Hela-lable - Calcein AM-488 nm green 3.1 x 10^7 / mL

Hela-lable- Life-Cell Tracker- -548 nm red 4.1 x 10^7 / mL

Assessing the viability of in-vitro Blood-Brain-Barrier models by analyzing a 3-D Blood-Brain-Barrier Organ Chip

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Abstract

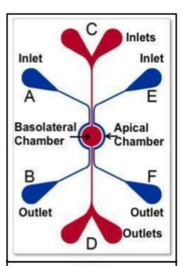
The blood-brain barrier (BBB) is a complex network of layers and channels that uses specialized endothelial cells with tight junction protein complexes and efflux transporters to regulate the transport of ions and molecules across the bloodstream into brain tissue. In addition, a basement membrane composed of robust extracellular matrix proteins, as well as pericytes and astrocyte glial cells, are critical in maintaining the integrity of the BBB.

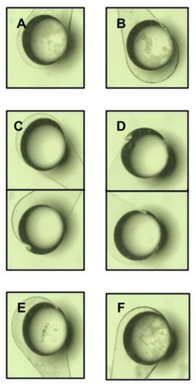
Although murine, in-vivo models have been historically used for study of the BBB, the biological differences between mice and humans, such as in the microglia of the two species, can make effective drugs in mice ineffective in humans. Because of this, 95% of drugs approved after animal testing have failed in humans.

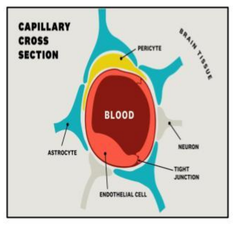
However, in-vitro 2-D and 3-D models provide a novel way for scientists to study the BBB. Although both models have their strengths and drawbacks, the 3-D organ chip model avoids many of the drawbacks of the 2-D model, such as the lack of an extracellular matrix and brain compartment, thereby allowing the 3-D chip to more accurately model the physiology of the human BBB, which has dramatic ramifications for disease modeling and drug testing.

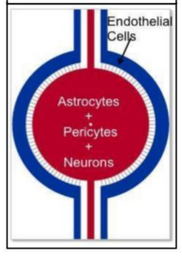
In this experiment, we used multiple SynVivo 3-D BBB model chips to simulate the diffusion of particles and drugs across the BBB. Furthermore, we specifically examined the health and integrity of both HeLa and murine primary endothelial cells once injected into the chip to better understand both the strengths and limitations of the organ chip model.

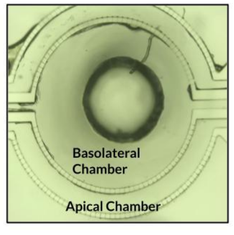
BBB Chip Structure





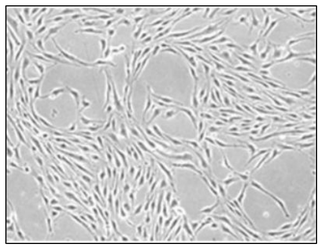






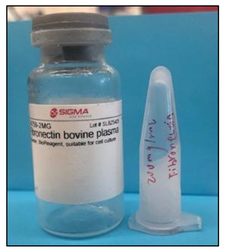
Methods

Both HeLa cells and murine endothelial cells were cultivated for use in the organ chip. Cells were cultured in fibronectin-coated T-25 flasks. Frozen cells were thawed to 37°C, mixed with cell-specific media in their respective flasks, and incubated at 37°C, 5% CO_2. Cells were cultured until the culture reached 70%-80% confluency. Any higher confluence would make the injection of cells into the chip harder due to tissue formation.



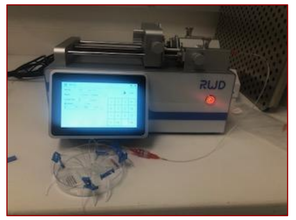
Rat endothelial cells at 70% confluency

To simulate the extracellular matrix of the BBB, the vessels of the 3-D organ-chip were coated with fibronectin, which is a major component of vascular endothelial networks. Frozen fibronectin was thawed to 37°C, then diluted with 1x PBS to a concentration of 200 µg/mL. All 4 channels of the organ chip were then perfused and coated with the fibronectin solution via syringe pump. The chip was incubated at 37°C to strengthen the coating.



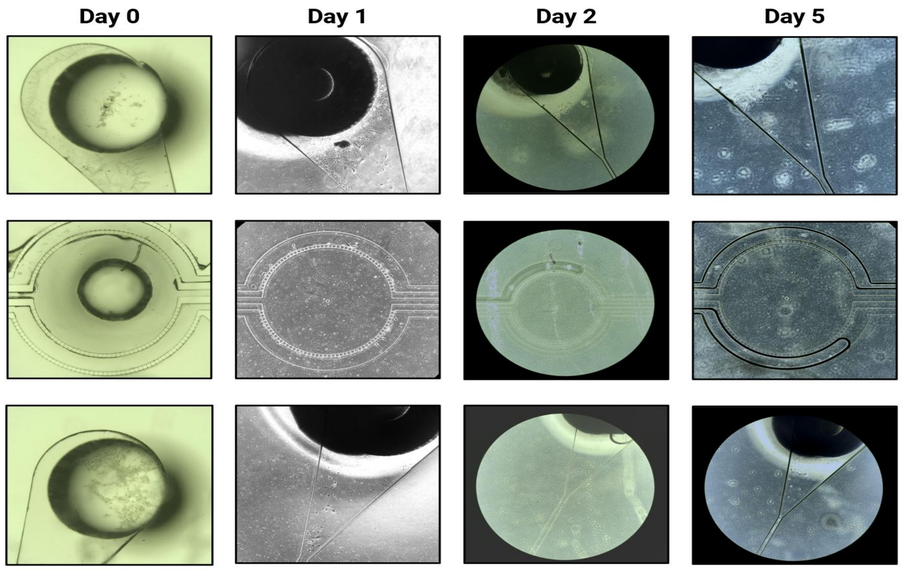
Undiluted and Diluted Fibronectin

Once the cell cultures reached 70%-80% confluency, they were injected into the organ chip. The cell culture was centrifuged at 200 x g for 5 minutes at 1500 rpm, forming a cell pellet. The pellet was resuspended in its respective cell medium at a concentration of 5 x 10^7 cells/mL. The solution was then injected into the organ chip via syringe pump.



The Syringe Pump

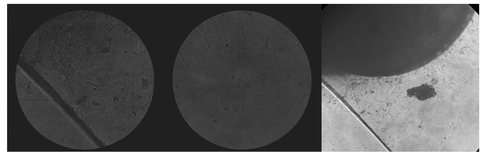
BBB Chip After Murine Endothelial Cell Culture Added



Cell death was mostly likely caused by a poor fibronectin coating inside the chip's vessels. Fibronectin simulates the extracellular matrix of the BBB and is critical to maintaining cell integrity.

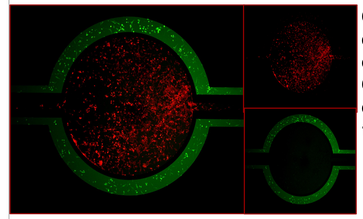
Another potential cause of cell death could be due to a failure to renew the basal media inside the chip's vessels. Basal media is critical to the growth of cells by providing essential molecules for their health.

A closer look at the Day 1 endothelial cells

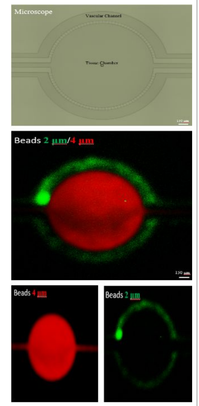


Fluorescent Staining of BBB Chip

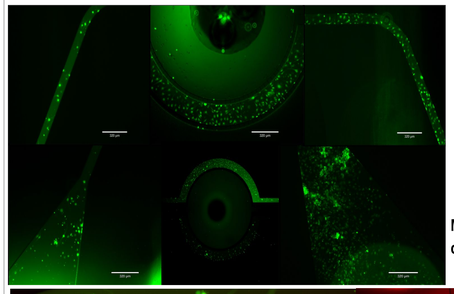
Two HeLa cell cultures were created. One was stained with green calcein and injected into the apical chamber, and the other was stained with CellTracker Red Dye and was injected into the basolateral chamber.



Calcein-stained cell density: 3.1 x 10^7 cells/ mL
CellTracker-stained cell density: 4.1 x 10^7 cells/ mL

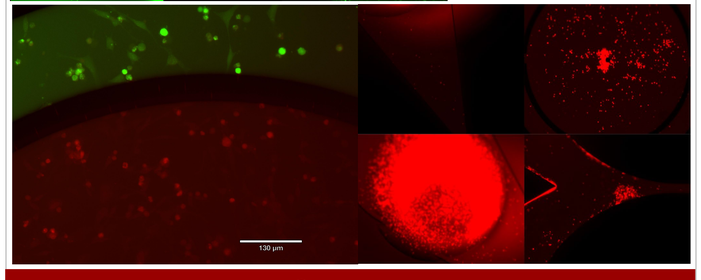


Murine primary endothelial cells were stained with green calcein and were injected into the apical chamber. HeLa cells were stained with CellTracker Red Dye and were injected into the basolateral chamber.



Calcein-stained bead density: 6.9 x 10^6 beads/ mL
CellTracker-stained bead density: 7.2 x 10^6 beads/ mL

Murine Endothelial Cell (MEC) density: 6.9 x 10^6 cells/mL



Summary

1. The organ-chip model is a viable way to model the human BBB, as it can sustain both human and murine cells within its vessels to model diseases and test drugs. The organ-chip can be quickly set-up and be ready for testing within a few days if high confluence cell cultures are already grown.
2. Once the cell culture is injected into the chip's vessels, the cells can rapidly die off without warning. This is a critical flaw in the organ-chip model. Although measures can be taken to maintain the health of the cells, they are nonetheless very vulnerable when compared to their in-vivo murine counterparts.
3. In our study, we successfully demonstrated that cells can thrive well enough inside the organ-chip for disease and drug testing. Further experimentation is needed to fully understand how analogous the chip is to the BBB of both mice and humans.

Thank you to Dr. Zhen Zhao and Tenghuan Ge for providing me the opportunity to work in their lab. I'd like to give special thanks to Feixiang Chen for mentoring and helping me with the organ-chip model and for providing me with so much help with the techniques of the lab.

CONTACT US

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