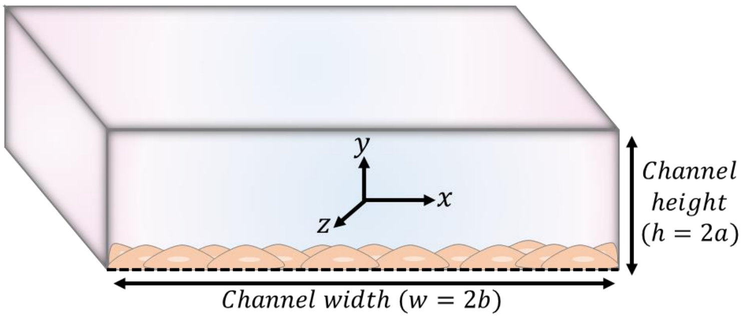
Supplementary data 2. Flow rate calculations

1. Theory

First, an orthonormal coordinate system was imagined in the channel cross section to determine the coordinates of a position relative to the center of the channel.



2a corresponds to the height of the channel in direction of the *y*-axis, *2b* is the width of the channel in direction of the *x*-axis, and the *z*-axis represents the direction of the flow.

Cells are seeded over the entire width of the channel and recover the membrane surface, that is to say in the following coordinates: $-b \leq x \leq b$ and $y = -a$ (endothelial cell thickness is negligible). According to their position in *x*, cells will undergo different shear stress values. Actually, shear stress (τ) depends on the culture medium viscosity (μ) and the shear rate ($\delta v/\delta y$) [23, 26]. The shear rate corresponds to the change in flow velocity according to the position channel. Near the side walls, the velocity tends toward zero. At the centre of the channel, the velocity is maximal.

$$\tau(x,y) = \mu \frac{\partial v(x,y)}{\partial y} \text{ (Equation 1)}$$

By detailing the derivative of the flow velocity, equation (1) becomes:

$$\tau(x,y) = \mu \left(-\frac{1}{\mu}\frac{\delta p}{\delta z} \sum_{n=0}^{\infty} \frac{(-1)^n b\pi}{(2n+1)^2} \left(\frac{2}{\pi}\right)^3 \frac{\sin a\left[(2n+1)\frac{\pi y}{2b}\right]}{\cos a\left[(2n+1)\frac{\pi h}{2b}\right]} \cos\left[(2n+1)\frac{\pi x}{2b}\right] \right) \text{ (Equation 2)}$$

$\delta p/\delta z$ is the change of pressure along the channel. This variable is unknown.

In a rectangular channel, the flow rate (Q) is expressed by the following equation [27]:

$$Q = -\frac{1}{\mu}\frac{\delta p}{\delta z} \underbrace{ab^3 \frac{4}{3}\left(1 - \frac{192}{\pi^5}\frac{b}{a} \sum_{n=0}^{\infty} \left\{\frac{1}{(2n+1)^5} \tan a\left[\frac{(2n+1)\pi a}{2b}\right]\right\}\right)}_{S} \text{ (Equation 3)}$$

$$= -\frac{1}{\mu}\frac{\delta p}{\delta z} S$$

We defined *S* to simplify the equation (3). $\delta p/\delta z$ is isolated as:

$$\frac{\partial p}{\partial z} = -\mu \frac{Q}{S} \text{ (Equation 4)}$$

Elimination of $\delta p/\delta z$ in equation (1) thanks to the equation (4) gives:

$$\tau(x,y) = \mu \left(-\frac{1}{\mu}\left(-\mu\frac{Q}{S}\right) \sum_{n=0}^{\infty} \frac{(-1)^n b\pi}{(2n+1)^2} \left(\frac{2}{\pi}\right)^3 \frac{\sin a\left[(2n+1)\frac{\pi y}{2b}\right]}{\cos a\left[(2n+1)\frac{\pi h}{2b}\right]} \cos\left[(2n+1)\frac{\pi x}{2b}\right] \right)$$

$$= \mu \frac{Q}{S} \sum_{n=0}^{\infty} \frac{(-1)^n b\pi}{(2n+1)^2} \left(\frac{2}{\pi}\right)^3 \frac{\sin a\left[(2n+1)\frac{\pi y}{2b}\right]}{\cos a\left[(2n+1)\frac{\pi h}{2b}\right]} \cos\left[(2n+1)\frac{\pi x}{2b}\right] \text{ (Equation 5)}$$

2. Relation between the shear stress and flow rate

In our device, the channel receiving cells has a height of 0.5 mm and a width of 5 mm. Cells
We first want to know the shear stress value for any flow rate at the centre of the membrane, i.e. for x = 0 and y = $-a$. For these coordinates, Equation (5) becomes:

$$\tau(x=0, y=-a) = \mu \frac{Q}{S} \sum_{n=0}^{\infty} \frac{(-1)^n b\pi}{(2n+1)^2} \left(\frac{2}{\pi}\right)^3 \frac{\sin a\left[(2n+1)\frac{-\pi a}{2b}\right]}{\cos a\left[(2n+1)\frac{\pi a}{2b}\right]} \cos(0)$$

$$= \mu \frac{Q}{S} \sum_{n=0}^{\infty} \frac{(-1)^n b\pi}{(2n+1)^2} \left(\frac{2}{\pi}\right)^3 \tan a\left[\frac{(2n+1)\pi a}{2b}\right] \text{ (Equation 6)}$$

In our device, the equation (6) can be resolved by using the following variables:

- Viscosity of the culture medium: $\mu = 0.00072$ N.s.m^{-2} (for the culture medium at 37 °C containing 10% of serum)
- Half channel height: $a = h / 2 = 0.0005$ m $/ 2 = 0.00025$ m
- Half channel width: $b = w / 2 = 0.005$ m $/ 2 = 0.0025$ m

We obtain the relation between shear stress (τ in N.m^{-2}) and flow rate (Q in m^3.sec^{-1}):

$$\tau\ (at\ center\ of\ the\ membrane\ in\ our\ device) = 271.12 \,.\, 10^{-9} \times Q$$

By converting the shear stress in dyn.cm^{-2} (1 N.m^{-2} = 1 Pa = 10 dyn.cm^{-2}) and the flow rate in mL.min^{-1}, the previous relation becomes:

$$\tau\ (at\ center\ of\ the\ membrane\ in\ our\ device)(\mathrm{dyn.cm^{-2}}) = \frac{Q\ (\mathrm{mL.min^{-1}})}{1.6266933}$$

Our pump system can drive the flow from 0.09 mL.min^{-1} to 52.5 mL.min^{-1}. The equation gives respective shear stresses of 0.055 and 32.3 dyn.cm^{-2}. For our experiments, we want to use a physiological shear stress of 10 dyn.cm^{-2}. We must configure the pump system at a flow rate of 16.3 mL.min^{-1} to apply a maximum shear stress of 10 dyn.cm^{-2} at the centre of the membrane.

3. Shear stress variation along the channel width

Then, we study the variation of the shear stress sensed by the cells according to their position on the membrane, i.e. for $-b \le x \le b$ and $y = -a$. For these coordinates, Equation (5) becomes:

$$\tau(x, y=-a) = \mu \frac{Q}{S} \sum_{n=0}^{\infty} \frac{(-1)^n b\pi}{(2n+1)^2} \left(\frac{2}{\pi}\right)^3 \tan a\left[\frac{(2n+1)\pi a}{2b}\right] \cos\left[(2n+1)\frac{\pi x}{2b}\right] \text{ (Equation 7)}$$

We resolve the equation (7) according to the flow rate configured for our experiments (16.3 mL.min^{-1}) and to the minimal and maximal flow rates that we can apply with our pump system (0.09 mL.min^{-1} and 52.5 mL.min^{-1}). **Table S2** gives the shear stress provided on cells according to their position on the membrane (i.e. for $|x| \le b$ and $y = -a$).

Distance from the center of the channel $|x|$ (10^{-3} m)	Shear stress (dyn.cm^{-2})		
	For the minimal flow rate of 0,09 mL.min^{-1}	For a flow rate of 16.3 mL.min^{-1}	For the maximal flow rate of 52.5 mL.min^{-1}
0,00 (center of the channel width)	0,0553	10,0010	32,2773
0,05	0,0553	10,0010	32,2773
0,10	0,0553	10,0010	32,2772
0,15	0,0553	10,0010	32,2772
0,20	0,0553	10,0010	32,2772
0,25	0,0553	10,0010	32,2771
0,30	0,0553	10,0009	32,2771
0,35	0,0553	10,0009	32,2770
0,40	0,0553	10,0009	32,2769
0,45	0,0553	10,0009	32,2768
0,50	0,0553	10,0008	32,2767
0,55	0,0553	10,0008	32,2766
0,60	0,0553	10,0007	32,2764
0,65	0,0553	10,0007	32,2762
0,70	0,0553	10,0006	32,2760
0,75	0,0553	10,0005	32,2757
0,80	0,0553	10,0004	32,2754
0,85	0,0553	10,0003	32,2750
0,90	0,0553	10,0001	32,2745
0,95	0,0553	10,0000	32,2739
1,00	0,0553	9,9997	32,2732
1,05	0,0553	9,9994	32,2722
1,10	0,0553	9,9990	32,2709
1,15	0,0553	9,9985	32,2691
1,20	0,0553	9,9978	32,2669
1,25	0,0553	9,9968	32,2639
1,30	0,0553	9,9956	32,2598
1,35	0,0553	9,9939	32,2544
1,40	0,0553	9,9916	32,2470
1,45	0,0553	9,9885	32,2370
1,50	0,0552	9,9843	32,2235
1,55	0,0552	9,9786	32,2050
1,60	0,0552	9,9708	32,1799
1,65	0,0551	9,9602	32,1456
1,70	0,0550	9,9457	32,0988
1,75	0,0549	9,9259	32,0348
1,80	0,0548	9,8988	31,9474
1,85	0,0546	9,8618	31,8279
1,90	0,0543	9,8111	31,6644
1,95	0,0539	9,7418	31,4408
2,00	0,0534	9,6470	31,1348
2,05	0,0527	9,5172	30,7158
2,10	0,0517	9,3394	30,1420
2,15	0,0503	9,0956	29,3553
2,20	0,0485	8,7611	28,2756
2,25	0,0459	8,3006	26,7896
2,30	0,0424	7,6639	24,7345
2,35	0,0375	6,7747	21,8646
2,40	0,0305	5,5070	17,7734
2,45	0,0199	3,6048	11,6342
2,50 (lateral border of the channel)	0,0000	0,0000	0,0000

Optimal shear stress $\pm$ 5 %

Table S2. Theoretical shear stress values sensed by the cells according to their position on the membrane in our device. Results are obtained for each flow rate value by resolving the equation (7) for $|x| \leq b$

(along the channel width) and y = - a (on the membrane receiving cells), according to the previous coordinate system. Our pump system delivers flow rates from 0.09 $mL.min^{-1}$ to 52.5 $mL.min^{-1}$. In our experiments, we want to apply a force of 10 $dyn.cm^{-2}$ on the cells, corresponding to a flow rate of 16.3 $mL.min^{-1}$.

As expected, the shear stress decreases when the x position approaches the side walls (at $|x|$ = 2,5 mm). Results are in green when the value corresponds to the shear stress at x = 0 with a variability of 5%. For example for a flow rate of 0.09 $ml.min^{-1}$, the shear stress at x = 0 is 0,0553, so all the values of 0,0553 $\pm$ 5% are in green. It is notable that all green values correspond to a x comprised between -2,05 and 2,05 mm. In other words, only the cells located at ≤ 0.45 mm from the side walls will undergo a lower shear stress than desired. This area of homogenous shear stress corresponds to 82% of the seeding cells.

4. Comparison with other BBB devices

By using the same procedure, we study the shear stress distribution on the other BBB fluidic devices. Two criteria were retained for the device selection: the reproduction of 2 channels separated by a straight interface seeded with brain endothelial cells, and a regular fluidic channel without variable dimensions. The **Table S3** reports all devices characteristics.
Briefly, according to each channel dimension, we calculate the flow rate to set up in order to have a shear stress of 10 $dyn.cm^{-2}$ on the cellular interface. Then, we simulate an application of the corresponding flow rate and we count all x positions where shear stress is 10 $dyn.cm^{-2}$ $\pm$ 5%. The number of x positions with a correct shear stress is then divided by the channel width to obtain a final percentage.

Publication		Dimensions (mm)			Flow rate ($ml.min^{-1}$) necessary for a shear stress of 10 $dyn.cm^{-2}$	% of cells exposed to the intended shear stress
Date	**Authors**	**Channel height (*h*)**	**Channel width (*w*)**	***h/w* ratio**		
2016	Shao et al. [30]	0,1	2	0,05	0,27	90%
Our device		0,5	5	0,1	16	82%
2020	Zakharova et al. [31]	0,05	0,5	0,1	0,13	82%
2015	Sellgren et al. [32]	0,15	1	0,15	0,28	72%
2018	Maoz et al. [33]	0,2	1	0,2	0,49	64%
2013	Griep et al. [34]	0,1	0,5	0,2	0,061	64%
2020	Ahn et al. [35]	0,1	0,4	0,25	0,047	56%
2016	Xu et al. [36]	0,2	0,4	0,5	0,16	35%
2019	Park et al. [37]	1	1	1	8,7	29%
2016	Walter et al. [38]	0,2	0,2	1	0,069	29%
2017	Adriani et al. [39]	0,92	0,19	4,8	0,54	27%
2019	Brown et al. [40]	0,2	0,1	2	0,026	27%

Table S3. Homogeneity of the shear stress distribution on BBB devices. The channel dimensions are taken by considering the cellular interface between the two channels. The flow rate to set up for a physiological shear stress of 10 $dyn.cm^{-2}$ on the cellular interface is measured as example. Based on this flow rate, the distribution of shear stress along the channel width is simulated. A variability corresponding of 5% of the intended shear stress value is taken.

These calculations highlight the variety of channel dimensions in BBB devices. Consequently, the percentage of cells submitted to the intended shear stress on the channel interface varies greatly. It is inversely proportional to the height/width ratio and independent of the flow rate. For squared channels, as in Park's and Walter's devices, only 29% of cells can be subjected to the intended shear stress. Rectangular channels benefit of better percentages. These results illustrate the importance of designing a low *h*/*w* channel ratio for an optimal distribution of shear stress. Our home-made device presents one of the most homogenous fluidic interfaces, as 82% of the seeded cells are theoretically cultured under homogenous shear stress (i.e. the intended shear stress $\pm$ 5%).