Application Note

Perfused primary human blood-brain barrier in MIMETAS OrganoPlates®

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Mimetas' OrganoPlates® are microfluidic cell culture plates that enable culturing of a range of miniaturized 3D organ and tissue models. Here we describe the development of a primary human model of the blood-brain barrier (BBB) comprising a microvessel of brain endothelial cells, supported by astrocytes and pericytes. The model shows expression of adherens- and tight junction proteins, barrier formation, and functional P-gp transport. The platform's compatibility with standard laboratory equipment and automation makes this perfused BBB-on-a-chip amenable to high-throughput screening purposes.

Perfused human BBB-on-a-chip comprising primary brain endothelial cells, astrocytes, and pericytes

Rat-tail collagen-I gel was seeded in the gel channel of the OrganoPlate® 3-lane (figure 1a) and polymerized at 37°C for 15 minutes. Primary human brain microvascular endothelial cells (HBMECs, Cell Systems) were detached from tissue culture flasks and seeded in the top channel to allow attachment to the ECM gel. Perfusion was started by placing the OrganoPlate® on a MIMETAS Perfusion Rocker™ to allow formation of an endothelial microvessel.

After the microvessel had formed, astrocytes and pericytes (ScienCell) were seeded in the bottom channel of the OrganoPlate® 3-lane to complete the BBB model (figure 1b-c). The endothelial microvessel shows expression of adherens junction proteins PECAM-1 and VE-cadherin and tight junction proteins Claudin-5 and ZO-1 (figure 1d-g). The expression and localization of these markers at the cell-cell interactions is indicative of barrier formation.

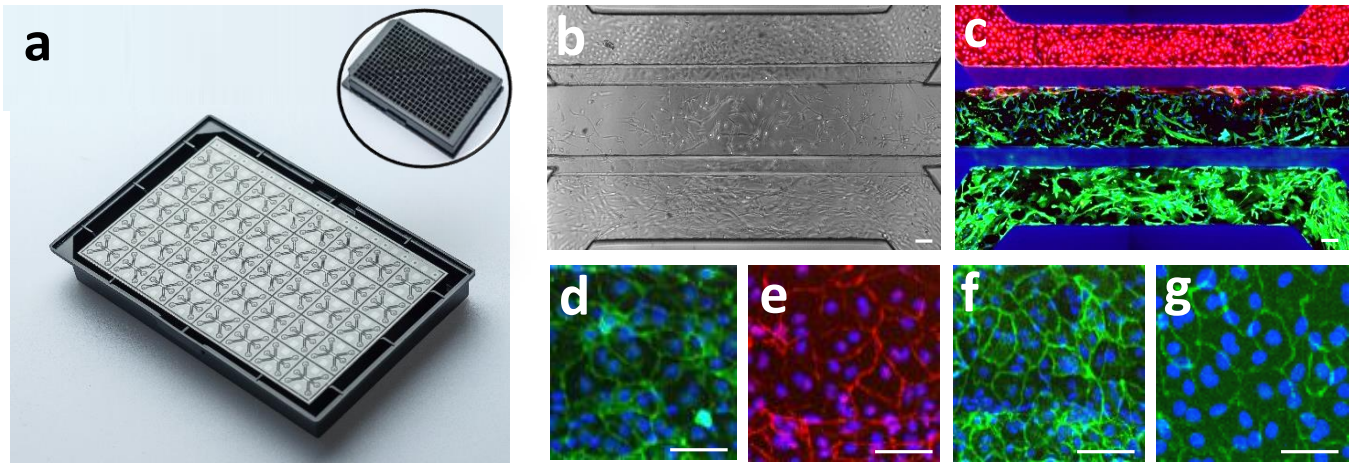

***Figure 1**: Perfused human BBB in OrganoPlates®. **(a)** The OrganoPlate® 3-lane comprises 40 microfluidic chips that can be used to culture miniaturized tissues and organs. **(b)** Phase contrast image of BBB on-a-chip in the OrganoPlate® 3-lane. The endothelial microvessel (top channel) is grown against an extracellular matrix gel (middle channel). Astrocytes and pericytes are added to the bottom channel and migrate through the gel to the endothelial vessel. Scale bar is 100 µm. **(c)** Fluorescent image showing a BBB culture of endothelial cells (red) and astrocytes and pericytes (green). Scale bar is 100 µm. **(d-e)** Staining of the endothelial microvessel for adherens junction markers (d) PECAM-1 and (e) VE-cadherin and tight junction markers (f) Claudin-5 and (g) ZO-1. Scale bars are 50 µm.*

Barrier integrity assay

Barrier function of the BBB model can be assessed using a real-time barrier integrity assay. In this assay, a fluorescent dye is perfused through the lumen of the model's endothelial vessel. In case of a leak-tight barrier, all dye is retained in the microvessel (figure 2a), while in case of a leaky barrier or a cell-free control, the fluorescent dye leaks into the adjacent ECM gel channel (figure 2b-c). The fluorescent intensity in both channels is monitored over time and is used to quantify barrier function in each condition (figure 2d).

Figure 2e shows that when the BBB model is treated to mimic ischemic stroke, its barrier function strongly decreases compared to control.

Transport assay

A different assay was employed to assess the function of one of the BBB's main efflux transporters, the P-glycoprotein (P-gp) transporter. Calcein-AM was perfused through the lumen of the BBB's endothelial vessel and taken up by the cells, making the cells green-fluorescent. In presence of a P-gp inhibitor, P-gp efflux of calcein is reduced, resulting in accumulation of green-fluorescent signal inside the cells (figure 3a). The endothelial cells forming the model's barrier were imaged using a FITC filter and the green-fluorescent signal per cell was calculated using an additional Hoechst staining to visualize the nuclei. Figure 3b shows that in presence of a P-gp inhibitor, the endothelial cells of the BBB model show increased green-fluorescence, indicative of functional P-gp transport.

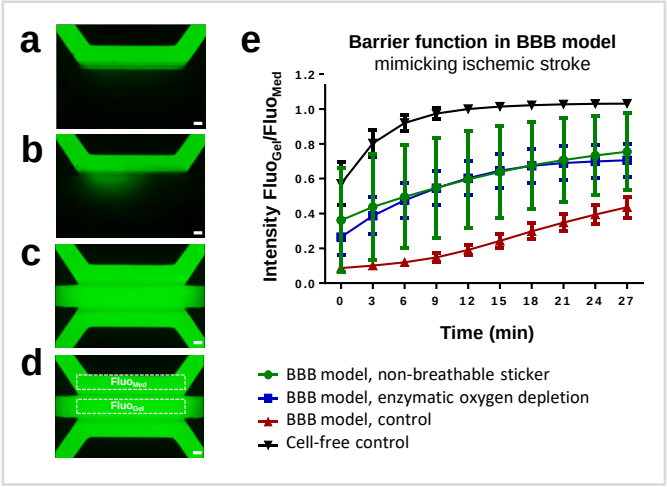

***Figure 2**: Barrier function in the BBB model. **(a-c)** Images taken during a barrier integrity assay of a (a) leak-tight barrier, a (b) leaky barrier, and (c) a cell-free control. Scale bars are 100 µm. **(d)** The fluorescent signal in the medium channel containing the endothelial vessel and the adjacent gel channel are measured over time to quantify barrier function. **(e)** Two methods to mimic ischemic stroke in the BBB model were applied and their effect on BBB integrity for 20kDa FITC-dextran was assessed.*

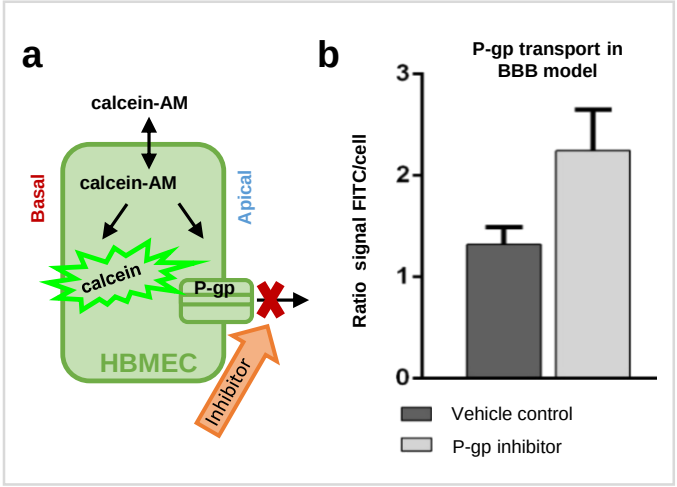

***Figure 3**: P-gp transporter activity in the OrganoPlate® BBB model. **(a)** Green-fluorescent calcein-AM is taken up in the BBB's endothelial cells. A compound is added to inhibit P-gp from effluxing calcein out of the cell, increasing the cell's intracellular fluorescence measured by FITC filters during imaging. **(b)** BBB endothelial cells treated with calcein-AM + P-gp inhibitor show higher intracellular green-fluorescence than cells treated with calcein-AM alone, indicative of P-gp function in the BBB model.*

Discussion & Conclusion

A perfused human BBB on-a-chip was developed in MIMETAS OrganoPlates®. The model shows presence of adherens and tight junctions and a functional barrier, which was compromised after mimicking ischemic stroke. In addition, the model shows presence and function of BBB efflux transporter P-gp. The platform's high-throughput nature (40 chips per OrganoPlate®) and its compatibility with standard laboratory equipment and automation make this BBB on-a-chip suitable for compound screening purposes.

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