Supplemental Information

A microfluidic cardiac flow profile generator for studying the effect of shear stress on the valvular endothelial cell

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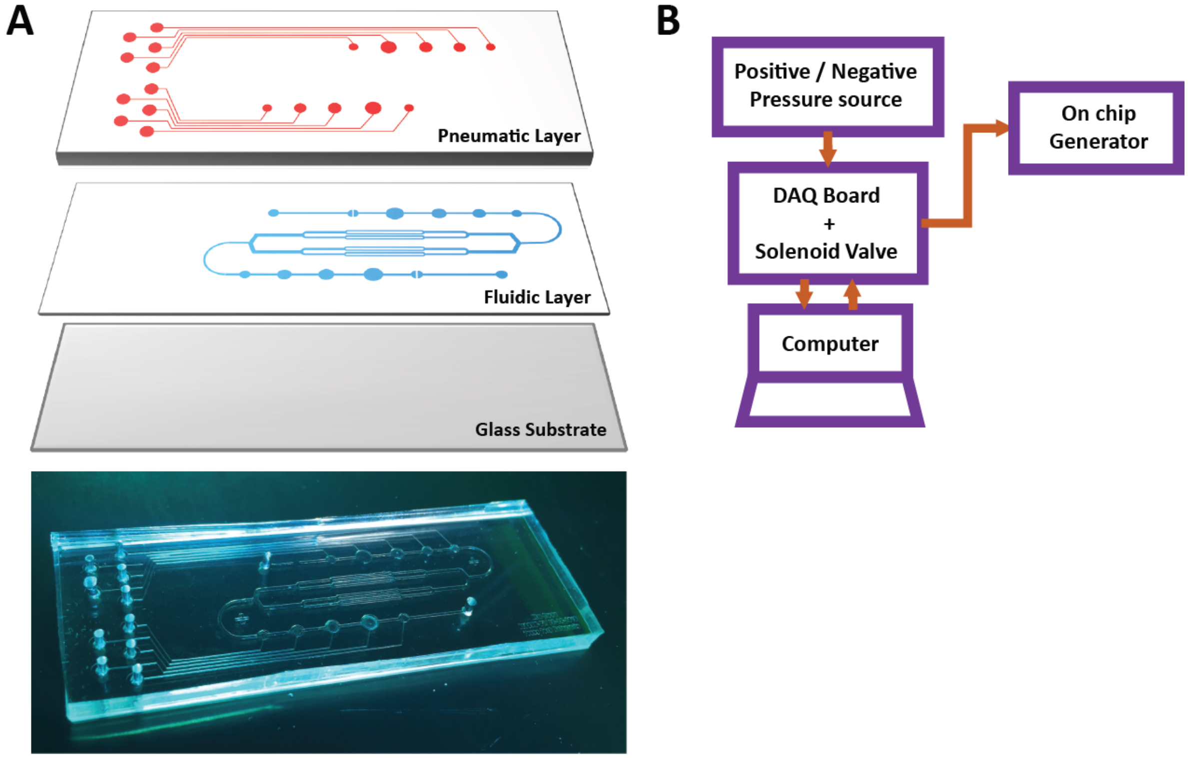


Figure SI 1. Schematic drawing of the entire system for microfluidic cardiac flow profile generator. (**A**) An integrated form of cardiac flow profile generator is divided by two layers including pneumatic and fluidic layers and bonded with glass substrate. (**B**) The generator is operated by applying a positive or negative pressure, and each pressure source is connected to solenoid valves and actuated by a programmed computer. A programmed cardiac sequence makes a cardiac-like shear stress pattern in on-chip generator to make a widely adjustable physiological range within a cell culturing bifurcating channel.

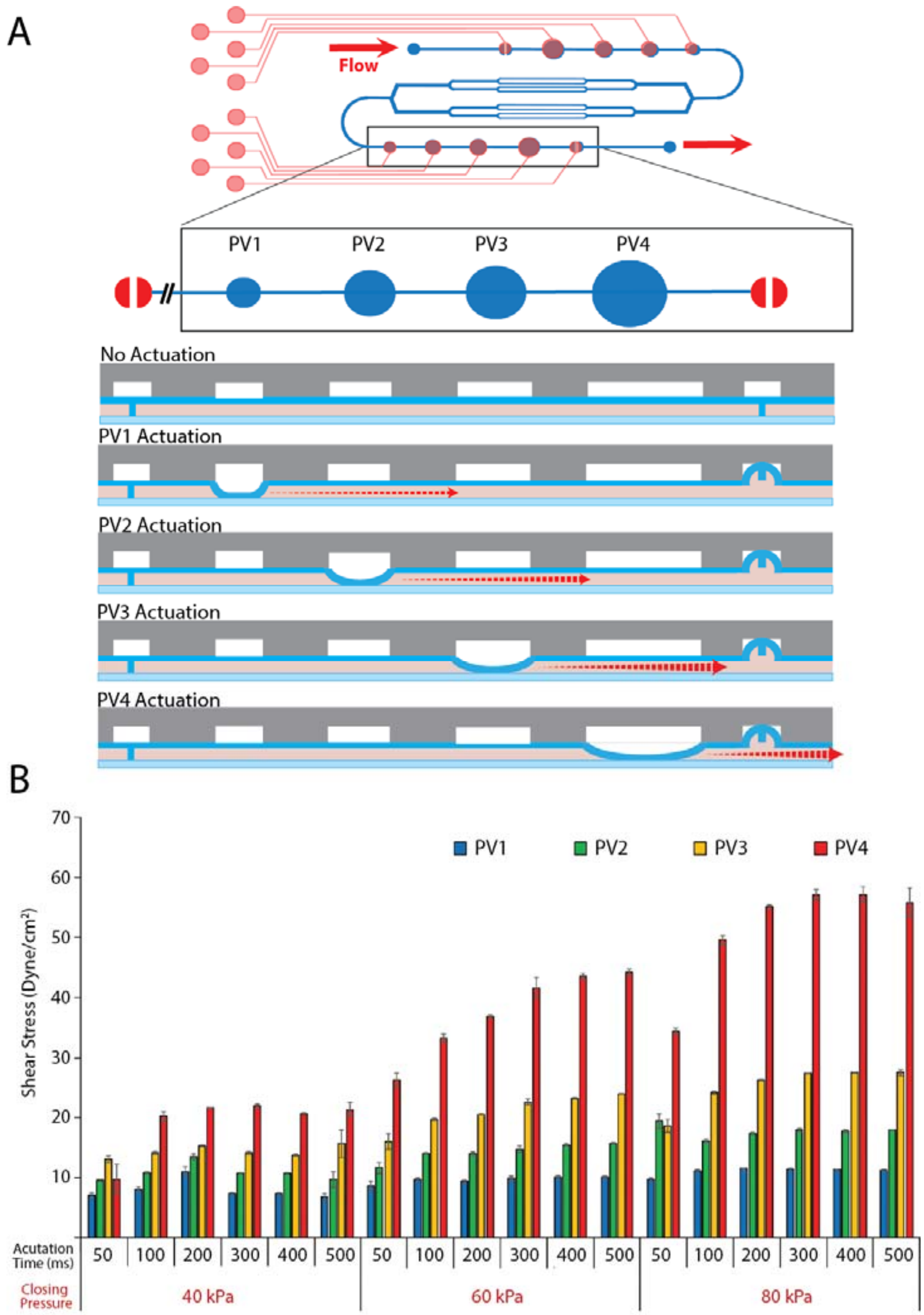
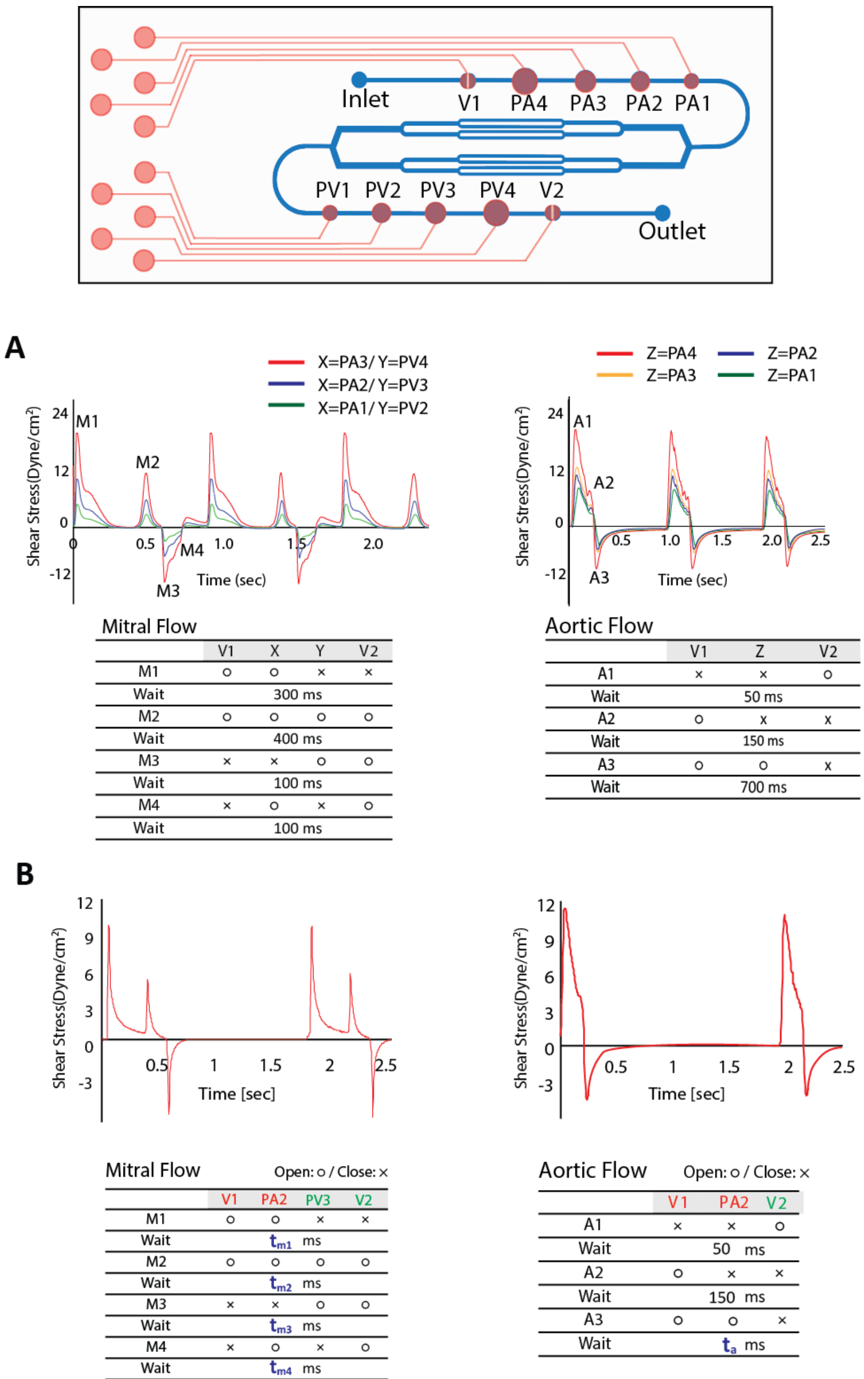


Figure SI 2. The figures represent (A) the pumping actuation sequence and (B) maximum shear stress per pump stroke graphs for characterization of PV1, PV2, PV3, and PV4 at 350 μm channel width. The maximum shear stresses are shown as functions of closing pressure (40, 60 and 80 kPa) and actuation time (50-1000 ms). Measured volumetric flow rate (Q) from each pump was plugged into the shear stress equation ($\tau = 6Q\mu/h^2w$) for a rectangular channel and generates various max shear stress ranges at 350 μm channel width (PV1: 7-11 dyne/cm^2, PV2: 9-17 dyne/cm^2, PV3: 13-27 dyne/cm^2, PV4: 26-56 dyne/cm^2).



Mitral Flow (A)

	V1	X	Y	V2
M1	o	o	×	×
Wait		300 ms		
M2	o	o	o	o
Wait		400 ms		
M3	×	×	o	o
Wait		100 ms		
M4	×	o	×	o
Wait		100 ms		

Aortic Flow (A)

	V1	Z	V2
A1	×	×	o
Wait		50 ms	
A2	o	x	x
Wait		150 ms	
A3	o	o	x
Wait		700 ms	

Mitral Flow (B) Open: o / Close: ×

	V1	PA2	PV3	V2
M1	o	o	×	×
Wait		t_{m1} ms		
M2	o	o	o	o
Wait		t_{m2} ms		
M3	×	×	o	o
Wait		t_{m3} ms		
M4	×	o	×	o
Wait		t_{m4} ms		

Aortic Flow (B) Open: o / Close: ×

	V1	PA2	V2
A1	×	×	o
Wait		50 ms	
A2	o	×	×
Wait		150 ms	
A3	o	o	×
Wait		t_a ms	

Figure SI 3. Validation of controlling (A) magnitude and (B) frequency levels for both aortic and mitral inflows. By selecting proper sets of pumps, a wide range of the shear stress magnitude can be reconstructed. In addition, by selecting actuation times, different heart rhythms can be demonstrated at 0.8Hz (t_{m1}=700, t_{m2}=400, t_{m3}=100, t_{m4}=100 ms/ t_a=1200ms), 1.2Hz (t_{m1}=300ms, t_{m2}=400, t_{m3}=100, t_{m4}=100 ms/ t_a=700ms), and 2Hz (t_{m1}=200ms, t_{m2}=200, t_{m3}=50, t_{m4}=50 ms/ t_a=250ms). All the flows were generated by programmed actuation sequence as shown in each table.

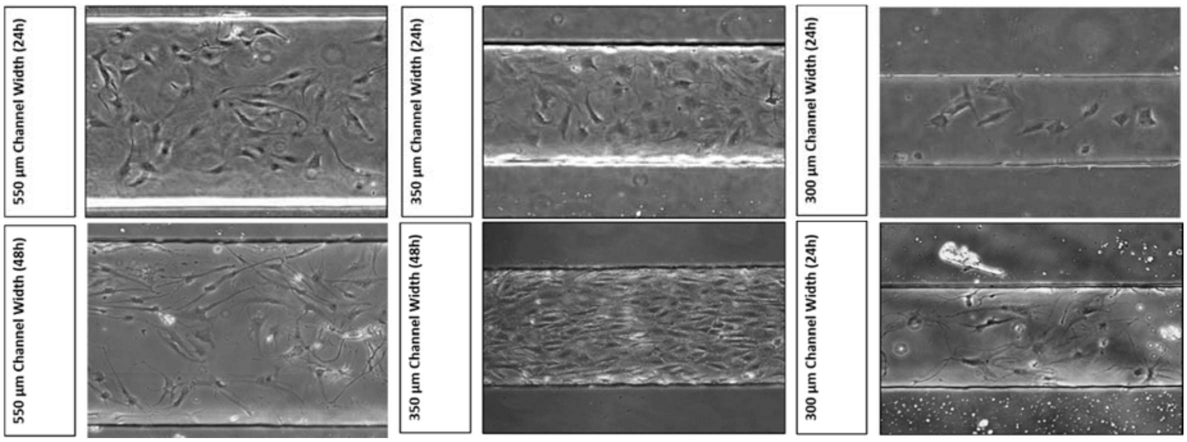


Figure SI 4. Initial assessment of PEVEC culture in a microfluidic channel. Among three different width microfluidic channels, 350 µm in width shows the best loading conditions for culturing the PAVECs.

Reynolds number, Womersley number, and Oscillatory Shear Index: Reynolds number was calculated using the normal definition: $Re = \frac{uL}{\nu}$ with u as the flow velocity, L as the characteristic length, and ν as the kinematic viscosity of the media. The height of the fluidic channel was used as the characteristic length and the kinematic viscosity of water at 20 Celsius were used for calculations in our fluidic channels. Womersley number was calculated as usually defined: $W = L\sqrt{\frac{\omega}{\upsilon}}$ with L again as the characteristic length, ω as the frequency, and ν again as the kinematic viscosity. Lastly, OSI was calculated as: $OSI = 0.5\left(1 - \frac{\left|\int_0^T WSS\ dT\right|}{\int_0^T |WSS|\ dT}\right)$ and the values referenced in the plain texted are calculated based on the shear stress presented in **Figure 3**. The dynamic viscosities used in these calculations are $1.00*10^{-6}$ m^2/s for water and media and $1.32*10^{-6}$ m^2/s for blood.

Table SI1: Various flow characteristic values (Re, W, OSI) with respect to characteristic length from previous valve studies.

Sources	***Re***	***W***	***OSI***	***L***
In-Vivo Aortic inflow	~849-4,100 [1]	21.15 [2] $	0.13-0.47 [3-4]	~22.5 mm [5]
In-Vivo Mitral inflow	~2,053 [2] #	~29.42 [2]$	0.051 [2]*	~31.3 mm [6]
Butcher & Nerem [6]	~2200	-	0	~3 mm
Mahler et al. [7]	~113-321	~1.12-5.64	0.5	~5 mm
Young et al. [8]	~6-129 x	-	0	59 µm
Aortic inflow in this work	**0.22-4.16**	**0.14-0.6**	**0.2-0.32**	**80 µm**
Mitral inflow in this work	**0.22-4.16**	**0.14-0.6**	**0.19-.28**	**80 µm**

Max values based on overall flow rate of the profile (4 L/min). Based on a 1.7 Hz frequency
* Estimated using Figure 5-1 of reference 1. x Experiments ran for 12 minutes only at high shear levels.

References

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