0	0.0	0.0	0.28 ± 0.08	0.23 ± 0.05

Quantitative analysis of intracellular VE-Cadherin signal and junctional VE-Cadherin signal at the bifurcation point (BP) and branched vessel (BV) after 1 hour and 6 hour simultaneous flow and direct current electric field (DC-EF) stimulation

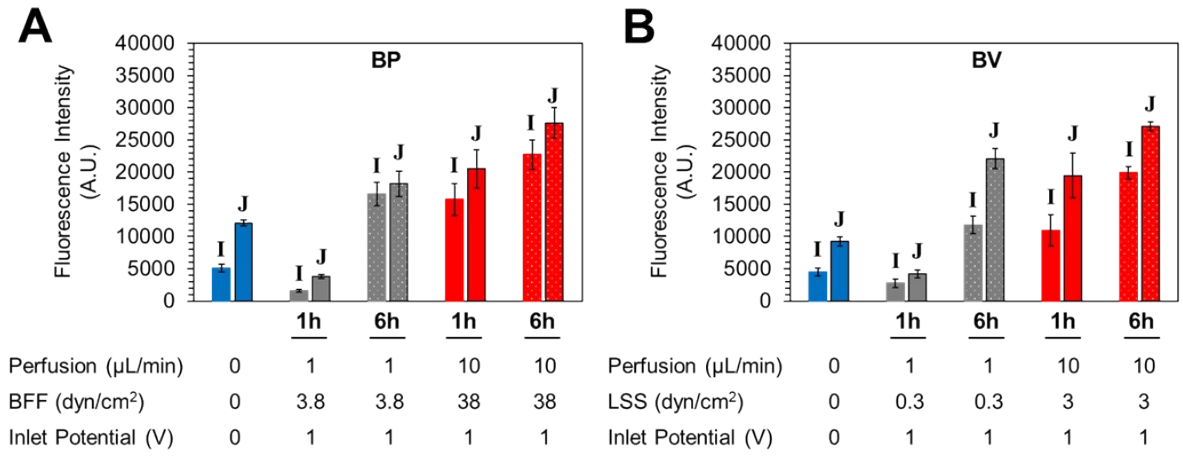

Figure S1 - Variation in endothelial hydraulic conductivity (L_P) corresponds to an increase or decrease in VE-cadherin junction protein expression at the bifurcation point (BP) and branched vessel (BV) apertures, under select stimulation conditions. (A) Quantitative analysis of intracellular VE-Cadherin signal (labelled as I) and junctional VE-Cadherin signal (labelled as J) at the BP after 1 hour and 6 hour of treatment with 70 V/m DC-EF alongside 3.8 dyn/cm^2 BFF (1 µL/min) and 38 dyn/cm^2 BFF (10 µL/min) in comparison to the static control condition. (B) Quantitative analysis of intracellular VE-Cadherin signal (labelled as I) and junctional VE-Cadherin signal (labelled as J) at the BV after 1 hour and 6 hour of treatment with 70 V/m DC-EF alongside 0.3 dyn/cm^2 LSS (1 µL/min) and 3 dyn/cm^2 LSS (10 µL/min) in comparison to the static control condition. The fluorescence intensity values are presented as the mean ± the standard error of the mean for each experimental test condition tested in 2 separate microfluidic devices as replicates.

Direct current electric field (DC-EF) induced increase in endothelial hydraulic conductivity (L_P) at the bifurcation point (BP) and branched vessel (BV) is reversible.

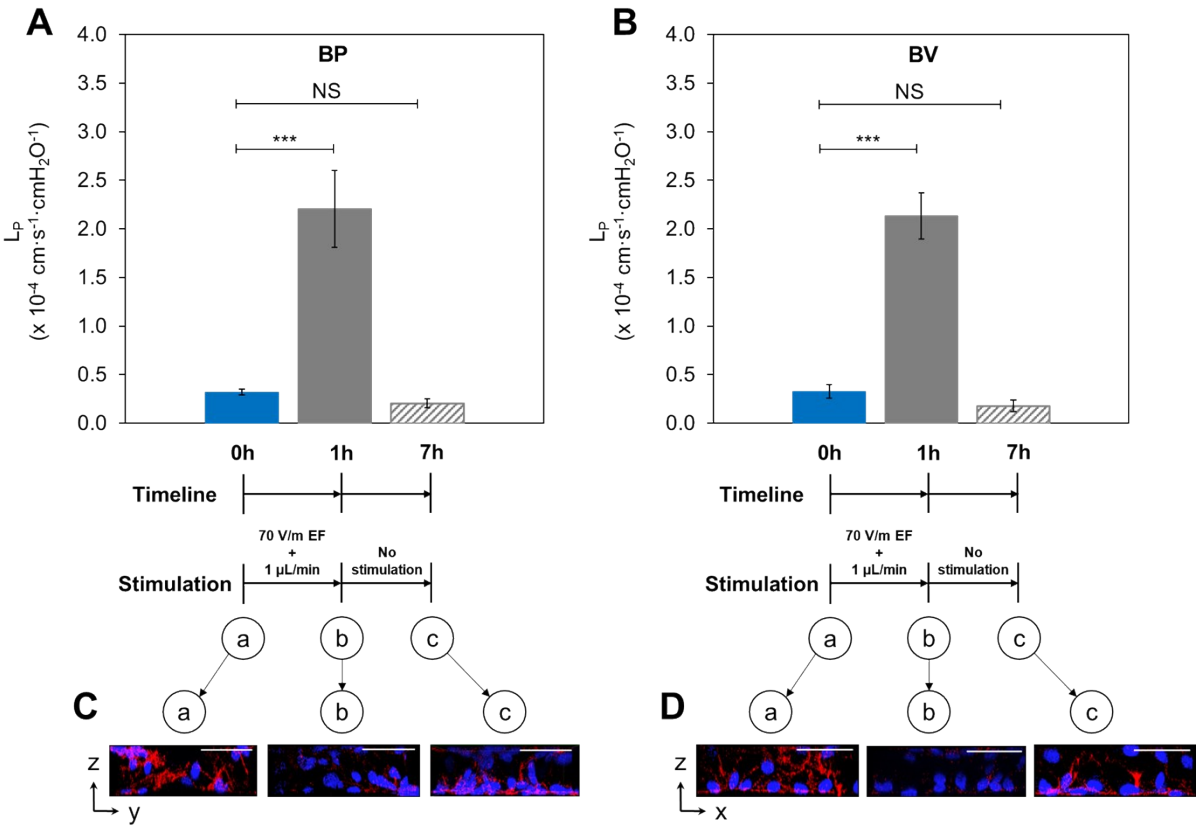

Figure S2 - Direct current electric field (DC-EF) induced increase in endothelial hydraulic conductivity (L_P) at the bifurcation point (BP) and branched vessel (BV) is reversible. (A) Quantitative effect of 70 V/m DC-EF co-applied alongside 3.8 dyn/cm^2 BFF (1 µL/min) on L_P immediately after the 1-hour stimulation (point b) and after 6 hours of no stimulation (point c), compared to static control condition (point a). (B) Quantitative effect of 70 V/m DC-EF co-applied alongside 0.3 dyn/cm^2 LSS (1 µL/min) on L_P immediately after the 1-hour stimulation (point b) and after 6 hours of no stimulation (point c), compared to static control condition (point a). Confocal microscopy images of the HUVECs at (C)the BP and (D) the BV [DAPI (Blue); VE-Cadherin (Red)]. Scale bars are 50 µm. * denotes $p < 0.05$, ** denotes $p < 0.01$, *** denotes $p < 0.001$, and NS denotes not significant. Error bars indicate ±SEM (Standard Error of the Mean).

Immunostaining of HUVECs at the bifurcation point (BP) and branched vessel (BV) aperture with VE-Cadherin

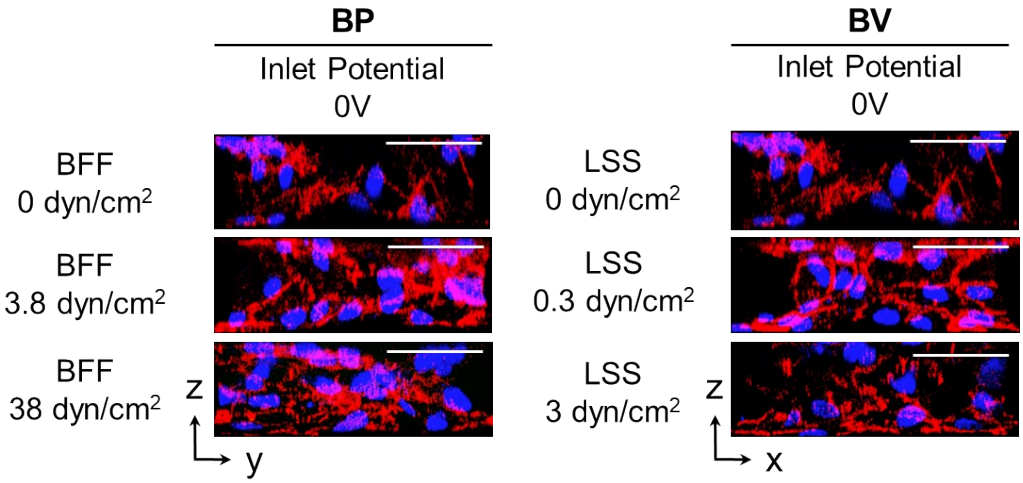

Figure S3 - Confocal microscopy images of the HUVECs at the BP and the BV at static conditions (0 dyn/cm^2 BFF and 0 dyn/cm^2 LSS) and flow only conditions (1 µL/min generating 3.8 dyn/cm^2 BFF and 0.3 dyn/cm^2 LSS; 10 µL/min generating 38 dyn/cm^2 BFF and 3 dyn/cm^2 LSS) after 1 hour of treatment. [DAPI (Blue); VE-Cadherin (Red)]. Scale bars are 50 µm.

Live/dead cell staining of HUVECs

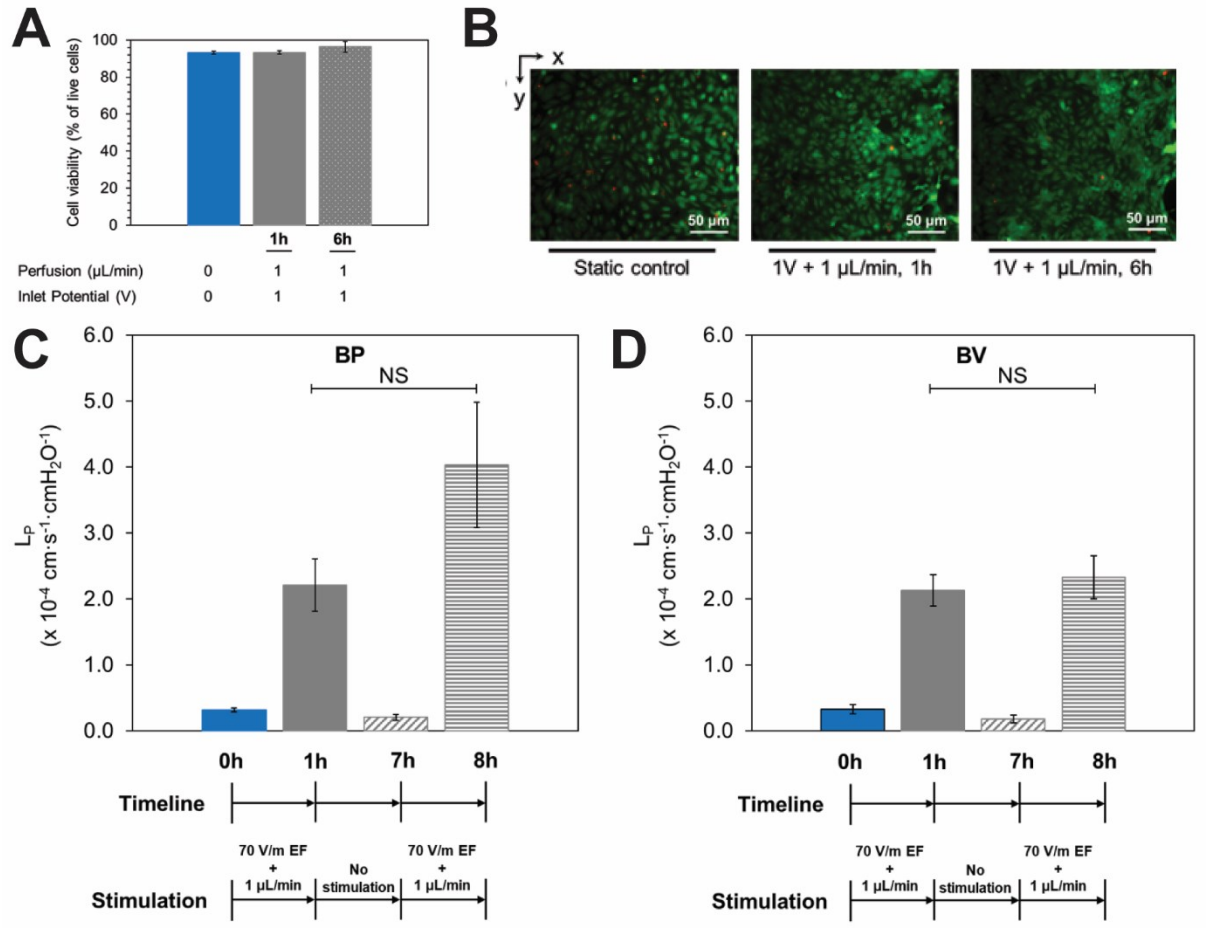

Figure S4 – HUVEC viability and barrier integrity when subjected to stimulation with DC-EF. (A) Quantitative analysis of viability rate of HUVECs subjected to 70 V/m DC-EF (Inlet Potential: 1V) co-applied alongside 1 µL/min perfusion after 1-hour and 6-hour stimulation, in comparison with static control condition. (B) Immunofluorescence images of the HUVECs depicting the live and dead cells in the microfluidic device after simultaneous 70 V/m DC-EF and 1 µL/min perfusion stimulation for 1 hour and 6 hours. [Live cells (Green); dead cells (Red)]. Scale bars are 50 µm. The cell viability values are presented as the mean ± the standard error of the mean for each experimental test condition tested in 2 separate microfluidic devices as replicates. (C) Quantitative effect of 70 V/m DC-EF co-applied alongside 3.8 dyn/cm^2 BFF (1 µL/min) on L_P immediately after the 1-hour of re-stimulation following 1 hour of stimulation with 70 V/m DC-EF co-applied alongside 3.8 dyn/cm^2 BFF (1 µL/min) and 6 hours of no stimulation, compared to static control condition. (D) Quantitative effect of 70 V/m DC-EF co-applied alongside 0.3 dyn/cm^2 LSS (1 µL/min) on L_P immediately after the 1-hour of re-stimulation following 1 hour of stimulation with 70 V/m DC-EF co-applied alongside 3.8 dyn/cm^2 LSS (1 µL/min) and 6 hours of no stimulation, compared to static control condition. At both BP and BV, HUVECs subjected to 1 hour of re-stimulation following 6 hours of incubation under no-stimulation elicited statistically comparable level of L_P compared to when stimulated for 1 hour. Collectively, these results confirm HUVEC viability when subjected to DC-EF in presence of fluid flow.

Quantitative analysis of intracellular VE-Cadherin signal and junctional VE-Cadherin signal at the bifurcation point (BP) and the branched vessel (BV) after 1 hour simultaneous flow and DC-EF stimulation in the absence and presence of Akt inhibitor.

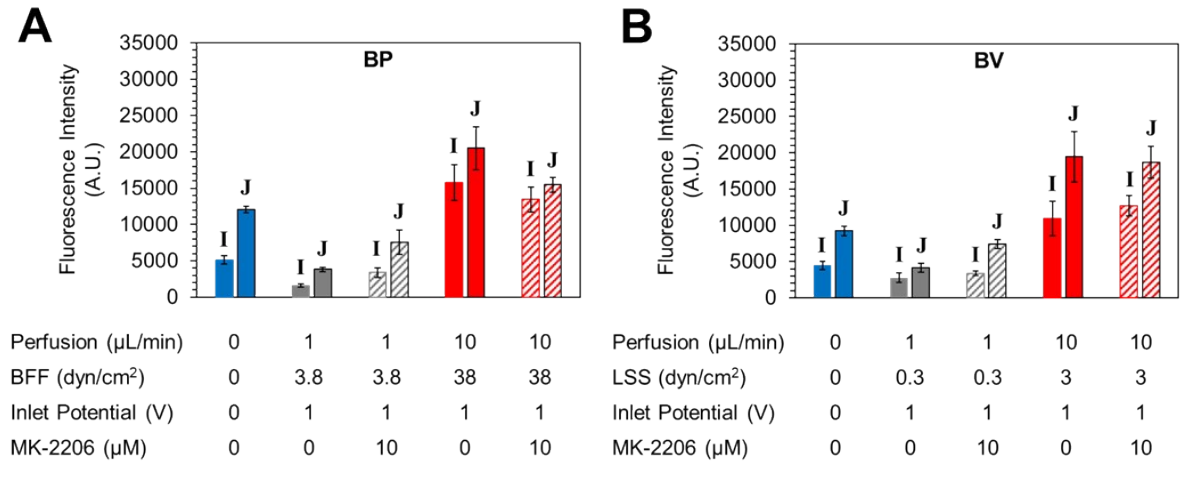

Figure S5 - Decrease in endothelial hydraulic conductivity (L_P) in the presence of Akt inhibitor corresponds to an increase in VE-cadherin junction protein expression at the bifurcation point (BP) and branched vessel (BV) apertures, under select stimulatory conditions. (A) Quantitative analysis of intracellular VE-Cadherin signal (labelled as I) and junctional VE-Cadherin signal (labelled as J) at the BP after 1 hour of treatment with 70 V/m DC-EF alongside 3.8 dyn/cm^2 BFF (1 µL/min) and 38 dyn/cm^2 BFF (10 µL/min), both in the absence and presence of Akt inhibitor MK – 2206 (10 µM), compared against the static control condition. (B) Quantitative analysis of intracellular VE-Cadherin signal (labelled as I) and junctional VE-Cadherin signal (labelled as J) at the BP after 1 hour of treatment with 70 V/m DC-EF alongside 0.3 dyn/cm^2 LSS (1 µL/min) and 3 dyn/cm^2 LSS (10 µL/min), both in the absence and presence of Akt inhibitor MK – 2206 (10 µM), compared against the static control condition. The fluorescence intensity values are presented as the mean ± the standard error of the mean for each experimental test condition tested in 2 separate microfluidic devices as replicates.

Immunostaining of HUVECs monolayer with PECAM-1

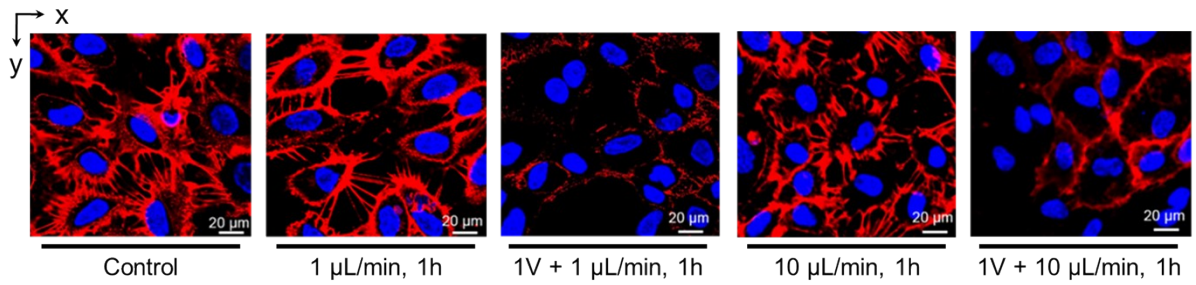

Figure S6 - Confocal microscopy images of the HUVECs immunostained with PECAM-1 antibody before and after stimulating the HUVECs with 1 µL/min or 10 µL/min perfusion both in the absence and presence of 70 V/m DC-EF (Inlet Potential: 1V, treated for 1 hour). Static control refers to "no flow and no DC-EF" condition. [DAPI (Blue); PECAM-1 (Red)].