# Supplemental Document A

**Design of Membrane Micro-Flows**

The design of microfluidic transwell insert was conceptualized using the hydraulic-electrical circuit analogy based on the similarities of the Hagen-Poiseuille's law for the flow of fluid and Ohm's law for the flow of current. For generating wide concentration gradients with the microfluidic transwell insert we desired a microfluidic circuit in which the flow delivered through the track-etched membrane was uniform along the length of the delivery microchannels. In the ideal scenario, shown in **Fig. A1(a)**, each pore of the track-etched membrane can be considered to have equal flow, so that multiplying the flow through an individual pore *Ip* by the number of pores we obtain the total membrane flow *Im*:

$$\mathrm{I_m} = \mathrm{I_p}\rho\mathrm{A} \tag{1}$$

where $\rho$ is the porosity of the membrane and $A$ is the area of the membrane. For uniform membrane flow the delivery channel must be designed to have significantly less hydraulic resistance than the membrane such that the pressure applied to each pore is equivalent. We can evaluate this situation by calculating the hydraulic resistances of our device. The hydraulic resistance of the track-etched membrane can be calculated from the equivalent resistance of the individual pores in parallel:

$$\frac{1}{R_{mem}} = \frac{1}{R_1} + \frac{1}{R_2} + \cdots + \frac{1}{R_n} \tag{2}$$

Knowing that individual pores of the track-etched membrane are largely homogenous in diameter and length due to the track-etching process, we assume

$$R_{pore} = R_1 = R_2 = R_n \tag{3}$$

Simplifying Eq. (2) and (3) we can define the resistance of the membrane:

$$\frac{1}{R_{mem}} = \frac{n}{R_{pore}}$$

$$R_{mem} = \frac{R_{pore}}{A\rho_{pore}}. \tag{4}$$

The resistance of an individual pore is calculated from the resistance of a circular pipe:

$$R_{pore} = \frac{8\mu L}{\pi R^4} \tag{5}$$

where $\mu$ is the viscosity, $L$ the thickness of the membrane, and $R$ the radius of the pore. Given the 1.0 µm pore diameter and 12 µm thickness of a BD falcon brand track-etched membrane used for the devices, we calculate the resistance of a single pore as:

$$R_{pore} = \frac{8\mu L}{\pi R^4} = \frac{8 \cdot 1\times10^{-3} \cdot 12\times10^{-6}}{\pi\,(500\times10^{-9})^4}$$
$$= 4.89\times10^{17}\,\frac{kg}{m^4 s} \tag{7}$$

For the microfluidic transwell insert, the area of the membrane that receives flow from one inlet is 8 mm long and 350 µm wide with circular ends. The nominal membrane area is $2.896 \times 10^{-6}$ $m^2$. Solving Eqs. (4) and (5) using the area of the membrane and the porosity, $1.6\times10^{10}$ pores $m^{-2}$, we calculate the resistance of the membrane for one inlet as:

$$R_{mem} = \frac{R_{pore}}{A\rho_{pore}} = \frac{4.89\times10^{17}}{2.896\times10^{-6} \cdot 1.6\times10^{10}}$$
$$= 1.06\times10^{13}\,\frac{kg}{m^4 s} \tag{8}$$

For the delivery microchannel we use the equation for the hydraulic resistance of a rectangular microchannel[71]:

$$R_d = \frac{12\mu L}{wh^3\left(1-\frac{h}{w}\left(\frac{192}{\pi^5}\right)\sum_{n=1,3,5}^{\infty}\frac{1}{n^5}\tan h\left(\frac{n\pi w}{2h}\right)\right)} \tag{9}$$

Calculating Eq. (9) for $w$ = 350 μm, $h$ = 200 μm, and $L$ = 8 mm, we determine that

$$R_d = 3.44 \times 10^{10}\,\frac{kg}{m^4 s} \tag{10}$$

Comparing the results of Eqs. (8) and (10) shows that resistance of the membrane is 3 orders of magnitude greater than the resistance of the delivery channel. By this design, the pressure along the length of the delivery channel, $P_{mem}$, is insensitive to the flow lost through the membrane and uniform. Therefore, we can simplify our hydraulic circuit model by ignoring the pressure drop in the low resistance delivery channel. The diagram in **Fig. A1(b)** shows how the hydraulic circuit is simplified by this situation. The actual microfluidic transwell insert shown in **Fig. A2** has two inlet and delivery channels, so we can model the flow by combining two circuits in parallel as shown in **Fig. A2**. Following a similar logic for the negligible resistance of the delivery channel compared to the membrane, we have designed a connection between the two inlets that is low resistance. This ensures that the membrane area supplied by each inlet is at the same pressure and simplifies the fluid routing with a common outlet.

While having the resistance of the delivery channels much higher than the membrane is desirable for uniform flow delivery, we also require non-trivial resistance at the outlet to drive enough flow through the membrane. The pressure at the membrane can be defined using the conservation of energy according to Kirchhoff's laws:

$$P_{mem} = I_{in}\frac{R_{out}(0.5)R_{mem}}{R_{out}+(0.5)R_{mem}} \tag{11}$$

For our design, we have an outlet microchannel with dimensions $w$ = 100 μm, $h$ = 75 μm, and $L$ = 6 mm. Solving Eq. (9) we calculate the outlet resistance to be:

$$R_{out} = 1.71 \times 10^{12}\,\frac{kg}{m^4 s} \tag{12}$$

Using the analogy to Ohm's law, we can rearrange Eq. (11) to solve:

$$I_{mem} = \frac{P_{mem}}{(0.5)R_{mem}} = I_{in}\frac{R_{out}}{R_{out}+(0.5)R_{mem}}$$

$$= I_{in}\frac{1.71 \times 10^{12}}{1.71 \times 10^{12} + 0.53\times 10^{13}} \tag{13}$$

$$I_{mem} \approx I_{in}\cdot 0.24$$

The hydraulic circuit model described here predicts that 24% of the flow applied by the syringe pump will escape through the membrane.

We can extend the analysis further to describe the membrane with Darcy's law:

$$I_{mem} = \frac{kAP_{mem}}{\mu L} \tag{14}$$

where for one delivery channel, $I_{mem}$ is the flow, $\mu$ is viscosity, $A$ is area of membrane, $L$ is thickness of the membrane, $P_{mem}$ is the pressure drop, and $k$ is the permeability parameter of the membrane. Rearranging Eq. (14) using Ohm's law we define $k$ as:

$$\frac{I_{mem}}{P_{mem}} = R_{mem} = \frac{\mu L}{kA}$$

$$k = \frac{\mu L}{R_{mem}A} \tag{15}$$

Simplifying Eq. (15) further using Eqs. (7) and (8), the permeability parameter can be described as a function of the porosity and pore radius as follows:

$$k = \frac{\pi \rho R^4}{8} \tag{16}$$

Solving with the 1.0 μm pore diameter and porosity of $1.6\times10^{10}$ pores $m^{-2}$, we can calculate the permeability parameter, $k$ = $3.927\times10^{-16}$ $m^2$. This parameter is important for modeling the bulk properties of the track-etched membranes since it describes hydraulic conductivity independent of the area and thickness of the membranes used. We used this parameter in FEM simulations to predict the full 3D flow and molecular transport of the microfluidic transwell devices.

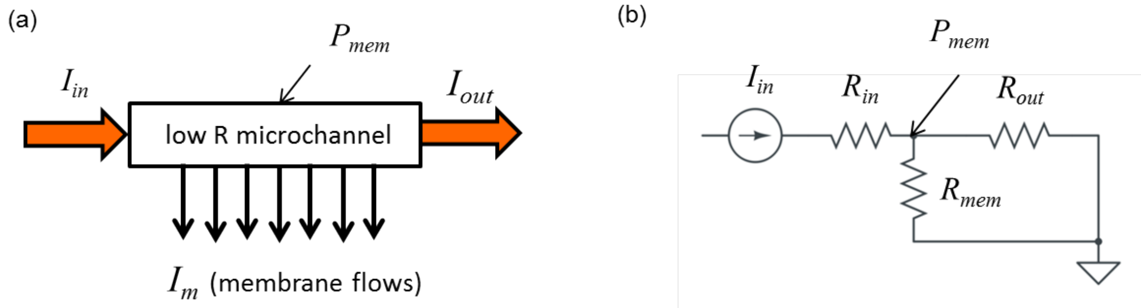


**Fig. A1** Hydraulic circuit for microfluidic transwell flows depicting the flow network for one inlet. Syringe pump driven flow is indicated by $I_{in}$. Flow entering the circuit at the inlet can either pass through the membrane or through the outlet of the device. (a) The membrane can be modeled as a series of parallel conduits that connect to ground in parallel with the resistance of the outlet microchannels. (b) The low resistance microchannel supplying flow across the membrane is 3 orders of magnitude lower in resistance compared to the membrane; therefore it is negligible and each pore can be treated as the same pressure. This simplifies the model to a single high resistance path in parallel with the outlet resistor.

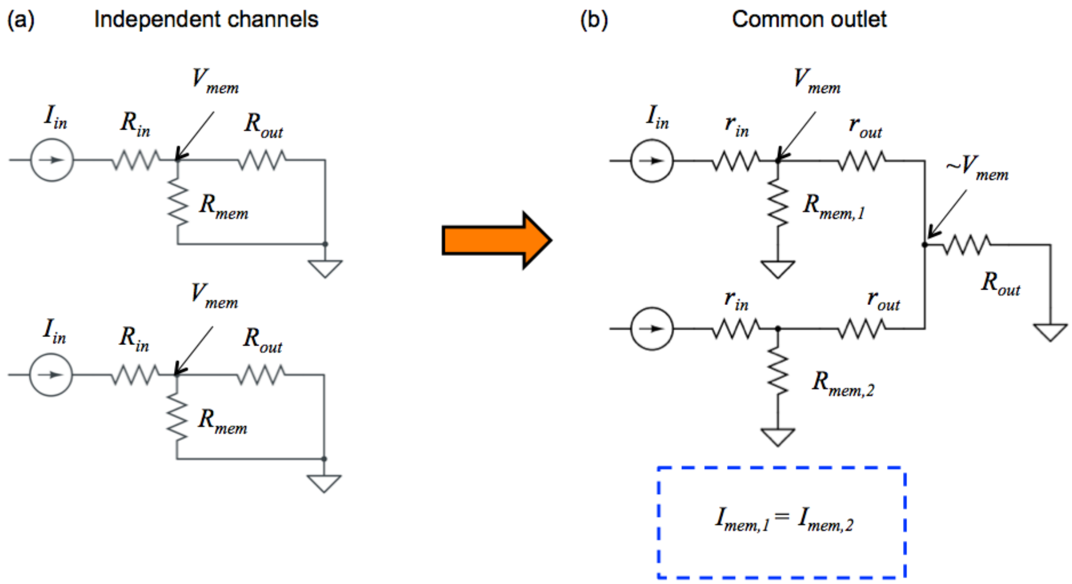


**Fig. A2** Hydraulic circuit for a microfluidic transwell device with 2 inlets and a common outlet. (a) Independent hydraulic networks. (b) Each network can be connected via a low resistance bridge to establish the same pressure at each portion of the membrane and ensure that equal flow is delivered.

71 K. W. Oh, K. Lee, B. Ahn and E. P. Furlani, Design of pressure-driven microfluidic networks using electric circuit analogy, Lab Chip, 2012, **12**, 515–545.