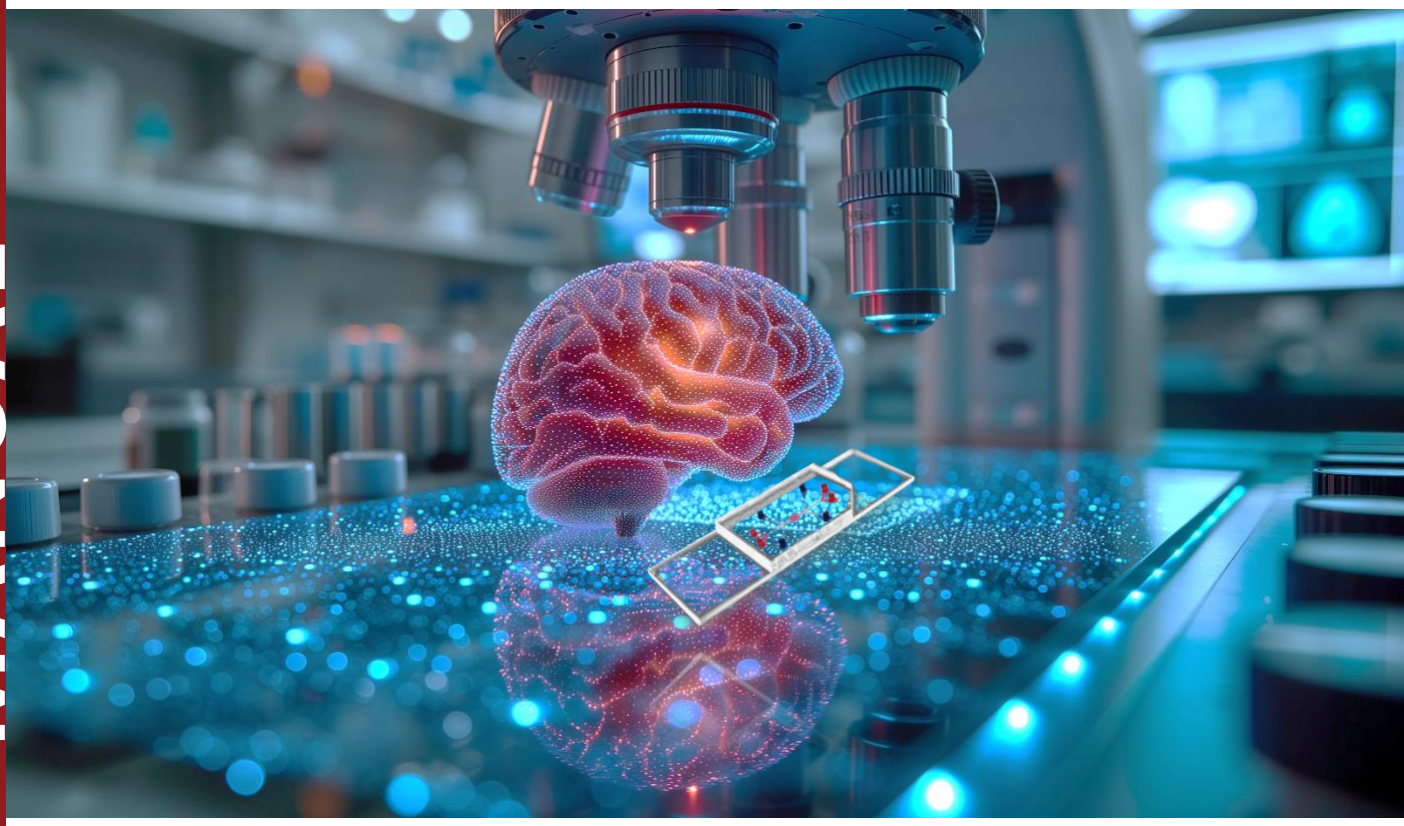
TECHNICAL MANUAL

SYNVIVO

REALISTIC.
DYNAMIC.

Organ-on-Chip Models



SynBBB Blood-Brain-Barrier Model

IDEALIZED CONFIGURATION

Linear and Radial

For Chips and Starter Kits:
Catalog #s 402002, 402006,
102005-SB3, 108011-SB3

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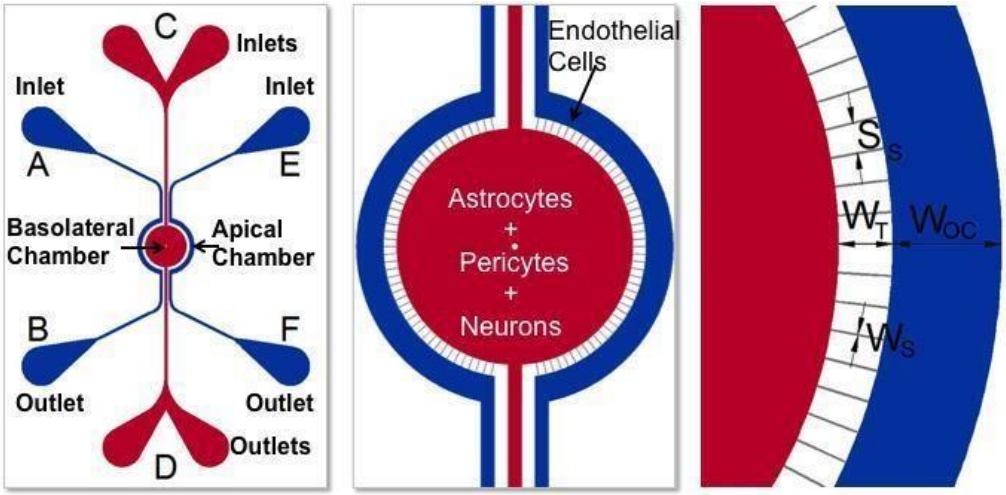
Introduction

SynVivo's SynBBB 3D blood brain barrier model recreates the *in vivo* microenvironment by emulating a histological slice of brain tissue cells in communication with endothelial cells across the blood brain barrier (BBB). Shear-induced endothelial cell tight junctions are easily achieved in the SynBBB model using physiological fluid flow. Formation of tight changes can be measured using biochemical assay. Side-by-side architecture makes visualization of the interactions between brain tissue cells and endothelial cells possible. The SynBBB Protocol can be modified to accommodate cell sources alternative to the ones presented in these instructions.



Apical channels (blue) are for culture of endothelial cells while basolateral chamber (red) is for culture of astrocytes and pericytes. A porous barrier enables communication between the vascular and tissue cells.

The SynBBB system is a highly versatile platform for investigation of:

- Drug permeability: Evaluate real-time permeability of therapeutics and small molecules across the endothelium of the BBB.
- Inflammation: Understand the underlying mechanisms of inflammatory responses on the regulation of the BBB.
- Cell migration: Visualize and quantify real-time migration of immune cells across the BBB.
- Omic changes: Perform genomic, proteomic and metabolic analysis on the BBB under normal conditions or in neurological disease.
- Neurotoxicity: Analyze toxicity effects of chemical, biological and physical agents on the cells of the BBB.

For further information on how the SynBBB model has been used in research, see the following supporting information:

1. Yuan H., M. W. Gaber, T. McColgan, M. D. Naimark, M. F. Kiani, and T. E. Merchant. 2003. Radiation-induced permeability and leukocyte adhesion in the rat blood–brain barrier: modulation with anti-ICAM-1 antibodies. *Brain Res*. 969: 59-69.
2. Prabhakarpandian, B., M. C. Shen, J. B. Nichols, I. R. Mills, M. SidorykWegrzynowicz, M. Aschner, and K. Pant. 2013. SyM-BBB: a microfluidic blood brain barrier model. *Lab Chip*. 13(6):1093-101.
3. Deosarkar, S. P., B. Prabhakarpandian, B. Wang, J. B. Sheffield, B. Krynska, and M. F. Kiani. 2015. A novel dynamic neonatal blood-brain barrier on a chip. *PLoS One*. 10(11):e0142725.
4. Terrell-Hall, T. B., A. G. Ammer, J. I. Griffith, and P. R. Lockman. 2017. Permeability across a novel microfluidic blood-tumor barrier model. *Fluids Barriers CNS*, 14(1):3.
5. Terrell-Hall, T. B., M. I. Nounou, F. El-Amrawy, J. I. G. Griffith, and P. R. Lockman. 2017. Trastuzumab distribution in an in-vivo and in-vitro model of brain metastases of breast cancer. *Oncotarget*. 8(48):83734-83744.

Primary Human Brain Microvascular Model Protocols

MONOCULTURE AND TRI-CULTURE

Workflow

The following workflow chart shows the basic steps of setting up the monoculture or tri-culture SynBBB model:

Monoculture SynBBB Model:

- Human brain microvascular endothelial cells (iXCells Biotech 10HU-051, Passage 1)

Tri-culture SynBBB Model:

- Human brain microvascular endothelial cells (iXCells Biotech 10HU-051, Passage 1)
- Human astrocytes (HA; ScienCell 1800, Passages 2 to 4)
- Human brain vascular pericytes (HBVP; ScienCell 1200, Passages 2 to 4)

Media and Reagents:

- 1X PBS without calcium or magnesium (Corning 21040CV)
- Poly-L-lysine (Millipore Sigma P4707)
- Astrocyte medium (ScienCell 1801)
- Pericyte medium (ScienCell 1201)
- Endothelial cell growth medium (R&D Systems CCM027)
- 0.05% and 0.25% Trypsin-EDTA (ScienCell 0183 & 0103)

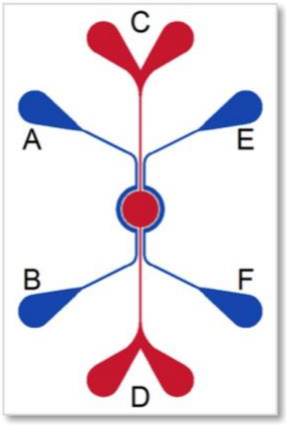
- Trypsin neutralization solution (ScienCell 0113)
- Fibronectin, human plasma (Millipore Sigma FC010)

Equipment:

- Pneumatic Primer with Multiple Port Manifold (Synvivo 205001)
- Inverted Microscope
- Syringe pumps: remote-head and multichannel syringe pumps
- Cell Culture Incubator
- Scissors
- Forceps

Establishing the Basement Membrane

The following protocol should be carried out within a laminar flow hood as much as possible to maintain sterility.

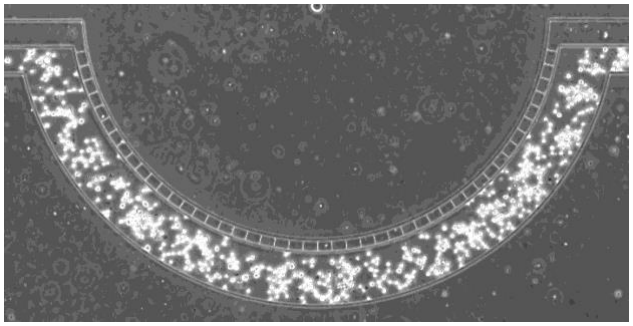


1. Place approximately 1-inch (2.54 cm) long segments of Tygon tubing into all the outlet ports (Outlets B, D, and F), and one inlet port (Inlet C) of the radial IMN device.
2. Dilute fibronectin in sterile 1X PBS to a final concentration of **200 µg/ml**.
3. Using a 1-mL syringe with approximately 5 inches of tubing attached, draw up the fibronectin solution into tubing.
4. Place the liquid-filled tubing into inlet port A.
5. Fill the channel with fibronectin.
6. Cut the inlet tubing.
7. Repeat process for the other channels (inlet ports C and E).
8. Place device in a 37 °C incubator for 1 hour before priming with serum free media.
9. After incubation, completely perfuse the device with serum-free cell media using the Pneumatic Primer (see **Pneumatic Primer-Technical Manual** for details).

Seeding the Vascular Channel with Endothelial Cells

1. Remove the T75 flask of passage 1 HBMVECs (85 to 95% confluent) from the incubator and aspirate the cell media from the flask.
2. Rinse the HBMVECs with 5 mL of room temperature 1X PBS and aspirate.
3. Add 3 mL of 0.05% trypsin-EDTA to the T75 flask and place the flask in a 37 C incubator for 2 minutes.
4. After 2 minutes, check the cells under a microscope for cell rounding and detachment. If the cells are not rounded, place the flask into the incubator for 1 additional minute.
5. Gently knock the flask to detach cells and add 6 mL of trypsin neutralizing solution then transfer the contents of the flask to a 15 mL conical tube.

6. Centrifuge the cells for 5 minutes at 200 g at room temperature.
7. Aspirate the supernatant and re-suspend the cell pellet in 2 mL of HBMVEC media.
8. Take a sample to count, and during counting, centrifuge the cells for 5 minutes at 200 g.
9. Resuspend the cell pellet in the volume of complete HBMVEC media to achieve $3x10^7$ cells/ml (typically 40 to 80 uL)
10. The cell solution should have the appearance of frosted glass, which will indicate a dense cell concentration.
11. Attach a 24 G needle and 8 in Tygon tubing to a 1 mL syringe and mount the empty syringe onto the remote head syringe pump.
12. Unclamp one outlet port of the vascular channel. Leave the other chambers clamped.
13. Place a drop of liquid beside the tubing for the inlet port of the vascular channel.
14. Load the Tygon tubing of the mounted syringe with the prepared cell concentrate.
    a. Using the "Fast Reverse" button on the pump user interface, draw up approximately 1 inch of cell concentrate into the Tygon tubing of the mounted syringe.
    b. Using the "Fast Forward" function on the pump user interface, push the cell mixture forward until the concentrate liquid is flush with the end of the tubing.
15. Insert the tubing into the inlet port - the drop of water will prevent air entering the device as the tubing is inserted.
16. Inject the cells into the device at a flow rate of 3 µl/min.
17. Once the vascular channel is filled with cells, stop the flow and clamp the outlet tubing. Cell density should be consistent across the whole channel (see figure below).
18. Carefully cut the inlet tubing, keeping the length of the inlet tubing as small as possible.
19. Place the device in a 37 °C incubator for 4 hours before proceeding to Establishing Flow Model.



Seeded HBMVECs in the vascular channel.

Establishing Flow Model

After 4 hours of incubation at 37 °C, check the SynBBB device under the microscope for cell attachment.

1. For each device, prepare a 1-mL syringe with complete HBMVEC media. Attach a length of Tygon tubing to the syringe that is long enough to reach from the syringe pump to the device inside the tissue culture incubator.
2. Mount the syringes onto the syringe pump and perfuse the Tygon tubing completely, ensuring no air bubbles are in the tubing.
3. Place a drop of water at the base of the inlet port tubing to be removed and remove the tubing with forceps.
4. Remove the clamp on the outlet port of the vascular channel. Keep all other ports clamped. To capture the effluent during the media change, a small tube can be placed under the outlet tubing to serve as a waste reservoir.
5. While holding the end of the Tygon tubing above the level of the SynBBB device, use the "Fast Forward" function on the syringe pump to push the media forward until a droplet forms at the end of the tubing.
6. Insert the tubing into the inlet port. The drop of water will prevent air from entering the device as the tubing is inserted.
7. Clean the fluid from the surface of the device.
8. Start the syringe pump program (see Pump Programming below)
9. Incubate the device overnight in a 37 °C incubator, 5% CO_2.

Pump Programming

Monoculture SynBBB Instructions

If desired, perfuse the central tissue chamber with astrocyte conditioned media (ACM) by hand via syringe before beginning the flow program. The following program will establish a monoculture SynBBB model over 48 hours:

Table 1. Programming step increases in flow rate (0.02-0.1 µl/min)

STEP	Flow Rate (µl/min)	Time (h:m:s)	Direction
1	0.02	2:00:00	INFUSE
2	0.03	2:00:00	INFUSE
3	0.04	2:00:00	INFUSE
4	0.05	2:00:00	INFUSE
5	0.06	2:00:00	INFUSE
6	0.07	2:00:00	INFUSE
7	0.08	2:00:00	INFUSE
8	0.09	2:00:00	INFUSE
9	0.1	8:00:00	INFUSE

At the end of the program, the SynBBB device is ready for assay.

Tri-culture SynBBB Instructions

The following program will begin flow overnight, then astrocytes and pericytes can be seeded into the tissue chamber the next day:

Table 2. Programming step increases in flow rate (0.02-0.0.05 µl/min)			
STEP	**Flow Rate (µl/min)**	**Time (h:m:s)**	**Direction**
1	0.02	2:00:00	INFUSE
2	0.03	2:00:00	INFUSE
3	0.04	2:00:00	INFUSE
4	0.05	12:00:00	INFUSE

When the flow program ends, continue to Seeding Astrocytes and Pericytes in the Tissue Chamber below.

Seeding Astrocytes and Pericytes in the Tissue Chamber

Dissociating astrocytes and/or pericytes from the flask:

1. Prepare two 50-mL conical tubes with 5 mL of FBS in each tube (label one for astrocytes and one for pericytes). Prepare 0.025% trypsin-EDTA (diluted in 1XPBS) for dissociation.
2. Following the manufacturer's dissociation instructions, add 10 mL of 0.025% trypsin-EDTA solution to each T-75 flask and observe under the microscope until cells round up (approximately 1 minute).
3. Add 5 mL of trypsin neutralizing solution to each flask and transfer solutions into their respective 50-mL conical tubes with FBS.
4. Add an additional 5 mL of trypsin neutralizing solution to each flask, and transfer into their respective 50-mL conical tubes.
5. Centrifuge for 5 minutes at 200 g and remove supernatant.
6. Resuspend cells in 4 mL of either astrocyte or pericyte medium.
7. Take a sample from each tube to count, and during counting, centrifuge the cells for 5 minutes at 200 g.
8. Calculate the total number of cells and media to resuspend each cell pellet to a final concentration of 1 x 10^7 cells/mL (typically 200 to 800 uL)
9. Resuspend astrocytes at 1 x 10^7 cells/mL in astrocyte media. Resuspend pericytes at 1 x 10^7 cells/mL in pericyte media.
10. Mix astrocytes to pericytes in a ratio of 2:1 (typically 200 µl astrocytes to 100 µl pericytes should be adequate) into a 1.5 mL sample tube.

Seeding Astrocytes & Pericytes:

1. Attach a 24 G needle and 8 in Tygon tubing to a 1 mL syringe and mount the empty syringe onto the remote head syringe pump.
2. Unclamp one outlet port of the tissue channel. Leave the other chambers clamped.
3. Place a drop of liquid beside the tubing for the tissue chamber inlet port and remove the tubing.
4. Load the Tygon tubing of the mounted syringe with the prepared cell concentrate.
    a. Using the "Fast Reverse" button on the pump user interface, draw up approximately 1 inch of cell concentrate into the Tygon tubing of the mounted syringe.
    b. Using the "Fast Forward" function on the pump user interface, push the cell mixture forward until the concentrate liquid is flush with the end of the tubing.
5. Insert the tubing into the inlet port - the drop of water will prevent air entering the device as the tubing is inserted.
6. Inject the cells into the device at a flow rate of 3 µl/min.
7. Once the tissue channel is filled with cells, stop the flow and clamp the outlet tubing. Cell density should be consistent across the whole channel
8. Carefully and quickly cut the inlet tubing off the syringe.
9. Incubate the device for 1 hour in a tissue culture incubator at 37 °C (5% CO_2) to allow the cells to attach to the device.
10. Once the astrocytes and/or pericytes have adhered to the tissue chamber (for 1 hour), re-attach the vascular channel to the HBMVEC media on the syringe pump, and begin the following program:

Table 3. Programming step increases in flow rate (0.05-0.0.1 µl/min)

STEP	Flow Rate (µl/min)	Time (h:m:s)	Direction
1	0.05	2:00:00	INFUSE
2	0.06	2:00:00	INFUSE
3	0.07	2:00:00	INFUSE
4	0.08	2:00:00	INFUSE
5	0.09	2:00:00	INFUSE
6	0.1	32:00:00	INFUSE

At the end of the flow program (approximately 72 hours after seeding HBMVECs), the SynBBB tri-culture model is ready for assay.