Electronic Supplementary Material (ESI) for Lab on a Chip
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SUPPLEMENTARY MATERIALS

1. Supplementary protocols

a. *Preparation of the cell culture micro-wells:*

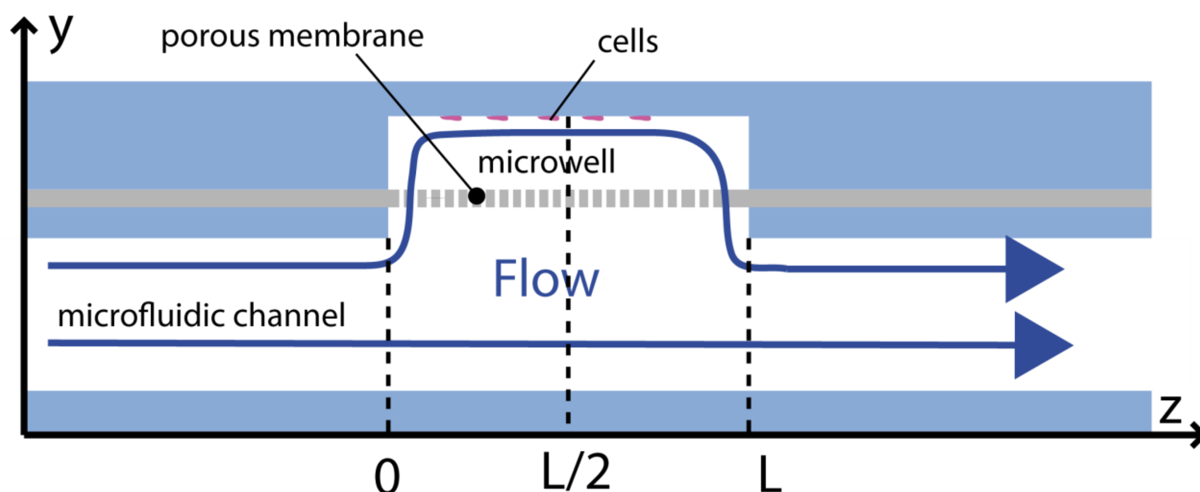
Micro-wells were prepared by classical UV lithography technique. Briefly, a NOA 81 droplet was deposited onto a flat PDMS surface structured with spacer pillars of the desired height, and a glass coverslip was placed on top to create a uniform layer. UV exposure (365nm, 10s, 8 mW/cm^2) is performed across a transparency mask through the coverslip to form a 1x1 mm^2 chamber (Fig. S2, C1). After exposure, the coverslip with reticulated chambers was removed from the PDMS (Fig. S2, C2) and non cross-linked NOA 81 was rinsed, first briefly with acetone and next with ethanol. A final UV exposure was performed to fully crosslink the glue. Before using them for cell culture, chambers were equilibrated into deionized water for 24 hours, sterilized and coated with adhesion proteins. They were then used with classical culture and labeling conditions (Fig. S2, C3). These structured coverslips could be washed after an experiment and used multiple times.

b. Spinal cord neurons culture in micro-wells

Neurons were prepared from spinal cord of rat embryo at E14-15, using procedures previously described (Levi et al., 1998). Drops of 50 µL of neurobasal medium with dissociated spinal cord neurons (concentration of 1.8*10^5 cells/ml) were deposited in a micro-well preliminarily coated with 80 µg/ml of poly-D-ornithine (Sigma). After sedimentation of the neurons, neurobasal medium supplemented with B27, L-Glutamine and antibiotics (Invitrogen) was added and the cells were grown in an incubator (37°C, 5 % CO_2) for 3 to 5 days prior to microfluidic experiments.

2. Supplementary material : derivation of equation (4)

In order to avoid fluid flows through the membrane, the hydrodynamic resistance of the entire membrane needs to be sufficiently high compared to the hydrodynamic resistance of the microfluidic channel below the membrane (supp. Figure 1). For cell-based assays, we take care that the flow speed above the cells is below 1 μm/s in order to avoid detrimental shear stress on the cells and alteration of the concentration landscape geometry (when compared to the purely diffusive model). Since the microwell above the membrane (supp. Figure 1) is closed by the membrane, we assume that there is no pressure gradient transverse to the membrane. Nevertheless there is a pressure drop along the direction of the flow, due to the hydrodynamic resistance of the microfluidic channel. This pressure drop generates a flow in the microwell in the same direction.



Supplementary figure 1 : sectionnal view of the microfluidic channel, the membrane and the microwell.

In order to estimate an upper limit of the flow over the cells, we place ourselves in the defavorable case where a fluid flow enters the microwell between z=0 and z=L/2 and exits the microwell between z=L/2 and z=L. Moreover we assume that the pressure drop between the entrance and the exit of the membrane is constant and equal to the pressure drop in the microfluidic channel between z=0 and z=L . We furthermore assume that the hydrodynamic resistance of the microwell is low compared to the resistance of the membrane. We can thus set an upper limit to the flux $Q_{microwell}$ in the microwell :

$$Q_{microwell} \leq \frac{P_c(0)-P_c(L)}{R_{membrane}+R_{microwell}} \approx \frac{P_c(0)-P_c(L)}{R_{membrane}}$$

with

$$R_{membrane} = \frac{R_{pore}}{wLd_{pore}}$$

where w is the width of the membrane opening (in the x direction on supplementary figure 1), R_{pore} is the hydrodynamic resistance of one cylindrical pore. $P_c(z)$ is the pressure in the microchannel at position z. We then can approximate the flow speed on the cells by dividing the flux by the microwell section :

$$V_{cells} = \frac{Q_{microwell}}{wh_{microwell}} = Ld_{pore}\frac{P_c(0)-P_c(L)}{R_{pore}h_{microwell}}$$

where $h_{microwell}$ is the height of the microwell and d_{pore} is the pore density of the membrane.

Since the flow in the microwell has to be small compared to the flow in the channel, the pressure difference in the microchannel between z=0 and z=L is equal to the product of the flux in the channel

Q_c and the hydrodynamic resistance of a section of length L of a rectangular channel $R_c(L)$. The hydrodynamic resistances of a cylindrical pore and of the rectangular channel can be calculated from there dimensions. We obtain a relation between the maximal velocity on the cells and the flux in the channel :

$$V^{\max}{}_{cells} = Ld_{pore} \frac{Q_c R_c(L)}{R_{pore} h_{microwell}}$$

with $R_{pore} = \frac{8\mu e}{\pi r^4}$ and $R_c(L) = \frac{12\mu L}{wh_c^3}$

where μ is the viscosity of the fluid (water), hc the height of the microchannel, e the thickness of the membrane and r the radius of the pores.

$$V^{\max}{}_{cells} = \frac{3Q_c L^2 d_{pore} \pi r^4}{2ewh_{microwell} h_c^3}$$

The flux in the channel can be estimated by the average flow speed in the channel divided by the channel section. This leads to equation 4 of the article :

$$V^{\max}_{cells} \approx V_c \frac{3\pi L^2 d_{pore} r^4}{2eh_{microwell} h_c^2}$$

This maximum velocity can also be linked to the pressure difference ΔP applied between the input and the output of the microchannel and the total length of the channel L_0

$$V^{\max}{}_{cells} = \frac{L^2 d_{pore} \pi r^4}{8\mu eh_{microwell} L_0} \Delta P$$

3. Supplementary movie M1

Fluorescence imaging of FITC streams alternatively flowing in 3 channels, each connected to a 100µm porous opening. By alternating the flow of solutes every 10 seconds from a channel to the next one, we generated a rotating gradient of fluorescent molecules in the chamber with an angular speed of $\omega \approx 0.2$ rad.s^{-1}. Scale bar 200 µm.