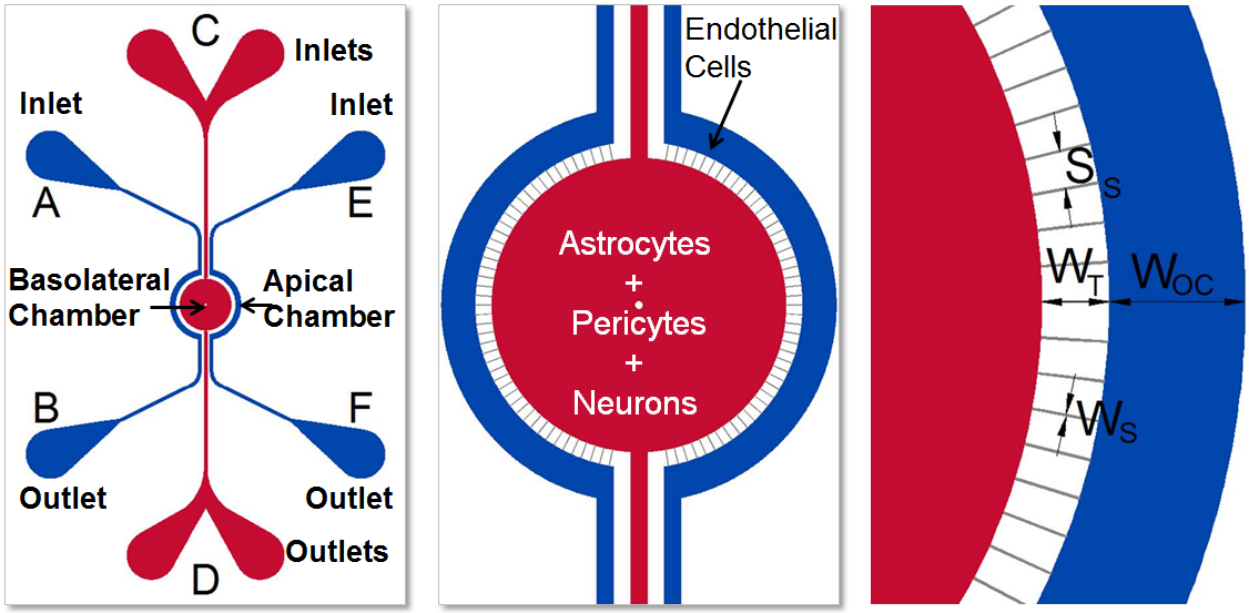
3D Blood Brain Barrier Model Using DESIGN 1 - OPT-A Chips – Technical Manual

Catalog # SY-102005



Schematic of the BBB Model Chip. Apical chamber (outer channels) are for culture of vascular (endothelial cells) while basolateral chamber (central chamber) are for culture of brain tissue cells (astrocytes, pericytes, neurons). Porous architecture enables communication between the vascular and tissue cells.

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Overview of the SynBBB Model

Delivery of neuroprotective or therapeutic agents to specific regions of the brain presents a major challenge, largely due to the presence of the Blood-Brain Barrier (BBB). Physiologically, the BBB consists of an intricate network of vascular endothelial cells (ECs) that isolate the central nervous system (CNS) from systemic blood circulation. Traditional blood brain barrier (BBB) assays, such as the Transwell® model have significant limitations such as lack of physiological shear stress, real-time visualization capability and large amount of consumables in addition to cumbersome protocols.

BBB model recreates the in vivo microenvironment by mimicking a histological slice of brain tissue cells in communication with endothelial cells across the BBB. **This is the only *in vitro* BBB model that allows**

- Accurate in vivo hemodynamic shear stress
- Real-time visualization of cellular and barrier functionality
- Significant reduction in cost and time
- Robust and easy to use protocols

This model has been successfully demonstrated for upregulation of tight junction molecules, functional assays (Prabhakarpandian et al., 2013) and validated against in vivo studies showing excellent correlation with permeation of small molecules (Deosarkar et al., 2015).

Material, Equipment and Cells Required

- BBB Chips* (Catalog # 102005): Use Ports A, C and E as Inlets and B, D and F as Outlets
- Pneumatic Primer Device (Catalog #205001) - optional
- 1 mL BD plastic syringes or other 1 mL syringes (Catalog # 203004)
- 24 gauge blunt tip needles (Catalog # 204002)
- Tygon microbore tubing, 0.02” ID X 0.06” OD (Catalog # 201005)
- Clamps (Catalog # 202003)
- Forceps
- Syringe Pump capable of flow rates from 10nl/min to 10ul/min
- Fibronectin
- Endothelial Cells (primary or cell line)
- Astrocytes (primary or cell line)

*** Store at Room Temperature. Shelf life is 6 months at room temperature**

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

Culture of Endothelial Cells in the Apical Channels

Shear-induced endothelial cell tight junctions, which cannot be achieved in the Transwell® model, are easily achieved in this model using fluid perfusion. Formation of tight junction changes can be measured using biochemical or electrical analysis (assessing changes in electrical resistance) with the SynVivo Cell Impedance Analyzer (Catalog # 304001).

Note: Most primary endothelial cells are usable only until passage 8. For endothelial cell lines, refer to vendor specifications for passage information. Most endothelial cell lines do not sustain shear stress that is required for formation of tight junctions.

A. Coating The Chip with Endothelial Cell Culture Matrix (e.g. fibronectin). This process is performed using Pneumatic Primer (Cat# 205001).

1. Place approximately 1 inch long segments of Tygon tubing into the outlet ports of the chip.
2. Draw 200ug/ml human fibronectin into a 1 mL syringe.
3. Using additional 1inch long segments of tubing, fill the chip with liquid by inserting the primed tubing into the inlet and pushing the solution through until the outlet tubing is filled.
4. Do this for all but one inlet port. For this last port, use a tubing approximately 2-3 inch long. When the chip is filled, unlock the needle from the syringe, leaving the needle attached to the tubing.
5. Clamp all tubing below the liquid line, except for the tubing with the needle attached.
6. Connect the chip to the Pneumatic Primer by locking the needle into the LuerLock connector on the box. *Note: Multiple chips can be primed simultaneously using the multiple port manifold (cat # 207001)*
7. Turn the knob on the controller box and adjust the pressure to ~5-7 psi. Apply the pressure for ~5-20 minutes. Chips will take at least 15 minutes to completely fill.
8. Turn off the pressure and cut the Tygon tubing connected to the Pneumatic Primer.
9. Allow the chip to incubate at 37° for a minimum of 1 hour before use.
10. Flush fresh media into chip just before seeding endothelial cells.

B. Culture of Endothelial Cells

1. Prepare the endothelial cell suspension for seeding. Endothelial cells should be dissociated, centrifuged and concentrated to approximately 5-8x10^6cells/ml in cell specific media.
2. Place a drop of water at the base of the inlet port tubing to be removed and gently remove the tubing from the port.

3. Remove the clamp on the outlet port. All channels should be clamped except for the channel being seeded.
4. Prepare a syringe and tubing with the previously prepared cell suspension and mount onto a syringe pump.
5. Ensure that the tubing is free of air bubbles and the cell mixture is flush with the end of the tubing
6. Insert the tubing into the port - the drop of water will prevent air entering the chip as the tubing is inserted.
7. Clean the fluid from the surface of the chip.
8. Begin the injection at 4-7 ul/min.
9. Watch the chip as the cells are flowing. Once the vascular channel is filled with cells, stop the flow and clamp the outlet tubing.
10. Carefully and quickly cut the inlet tubing, keeping the length of all the tubing equal.
11. Allow the cells to attach for at least 4 hours before changing media.
    a. Many endothelial cells can be incubated overnight before a media change is required.
12. Allow the cells to grow approximately 24 hours with at least 1 media change before running migration assay.
    a. It should be noted that some endothelial cells may need up to 72 hours (with daily media changes) before they can withstand flow.

    b. To set up a media change program using a syringe pump, use the following steps:
        i. Program the pump to flush out the vascular channel without cells every 3 hours at 2µl/min for 3 minutes to refresh the media.
            1. Program summary: Media Change
                a. Step 1: Constant Rate
                    i. Mode: Infuse
                    ii. Set rate: 2 µl/min
                    iii. Time: 0:03:00 (3 minutes).
                b. Step 2: Pause
                    i. Mode: Pause
                    ii. Target time: 3:00:00 (3 hours)
                c. Step 3: Repeat from Step 1
13. For complete confluent monolayers, introduce flow using programmable syringe pump with the flow rate climbing from 0.01µl/min to 1µl/min over a 24-hour period (Table 1).

    a. The program example uses the “Step” feature of the pump, which infuses media at a user-defined flow rate over a specific period of time.

Table 1: Step programming to increase the flow rate from 0.01 to 1 µL/min over 24 hours

SEQ	Flow Rate (µl/min)	Time (h:m:s)	Direction
1	0.01	2:00:00	INFUSE
2	0.025	2:00:00	INFUSE
3	0.05	2:00:00	INFUSE
4	0.075	2:00:00	INFUSE
5	0.1	2:00:00	INFUSE
6	0.2	2:00:00	INFUSE
7	0.3	2:00:00	INFUSE
8	0.4	2:00:00	INFUSE
9	0.5	2:00:00	INFUSE
10	0.6	2:00:00	INFUSE
11	0.8	2:00:00	INFUSE
12	1	2:00:00	INFUSE

Co-Culture of Endothelial Cells with Tissue Cells or Conditioned Media

Interactions between brain tissue cells and endothelial cells are readily visualized in the SynVivo-BBB model. Endothelial cells are cultured under flow in the vascular channel, and the tissue chamber is cultured with primary brain cells, such as astrocytes. Endothelial cells co-cultured with astrocytes form significantly tighter cell junctions compared to mono cultured endothelial cells.

1. All tubing should be clamped before beginning this procedure.
2. Astrocytes should be dissociated centrifuged and concentrated to approximately 1-3x10^7 cells/mL.
3. Place a drop of water at the base of the inlet port (C) tubing to be removed.
4. Gently remove the tubing - the water should cover the port once the tubing is removed, and should remain there until new Tygon tubing is inserted into the port. This step will prevent air from entering the chip.
5. Remove the clamp on the outlet port (D).
6. Prepare a syringe and tubing with the cell suspension and mount onto a syringe pump.
7. Ensure that the tubing is free of air bubbles and allow a convex meniscus to form at the end of the tubing.
8. Insert the tubing into the port (C)—the meniscus and the drop of water will form a bridge to prevent air from entering the chip as the tubing is inserted.
9. Clean the fluid from the surface of the chip.

10. Begin the injection at 5 µL/min.
11. Observe the chip as the cells are flowing. Once the tissue chamber is filled with cells, clamp the outlet tubing (port D) and stop the flow.
12. Cut the inlet tubing approximately 1 inch above the chip, and then clamp. This will help disperse the cells more evenly throughout the vascular chamber.
13. Allow astrocytes to attach for at least 4 hours before changing media.
14. Change media regularly until cells are confluent or time of experiments.
15. For conditioned media from astrocytes or neurons, repeat above process without cells.

Fluorescence Tagged Small Molecule Permeability Assay

Unlike BBB models that are arranged in top to bottom architecture (i.e., Transwell), small molecule transport can be assessed and quantified in real-time across the SynBBB model due to its side-by-side architecture. A fluorescently labeled drug molecule of interest is perfused through the vascular channels at physiological flow rate. Real-time videos are acquired and analyzed to calculate the rate of permeability into the tissue chamber. Different rates of permeability are observed across the BBB due to tight junctions of endothelial cells. Permeability through the vascular channel into the tissue chamber is measured by observing diffusion of a fluorescently labeled molecule. The permeability is related to the rate of change of fluorescent intensity in the tissue chamber by the equation [1]:

$$P = (1 - H_{CT}) \frac{1}{I_{V0}} \frac{V}{S} \frac{dI_t}{dt} \qquad (1)$$

H_{CT} is the hematocrit count (equal to 0 for in vitro measurements), I_{v0} is the fluorescent intensity in the apical channel containing the endothelial cells, V/S is the ratio of apical volume to surface area (in this case, calculated for a 200 µm wide and 100 µm height vascular channel), and I_t is the fluorescent intensity in the basolateral chamber.

Parameter	Description	Value
H_{CT}	Local Hematocrit	0 (in vitro)
I_{V0}	Apical Intensity	Calculated below
V/S	Ratio of Apical Volume to Surface Area	0.1 cm
dI_t/dt	Change in Basolateral Intensity over Time	Calculated below

A. Video Acquisition

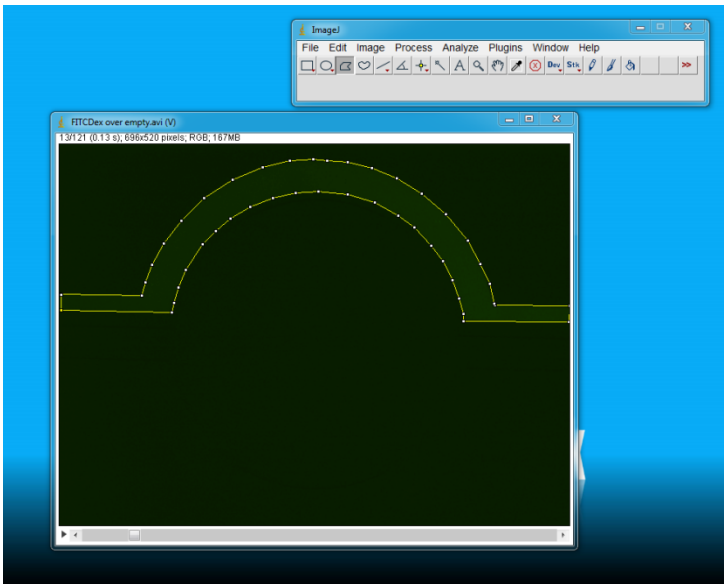
1. Prepare a desired concentration fluorescent-tagged molecule (e.g. 0.1 mM FITC-Dextran (3kDa)) solution in PBS, and load the solution into a 1 cc syringe with a 24 gauge needle

attachment with Tygon tubing. Set the syringe on the syringe pump and fully perfuse the tubing with the solution.

2. Place the cell-coated chip onto the stage of an epifluorescence microscope. Place a drop of water around the inlet tubing, and remove tubing.
3. Set the camera exposure, and stage of the microscope to capture both the apical and basolateral channels of the chip in one image.
4. Interface the perfused tubing containing the fluorescent small molecule to the chip, and begin flow at 0.1 uL/min. Immediately begin to acquire images every 30 s for 1 hour. (Note: close the fluorescence shutter between image acquisitions in order to prevent photobleaching).
5. Save the image stack as an AVI.

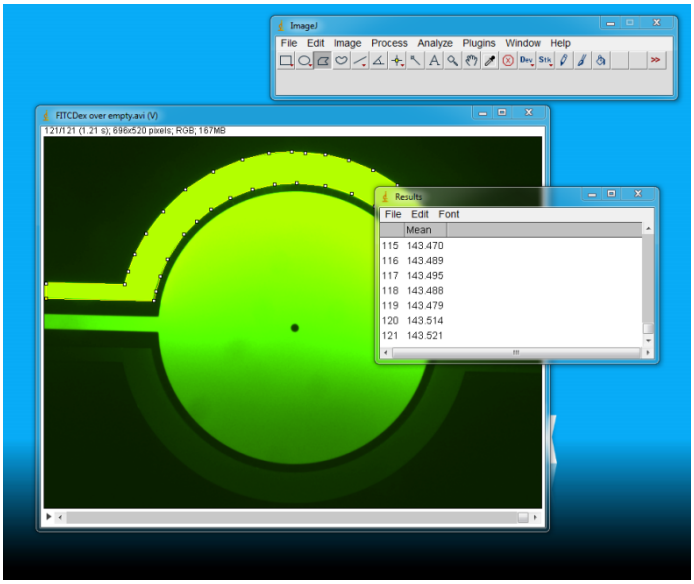
B. **<u>Video Analysis using ImageJ</u>**

1. Open ImageJ and import the acquired video as an AVI:
   File→Import→AVI
   Select video file
   In the AVI Reader box, check "Use Virtual Stack" and click OK
   The horizontal scroll bar will allow you to scroll through the stack
2. Using polygon selections tool, carefully outline the vascular channel geometry. You may need to scroll to a high contract section to clearly see the outline.

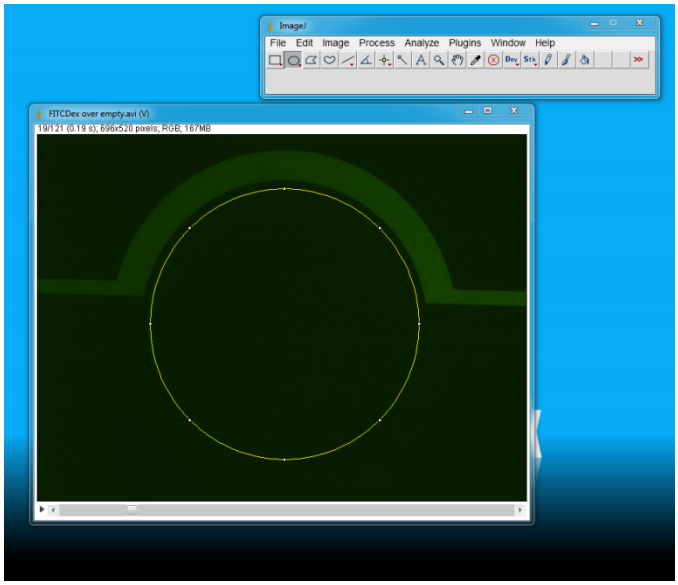


3. Set the Measurements to measure the mean value of the selection:
   Analyze→Set Measurements
   Select "Mean Gray Value"
   Under "Redirect to", select the file name
4. Analyze the mean gray value of the stack:

Plugins→Stacks→Measure Stack
Copy data from "Results" window to Excel for analysis. I_{vessel} will be generated from this data.

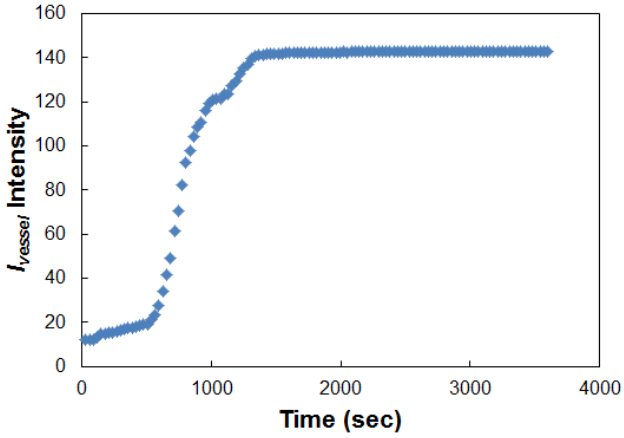


5. Deselect the vascular chamber, and select the tissue chamber using the elliptical selections tool.

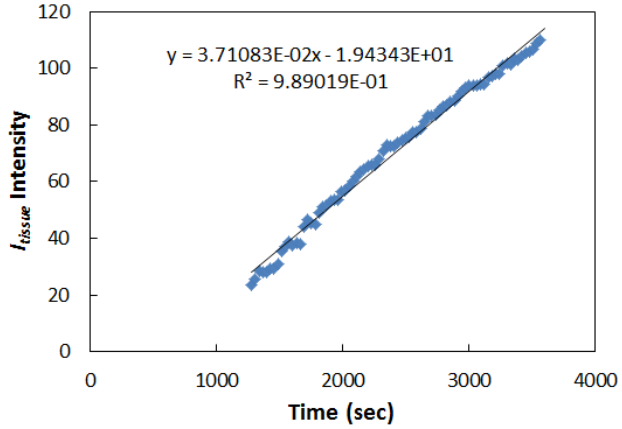


6. Repeat gray value measurement of the stack:
Plugins→Stacks→Measure Stack
Copy data from "Results" window to Excel for analysis. I_{tissue} will be generated from this data.

7. There should be three columns of data in Excel: the frame number, the I_{vessel} data and the I_{tissue} data. Calculate time in seconds by multiplying each frame number by 3600 and dividing by the number of frames (121).
8. Plot I_{vessel} vs. time in an Excel scatter plot. The plot should show an increase in I_{vessel} intensity, and then a plateau. Note the time point at the beginning of the plateau. The average gray value intensity of the plateau area will be I_{v0} in Equation [1]. In this example, I_{v0} = 142.5.



9. Next, find the slope of I_{tissue} vs. time. Only use data in the time range specified by the I_0 average above. Plot I_{tissue} data in the y-axis, and time in the x-axis. Display the trend line with its equation on the graph to determine the slope.



10. The slope of I_{tissue} is equal to I_t/dt in Equation [1] to calculate permeability in cm/s. From the example above, It/dt =3.7 x 10^{-2} s^{-1}. For the given example, Equation 1 is calculated:

$$P = (1-0)\frac{1}{140.5}(0.1\ cm)(3.7\text{x}10^{-2}s) = 0.0121\ cm/s$$

References

[1] Yuan H., Gaber M. W., McColgan T., Naimark M. D., Kiani M. F., Merchant T. E. Radiation-induced permeability and leukocyte adhesion in the rat blood–brain barrier: modulation with anti-ICAM-1 antibodies. Brain Res 969, 59-69 (2003).

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