Supplementary Information (SI) for Lab on a Chip.

Microfluidic Artery-on-a-Chip Model with Unidirectional Gravity-Driven Flow for High-Throughput Applications

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Supplemental Methods

Microfluidic Flow Simulation

Python code was created to simulate the fluid flow in the unidirectional and bidirectional chips.

Simulation Process

1. Initialization

Start with initial volumes V1 and V2 in the two reservoirs, converted from microliters to cubic meters:

- $volume1[0] = V1 \times 10^{-9}$
- $volume2[0] = V2 \times 10^{-9}$

2. Height Calculation

Calculate the height difference between the top of the two column of liquid, taking into account the tilt of the column themselves:

- Reservoir Height: $h_{reservoir} = \sqrt{(V \times \sin(2\beta) / w_{well})}$

  where V is the volume of the column, β is the plate tilt and w_{well} is the well width

- Tilt Height: $h_{tilt} = \sin(\beta) \times D$

  where D is the distance between the wells

- Total Height Difference: $H_{tot} = h_{tilt} + (h_{reservoir1} - h_{reservoir2})$

3. Pressure and Flow Calculation

Calculate the pressure difference and flow volume:

- Pressure Difference: $P_{diff} = \rho \times g \times H_{tot}$
- Resistance of the microfluidic channel: $R_{hyd} = 12*\mu*L/((1-0.63*(h/w))*(h^3)*w)$
    - L is length of channel, μ is dynamic viscosity, h is height of the channel, and w is width of the channel. Equation is true when h << w.
- Volumetric flow: $Q_{flow} = P_{diff} / R_{hyd}$

  $V_{flow} = Q_{flow} \times T_{step}$

4. Volume Update

Update the reservoir volumes iteratively:

- $volume1[i] = max(0, volume1[i-1] - V_{flow})$
- $volume2[i] = V1 + V2 - volume1[i]$

5. Flow Rate and Shear Stress Calculation

Calculate shear stress:

- Shear Stress (dyn/cm2): $\tau = (60 \times \mu \times Q_{flow}) / (w \times h^2)$

6. Unit Conversion and Plotting

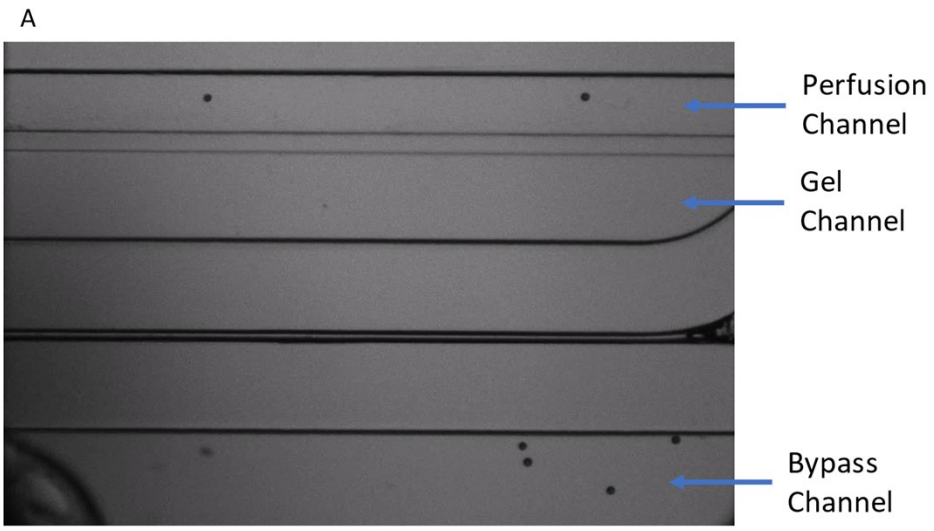
Convert flow rates to µL/min and volumes to µL, then plot volume, flow rate, and shear stress over time for analysis.

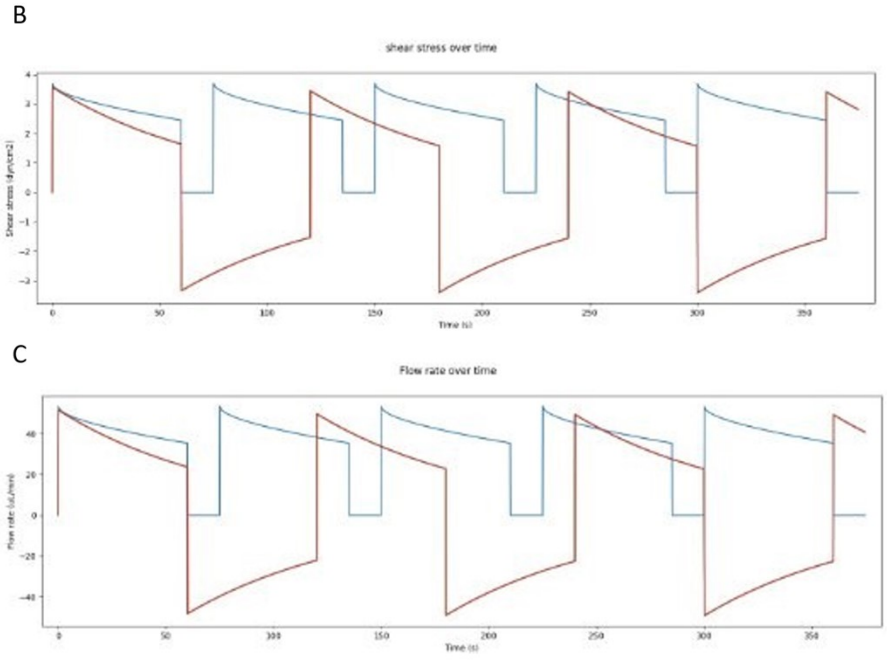
OrganoPlate 2-lane-48 UF and 2-lane-48 UF Bidirectional Settings

		Unidirectional	Bidirectional
Medium Volumes			
Growth settings	(µl)	100	50
Experimental settings	(µl)	40	40
Rocker Tilt Direction	Settings		
Negative	Tilt (°)	25	25
	Dwell time (second)	60	60
	Transition time (second)	5	5
Positive	Tilt (°)	25	25
	Dwell time (second)	15	60
	Transition time (second)	5	5

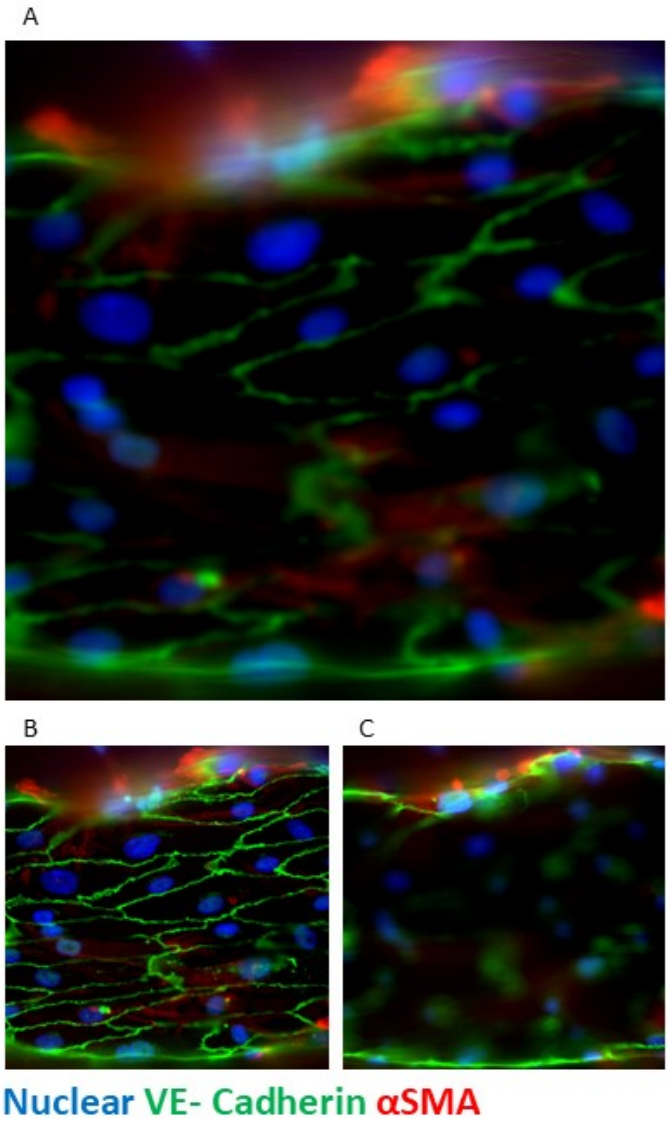
Supplementary Table 1: Settings to induce Unidirectional versus Bidirectional Flows

Supplemental Results

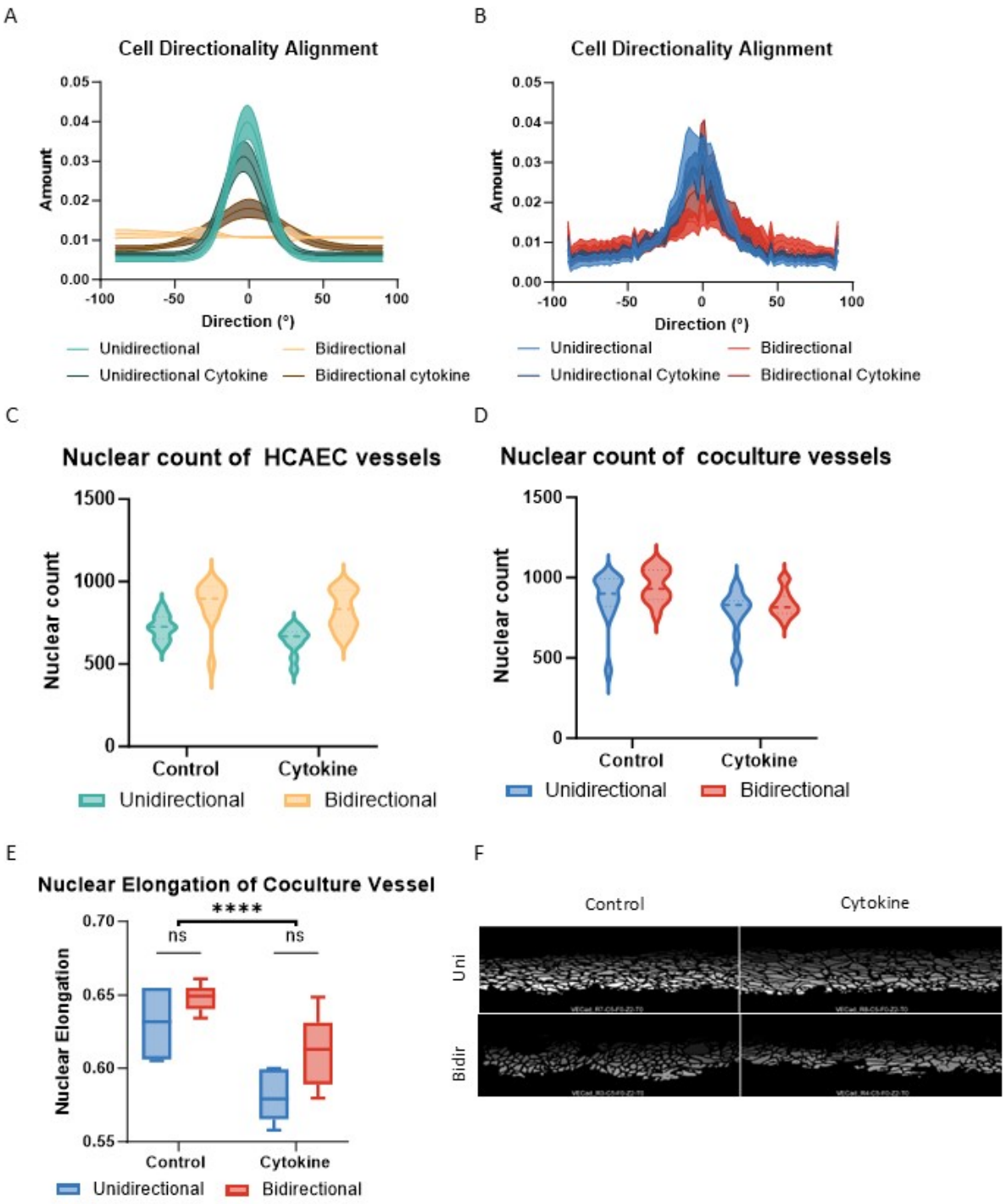




Supplemental Figure 1: Flow in the Unidirectional and Bidirectional plates **(A)** Brightfield video followed by fluorescent video illustrating unidirectional flow through the top microfluidic channel of OrganoPlate 2-lane UF using fluorescent beads. The channel at the bottom of the frame is the bypass channel to allow the beads to return to the perfusion channel. **(B)** Simulated shear stress over time in the Unidirectional (blue) and Bidirectional (red) overlay. **(C)** Simulated flow rate overtime in the Unidirectional (blue) and Bidirectional (red) overlay. Note: for the Unidirectional simulation, temporary reversal of flow in the perfusion channel due to the movement of the liquid already in the channel during the changing of rocking position is not included in the graph.



Supplemental Figure 2. HCAEC/HCASMC vessel under unidirectional flow without addition of cytokines with **(A)** Video stepping through from the bottom to middle of HCAEC/HCASMC vessel for 50 z slices. 60x magnification, z step = 2 µm **(B)** Z-step slice 7 **(C)** Z-step slice 25



Supplemental Figure 3. (A) Quantification of CD31 alignment in the direction of the flow of HCAEC monoculture vessels with and without the addition of cytokines **(B)** Quantification of CD31 alignment in the direction of the flow of HCAEC/HCASMC vessels with and without the addition of **C)** Nuclear count of HCAEC monoculture vessel **(D)** Nuclear count of HCAEC/HCASMC vessel **(E)** Nuclear elongation of coculture vessels. **(F)** Mask of bottom slice of VE Cadherin segmented vessels with IN Carta.