Simulating Small Molecule Transport Across the Blood-Brain Barrier Using COMSOL Multiphysics

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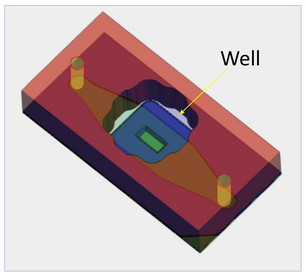
Abstract

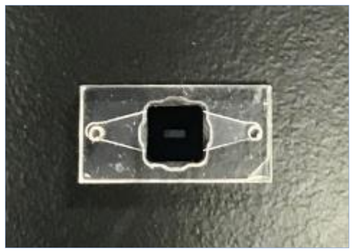
The blood-brain barrier (BBB), a unique and highly selective semipermeable barrier, can become "leaky" in stressed states, increasing in permeability and leading to unwanted solutes from the blood entering the extracellular fluid of the central nervous system. We are developing an in situ assay using microfluidic devices containing an ultra-thin, nanoporous silicon membrane to assess the permeability of a well established BBB cell line, human cerebral microvascular endothelial cell monolayer (hCMEC/D3). We use widefield fluorescence microscopy to measure diffusion of FITC-Dextran (a fluorescent probe) across the cell monolayer and nanoporous membrane and into the basal channel of the device over time, and we wanted to see whether the assay would support the device being reversed so that the cell monolayer could grow in a more uniform way. We used COMSOL to understand these time-based diffusion patterns in the reversed orientation of the chip, compared to the control in the normal orientation, and varied the permeability of the membrane, which simulates different amounts of cell growth and tight junction maturation. We found that the diffusion patterns in the reversed model were like those of the control, which supports further experimentation on this modification to the assay.

Devices and Techniques

ALine ™ modular devices are used in the experiments. These are shown below.



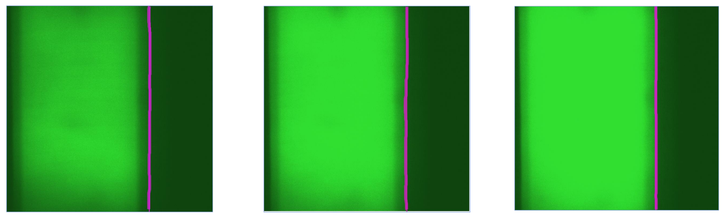
ALine modular device, trench up orientation, bottom channel highlighted in yellow (well is the rest of the device)



Fully assembled Aline Modular Device, trench down orientation

To assess diffusion of FITC-Dextran, we used widefield fluorescence microscopy. Measurements of the fluorescence 50 µm from the edge of the trench were taken at different time points, standardized against the source intensity, and then plotted accordingly as a percentage of source intensity.

Devices and Techniques (contd.)

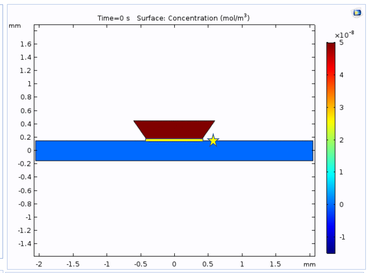


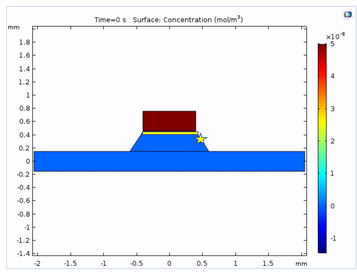
Fluorescence microscopy images taken one minute apart from each other. Fluorescence is averaged along the displayed magenta line, divided by the source intensity, and plotted against time (in minutes) through use of a MATLAB script.

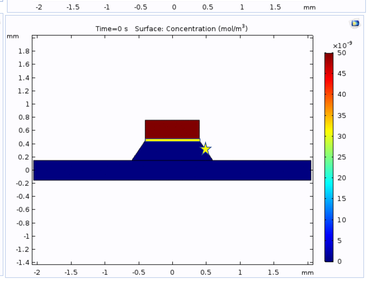
COMSOL Models

At the right is a cross-sectional model of the chip in the normal orientation with the trench facing up (dark red part).

The simulation is a 2D slice of the device with FITC-Dextran concentrated in the well (dark red source concentration) and diffusing into the bottom channel. The cell monolayer and nanomembrane is represented by a small, yellow rectangle forming the boundary between the source and the channel in all three images.







The two bottom images display a 2D slice of the reversed, trench-down orientation. The simulation was carried out with varying permeability values of the membrane and cell monolayer to correspond to permeability values of experimental data. Measurements were taken at the point marked by the stars. The image on the left is the cell free model with no effective resistance to diffusion and the image on the right has a P value of 1E-5 cm/s, which corresponds to the experimental permeability of a layer of hCMEC/D3 . Diffusion coefficient of 40 kDa FITC-Dextran used = 7.44 * 10^{-11} m^2/s

Data and Analysis

The following equations are used to model diffusion through our system with a semi-infinite medium and a constant source. C refers to dye concentration whereas I represents fluorescence intensity in the experimental model.

$$C_x(t) = C_o\, erfc\left(\frac{x}{2\sqrt{Dt}}\right)$$

Equation 1

$C_x(t)$ is concentration at a specific distance from the source at time t, C_o is the source concentration, x is the distance from the source at which concentration is measured, D is the diffusion coefficient of the FITC-Dextran through the medium, and t is time in seconds.

Data and Analysis (contd.)

$$I_x(t) = I_o\, erfc\left(\frac{x}{2\sqrt{Dt}}\right)$$

Equation 2

$I_x(t)$ is fluorescence intensity within the device at the measured point, and I_o is the initial intensity. This is related to the previous equation through the Beer-Lambert's Law, which allows us to make an equivalence between concentration and intensity and therefore determine concentration from our image readings.

The following graphs chart concentration as a percentage of initial concentration vs. time in minutes (data from the COMSOL Simulations and analytical solutions).

Concentration vs. Time 50 microns from edge

$C_x(t)$ (mol/m^3)

Time (minutes)

Conclusions

According to the graph above, the analytical solutions do not match the COMSOL solutions. This is likely because the equations model diffusion at a point straight down from the membrane, while the simulations collected data 50 microns from the edge of the membrane. Data is collected here, as the silicone chip support blocks light from the well, and dye in the bottom channel alone can be measured. These simulations can then be used to create look-up tables to match experimental data to the appropriate permeability value, with more accuracy than the analytical solution could provide.

While there isn't a large deviation between the traditional orientation measurements and the reversed orientation measurements, the slight differences justify the need for separate look-up tables for trench-down studies. However, the similarities justify that this orientation can be used. This is optimal since it allows for the cells to grow in a more uniform layer.

COMSOL is critical to this work, allowing for a true vision of what is going on in terms of diffusion 50 µm from the edge of the window, rather than 50 µm below the window. The assay will be adjusted accordingly to optimize data collection and validate COMSOL simulations.

Acknowledgements

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