Electronic Supplementary Material (ESI) for Lab on a Chip.
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Supporting Information

Microfluidic organ-on-chip system for multi-analyte monitoring of metabolites in 3D cell cultures

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Computational mass transport modelling

Simulation model and parameters

We performed modelling of the mass transport in the microfluidic organ-on-chip system to complement our experimental mass transport studies. We used COMSOL Multiphysics 4.2 for finite element analysis. The modelled geometry consisted of the three fluidic channels and the two cell compartments of the system without all the inlets, and was represented by a horizontal plane [Fig. S1a]. A cross-sectional vertical plane is shown in Fig. S1b.

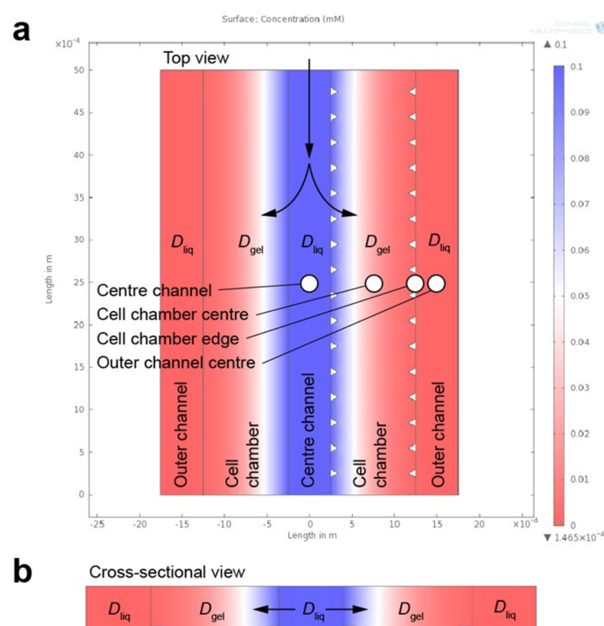

Fig S1 *Geometry of the microfluidic organ-on-chip system as a computational simulation model. Substances are introduced via the center fluidic channel and diffuse into the gel-filled cell chambers and liquid-filled outer channels. Concentration values are extracted at the indicated points.*

The liquid in the centre channel is exchanged in around 20 s at 2 µl min^{-1} flow rate. So, the mass transport by fluidics can be considered instantaneous, and overall mass transport is primarily limited by diffusion. We therefore only modelled diffusion with the "Transport of Diluted Species" module. The liquid channels were attributed one diffusion coefficient, D_{liq}, and the gel-filled chambers another one, D_{gel}, with D_{gel} being 0.75 D_{liq}. This ratio was based on our previous experimental estimations of diffusion coefficients in Matrigel

and liquids. We recapitulated the practical experiment shown in Fig. 2. The system was empty for 20 min. Then, 100 µM concentration of species was introduced from the centre channel for 60 min, before zero concentration was introduced from the centre channel again. To account for the fluidic exchange, we used
30 a transition zone of 20 s for the concentration change. Concentrations were monitored at four locations [Fig. S1a]: in the centre channel, in the centre of the cell chamber (500 µm from the centre channel), at the outer edge of the cell chamber (1000 µm from the centre channel), and in the centre of the outer channel (1250 µm from the centre channel). The overall behaviour is shown in Fig. S2a for diffusion into the system via the centre channel, and in Fig. S2b for diffusion out of the system via the centre channel.

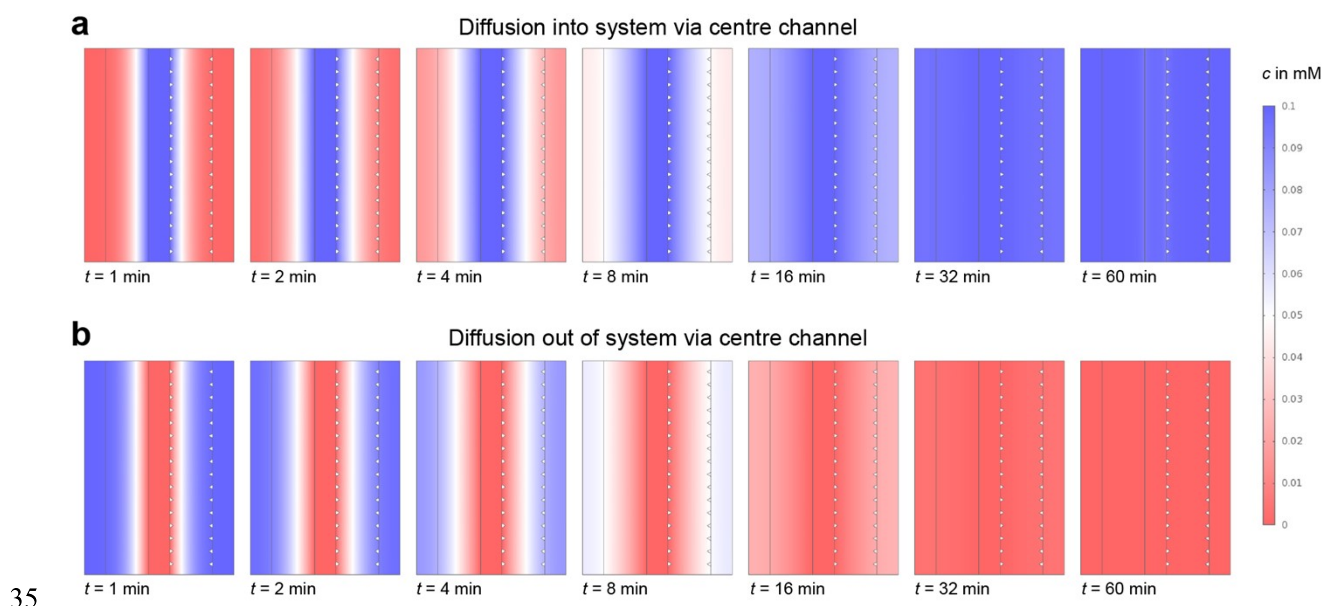

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Fig S2 *Simulation results for time-dependent diffusion of 100 µM of the small molecule hydrogen peroxide from the centre fluidic channel into the gel-filled cell chambers and outer fluidic channels, and vice versa.* ($D_{liq} = 2 \cdot 10^{-9}$ m^2 s^{-1})

Geometry considerations

The results from the two geometries allow important conclusions about the barrier structures. In Fig. S1a,
40 one half was modelled with the posts and one without. It can be observed that the substance diffuses almost symmetrically in both directions. Therefore, regarding diffusional mass transport, the existence of the vertical posts as barrier structures to contain the ECM gels has a negligible influence. Fig. S1b includes the continuous step at the bottom which serves as a barrier structure for the filling procedure. The diffusion profiles in Fig. S1b show only minimal distortion caused by the step. Thus, a horizontal plane study
45 as in Fig. S1a is sufficient to describe the mass transport in the system.

Mass transport simulation vs. experimental data

This section is found in the main article [Fig. 2c].

Mass transport of small vs. larger molecules

This section is found in the main article [Fig. 2d].

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Concentration change at different locations for various diffusion coefficients

From the simulation results, it becomes evident that the used parameters, especially for the diffusion coefficient, strongly influence the result. Furthermore, effective diffusion coefficients are difficult to measure precisely, and values from the literature often vary strongly. Here, we simulated the concentration

change at different locations in the organ-on-chip system using different diffusion coefficient combinations. They ranged from slightly higher than expected for small molecules such as oxygen and hydrogen peroxide (D_{liq} = 2.50 · 10^{-9} m^2 s^{-1}) to the values for the larger compound doxorubicin (D_{liq} = 0.58 · 10^{-9} m^2 s^{-1}) [1-3]. We simulated two locations in the organ-on-chip system, the centre of the cell chamber and the outer edge of the cell chamber.

Fig. S3a shows that for the centre of the chamber, 50% of the concentration change is reached between 2.6 and 8.1 min for all diffusion coefficients. 90% of the concentration change is reached between 14.9 and 49.2 min. Fig. S3b shows that for the outer edge of the chamber, 50% of the concentration change is reached between 6.6 and 20.9 min for all diffusion coefficients. 90% of the concentration change is reached between 19 and over 60 min.

Regarding the stop/flow protocols used in the cell culture experiments, we can conclude that flow phases of 30 min are sufficient to exchange almost all of the concentration of the small metabolite molecules in the entire cell compartment. For slower diffusing molecules such as the drug doxorubicin, it will take over an hour, one stop and one flow phase, for a full concentration exchange. This mass transport situation has to be taken into account when considering the measurement of fast cellular responses. Consequently, using also the outer channels as fluid supply shortens the diffusion times to the situation in Fig. S3a.

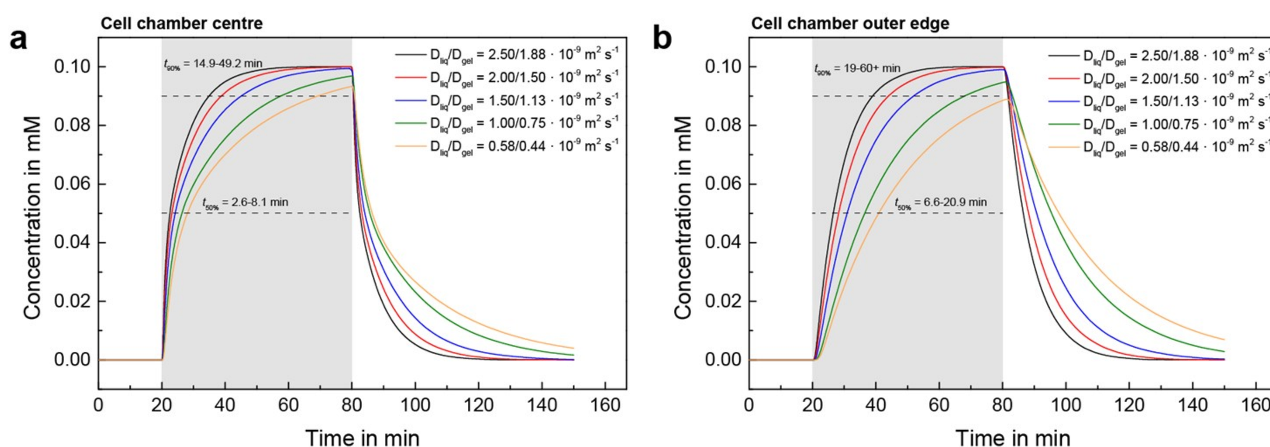

Fig S3a *Simulation results of concentration changes in the centre of the cell chamber for various diffusion coefficients.* ***b*** *Simulation results of concentration changes at the outer edge of the cell chamber for various diffusion coefficients.*

Diffusion times for various diffusion coefficients

The diffusion times extracted from the data in Figs. S3a,b are summarized in Fig. S4. The diffusion time for different locations in the system and a different extent of concentration exchange is shown dependent on the diffusion coefficient. Small molecules, such as oxygen and hydrogen peroxide, fall in the range of 1.5 to 2.0 · 10^{-9} m^2 s^{-1} and result in diffusion times of less than 30 min, which is the typical duration of one flow phase. Larger molecules such as the drug doxorubicin are found on the left-hand side of the graph and may require over 60 min of diffusion time.

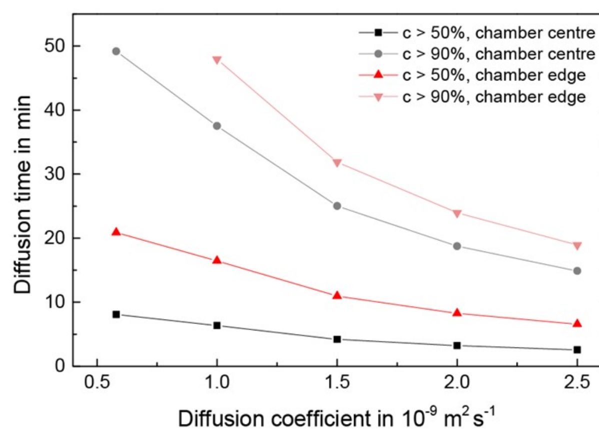

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Fig S4 *Simulated diffusion times dependent on the diffusion coefficient for different locations in the cell chamber of the organ-on-chip system and a 50%/90% concentration change. Small molecules such as oxygen or hydrogen peroxide are found on the right-hand side, larger molecules such as doxorubicin are found on the left-hand side of the graph.*

References

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