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Title: Establishing a Microfluidic Co-Culture Blood–Brain Barrier Model Using Human Brain Endothelial Cells and Astrocytes

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. Filming location: Will the filming need to take place in multiple locations? **Yes—office and lab**

In the same building, one floor apart, 2 minute walk. Lift is available.

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 30

Number of Shots: 56

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Sarah Boardman:** Our research focuses on a 3D flow-based microfluidic BBB model, including endothelial cells and astrocytes, to investigate cellular mechanisms.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Sarah Boardman:** This platform is suitable for investigating how the BBB reacts to shear flow, pathogens, and inflammatory responses.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Sarah Boardman:** This protocol allows users to assess barrier integrity via TEER, immunofluorescent staining, and qPCR, as well as determine the fluid mechanics.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.4. **Sarah Boardman:** Further techniques, such as supernatant analysis of biomarkers and permeability to fluorescent tracers, are possible using this model.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Sarah Boardman:** Future work aims to integrate more cell types to produce an enhanced BBB model to understand cellular interactions in disease.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

NOTE: The authors would like to send their own headshots.

Videographer: Obtain headshots for all authors available at the filming location.

Promotional Interview Questions:

Videographer: Please capture these shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Statements will be presented live by the authors, sharing their spontaneous perspectives.

Authors NOTE: we ran out of time and our co-authors who would like to shoot the additional promotional QandA 1.6 and 1.7 below were no longer available for filming today. We will send those videos to you separately.

How could this blood–brain barrier model ultimately be used to study neurological disease, infection, or the ability of potential therapeutics to reach the brain?

- 1.6. **Enter author name:** (authors will present their testimonial statements live)
 - 1.6.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

The blood–brain barrier is particularly difficult to recreate in the lab. What aspects of the human blood–brain barrier does this microfluidic model capture that are difficult to reproduce with traditional static models?

- 1.7. **Enter author name:** (authors will present their testimonial statements live)
 - 1.7.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

NOTE: Cordelia's Testimonial best takes are 3, 4 & 5. Don't use the other takes.

What is the key takeaway you hope viewers remember after watching your video article?

- 1.8. **Cordelia Dunai, Postdoc at University of Liverpool**: (authors will present their testimonial statements live)
 - 1.8.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

How does the video format of your article help you to share your work with the broader scientific community?

- 1.9. **Sarah Boardman, Ph.D. student at U of Liverpool**, : (authors will present their testimonial statements live)
 - 1.9.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

2. Microfluidic Chip Tubing Setup

Demonstrator: Sarah Boardman

2.1. To begin, cut the outlet tubing to a length of 10 centimeters [1]. Insert Tygon tubing into the three outlet ports and three electrode ports of the microfluidic chip using sterile tweezers [2].

2.1.1. WIDE: Talent cutting outlet tubing to a length of 10 cm

2.1.2. Talent inserting tubing into the outlet ports of the microfluidic chip using sterile tweezers.

2.2. Insert an electrode into the tubing to determine the required tubing length for the electrode ports [1]. Mark the appropriate position, remove the electrode [2], and cut the tubing at the marked location [3].

2.2.1. Talent inserting an electrode into tubing. NOTE: Shots 2.2.1, 2.2.2 & 2.2.3 were combined together. Take 4 is the shot to use.

2.2.2. Talent marking the tubing at the appropriate position and removing the electrode.

2.2.3. Talent cutting the tubing at the marked location.

NOTE: Insert a title card: **Perfusion of the Chip with PBS**

2.3. To perfuse the chip, attach approximately 24 centimeters of Tygon tubing to a 1-milliliter syringe needle [1]. Aspirate PBS into the syringe and tubing until the system is filled and free of visible air bubbles [2].

2.3.1. Talent attaching Tygon tubing to a 1-milliliter syringe needle. NOTE: Shots 2.3.1 & 2.3.2 were combined together

2.3.2. Talent aspirating PBS into the syringe and tubing.

2.4. Using sterile tweezers, insert the PBS-filled tubing into one outer apical-channel inlet port [1]. Gently depress the syringe plunger until approximately three drops of PBS emerge from the outlet tubing [2]. Then, apply a clamp to the outlet tubing [3].

2.4.1. Talent inserting the PBS-filled tubing into the outer apical-channel inlet port using sterile tweezers. NOTE: Shots 2.4.1, 2.4.2 & 2.4.3 were combined together.

2.4.2. Talent gently depressing the syringe plunger.

2.4.3. Talent applying a clamp to outlet tubing.

2.5. Continue depressing the syringe plunger until PBS fills the electrode chamber and electrode-port tubing, causing approximately three drops to emerge from the electrode-port tubing [1]. Apply a clamp to the electrode-port tubing [2]. Then, cut the inlet tubing, leaving approximately 8 centimeters attached to the chip and clamp [3].

2.5.1. Talent gently depressing the syringe plunger until the electrode chamber is filled. **NOTE: Shots 2.5.1, 2.5.2 & 2.5.3 were combined together.**

2.5.2. Talent applying a clamp to the electrode-port tubing.

2.5.3. Talent cutting inlet tubing and applying a clamp.

2.6. After perfusing the outer apical and inner basolateral channels, keep the basolateral tubing connected [1] and remove only the syringe barrel for subsequent pneumatic primer connection [2].

2.6.1. Shot of the tubing attached to the syringe connected to the basolateral channel. **NOTE: Shots 2.6.1 & 2.6.2 were combined together.**

2.6.2. Talent removing the syringe barrel from the needle assembly.

3. Chip Priming with Nitrogen Gas

3.1. Attach the pneumatic primer to the nitrogen-gas cylinder [1]. Then, attach the multiport manifold to the pneumatic primer to enable simultaneous priming of multiple chips [2].

3.1.1. Shot of the pneumatic primer connected to the nitrogen-gas cylinder.

3.1.2. Shot of the multiport manifold connected to the pneumatic primer.

3.2. Next, connect the chip to the manifold by securing the syringe needle attached to the chip tubing into the luer-lock connector closest to the pneumatic primer [1]. Connect additional chips to downstream luer-lock connectors as required [2].

3.2.1. Talent securing the syringe needle attached to the chip tubing into the luer-lock connector closest to the pneumatic primer. **NOTE: Shots 3.2.1 & 3.2.2 were combined together. Use Take 2. Don't use Take 1; the author told me there is an error during the process of this take.**

3.2.2. Talent connecting additional chips to downstream luer-lock connectors.

3.3. Then, open the nitrogen-gas cylinder regulator and maintain the inlet pressure below 20 pounds per square inch [1]. Set the pneumatic-primer pressure to 7.5 pounds per square inch and prime the chips for 25 to 30 minutes [2]. Disconnect the chips from the manifold [3] and inspect the channels under a microscope for residual air bubbles [4].

3.3.1. Talent opens the nitrogen-gas cylinder regulator and sets the pressure to 20psi/inch.

3.3.2. Talent setting the pneumatic primer regulator to 7.5psi/inch

3.3.3. Talent disconnecting the chips from the manifold.

3.3.4. LAB MEDIA: Pre-priming-(air-bubbles)-x10-mag.jpg, Pre-priming-(air-bubbles)-x4-mag.jpg, Post-priming-(w_o-air-bubbles)-x10-mag.jpg, Post-priming-(w_o-air-bubbles)-x4-mag.jpg. **NOTE: Two sets of images are provided just at different magnifications. Either of these would be fine to use, pick the preferred magnification.**

4. Measurement of Background Transendothelial Electrical Resistance (TEER)

4.1. Remove the humidity box containing the microfluidic chip coated with laminin, Matrigel, and fibronectin from the incubator [1] and disinfect the external surface with 70% ethanol [2].

4.1.1. Talent removing the humidity box containing the microfluidic chip from the incubator. **NOTE: Shots 4.1.1 & 4.1.2 were combined together.**

4.1.2. Talent disinfecting the external surface of the humidity box with 70 percent ethanol.

4.2. Once the humidity box is placed in a biosafety cabinet, place the chip on a hot plate set to 37 degrees Celsius for 10 minutes before measurement [1]. Then, remove the clamps from the electrode-port tubing [2].

4.2.1. Talent placing the chip on a hot plate. **NOTE: Shots 4.2.1 & 4.2.2 were combined together.**

4.2.2. Talent removing the clamps from the electrode-port tubing.

4.3. Disinfect two electrodes using 70 percent ethanol [1]. Insert one electrode into the apical electrode port tubing and another into the shared basolateral port tubing [2]. Advance the electrodes until the tips contact the glass base [3].

4.3.1. Talent disinfecting two electrodes with 70 percent ethanol.

4.3.2. Talent inserting an electrode into the tubing of apical electrode port and basolateral electrode port. **NOTE: Shots 4.3.2 & 4.3.3 were combined together. Take 2 will need editing towards the end when Sarah (author) shows the bottom of the base. Maybe a cross dissolve transition would work here**

4.3.3. Talent advancing the electrodes until the tips contact the glass base of the electrode chambers.

4.4. Attach the micro-grabbers to the electrodes [1]. Measure TEER for both apical channels at 500 hertz, 1 kilohertz, and 10 kilohertz. Record at least three measurements at each frequency [2]. If performing calibration, connect micro-grabbers to resistors instead [3].

4.4.1. Talent attaching the micro-grabbers to the electrodes.

4.4.2. Talent measuring TEER for the apical channels with the impedance analyzer.

- 4.4.3. Talent showing micro-grabbers attached to resistor.
- 4.5. Remove the electrodes carefully to minimize fluid displacement and prevent air bubbles from forming [1]. Disinfect the electrodes with 70 percent ethanol before reuse [2-TXT].
 - 4.5.1. Talent carefully removing the electrodes from the electrode-port tubing. **NOTE: Shots 4.5.1 & 4.5.2 were combined**
 - 4.5.2. Talent disinfecting the electrodes with 70 percent ethanol. **TXT: Similarly, measure TEER during cell culture**

5. First Cerebral Microvascular Endothelial Cell Seeding Under Inverted Conditions

- 5.1. Transfer the filtered cell suspension to a sterile microcentrifuge tube for immediate use [1] and store it in an incubator at 37 degrees Celsius and 5% carbon dioxide between chip seedings [2].
 - 5.1.1. Talent transferring the filtered cell suspension into a sterile microcentrifuge tube. **TXT: Cells used: hCMEC/D3**
 - 5.1.2. Talent placing the tube in the incubator.
- 5.2. Attach 15 centimeters of Tygon tubing to a 1-milliliter syringe and needle [1].
 - 5.2.1. Talent attaching 15 centimeters of Tygon tubing to a 1-milliliter syringe and needle.
- 5.3. Remove the endothelial cell suspension and the humidity box containing the coated microfluidic chip from the incubator [1]. Disinfect the external surfaces of the microcentrifuge tube and the humidity box with 70% ethanol before placing them in the biosafety cabinet [2].
 - 5.3.1. Talent removing the endothelial cell suspension and the humidity box containing the coated microfluidic chip from the incubator. **NOTE: Shots 5.3.1 & 5.3.2 were combined together.**
 - 5.3.2. Talent disinfecting the external surfaces of the microcentrifuge tube and humidity box with 70 percent ethanol.
- 5.4. Mix the endothelial cell suspension by gentle inversion or flicking immediately before aspiration [1].
 - 5.4.1. Talent gently inverting or flicking the microcentrifuge tube containing the endothelial cell suspension. **NOTE: Shot 5.4.1 Take 1 is the better take to use due to the focus.**
- 5.5. Aspirate the endothelial cell suspension into the tubing and syringe until approximately 100 microliters remain within the syringe barrel [1]. Return the remaining cell suspension to the incubator between chip seedings [2].

- 5.5.1. Talent aspirating the endothelial cell suspension into the tubing and syringe.
NOTE: Shot 5.5.1 Take 3 is the better shot to use. The others are unusable due to bad focus.
- 5.5.2. Talent placing the remaining cell suspension into the incubator.
- 5.6. Then, remove the clamp from the outlet tubing connected to one outer apical channel [1].
 - 5.6.1. Talent removing the clamp from the outlet tubing connected to the outer apical channel. **NOTE: Shots 5.6.1 & 5.7.1 were combined together**
- 5.7. Apply a drop of endothelial cell culture medium to the inlet port, then gently remove the existing tubing [1].
 - 5.7.1. Talent applying a drop of endothelial cell culture medium to the inlet port and removing the tubing
- 5.8. Gently depress the syringe plunger until a small meniscus forms at the tubing outlet [1].
 - 5.8.1. Talent gently depressing the syringe plunger until a small meniscus forms at the tubing outlet.
- 5.9. Insert the tubing into the inlet port connected to the apical channel [1]. Slowly depress the syringe plunger until approximately three drops of endothelial cell suspension emerge from the outlet tubing [2]. Reapply the clamp to the outlet tubing [3].
 - 5.9.1. Talent inserting the tubing into the inlet port connected to the apical channel. **NOTE: Shots 5.9.1, 5.9.2, 5.9.3 & 5.10.1 were combined together**
 - 5.9.2. Talent slowly depressing the syringe plunger until endothelial cell suspension droplets emerge from the outlet tubing.
 - 5.9.3. Talent reapplying the clamp to the outlet tubing.
- 5.10. Cut the inlet tubing to leave approximately 5 centimeters attached to the chip and apply a clamp [1].
 - 5.10.1. Talent cutting the inlet tubing to leave approximately 5 centimeters attached to the chip and applying the clamp.
- 5.11. Similarly, seed the second apical channel and use a microscope to confirm that endothelial cell coverage is at least 70 percent and evenly distributed [1].
 - 5.11.1. LAB MEDIA: (Figure 3A).
- 5.12. If needed, gently adjust the tubing with tweezers under the microscope until cells are evenly distributed [1]. **NOTE: The VO is edited for the deleted shot**
 - 5.12.1. Talent adjusts the attached tubing while observing the cells under the microscope.
 - ~~5.12.2. SCREEN: Shot of the evenly adjusted cells throughout the channel.~~ **NOTE: Not**

required, same as 3.11.1

~~Videographer: Please record the computer screen for this shot~~

- 5.13. Place the seeded chips in an inverted orientation inside the humidity box with the glass surface facing upward and the PDMS surface facing downward [1].
 - 5.13.1. Talent placing the seeded chips in an inverted orientation inside the humidity box. NOTE: Shots 5.13.1, 5.14.1 & 5.14.2 were combined together.
- 5.14. Secure the chip with masking tape attached to both ends of the glass slide and fixed to the inside of the humidity box lid [1]. Close the humidity box lid carefully, ensuring that all tubing remains inside the container [2].
 - 5.14.1. Talent securing the chip to the inside of the humidity-box lid using masking tape.
 - 5.14.2. Talent carefully closing the humidity-box lid with all tubing positioned inside the container.
- 5.15. Transfer the humidity box to an incubator at 37 degrees Celsius and 5 percent carbon dioxide for at least 4 hours [1].
 - 5.15.1. Talent placing the humidity box into the incubator.
- 5.16. After the second endothelial cell seeding, connect the chip to the syringe-pump system, seed the astrocytes, and perform immunofluorescence staining [1].
 - 5.16.1. Shot of the chip connected to the syringe pump medium replacement system.

Results

6. Results

6.1. The tight junction protein ZO-1 (*ZO-One*) was observed throughout the hCMEC/D3 (*H-C-M-E-C-D-Three*) endothelial cell layer within the apical channels [1], while the endothelial cell marker CD31 (*C-D-Thirty-One*) was also detected within the endothelial cell population [2].

6.1.1. LAB MEDIA: Figure 6A. *Video editor: Highlight the red staining labeled 'ZO-1' around the apical channel perimeter.*

6.1.2. LAB MEDIA: Figure 6A. *Video editor: Highlight the green staining labelled 'CD31' within the channel.*

6.2. Human primary astrocytes cultured within the basolateral chamber expressed GFAP (*G-F-A-P*), confirming astrocyte identity under the culture conditions used in this study [1].

6.2.1. LAB MEDIA: Figure 6A. *Video editor: Highlight the yellow staining labelled 'GFAP' within the central chamber.*

6.3. Immunofluorescent images revealed astrocytic processes extending through micropillars separating the apical and basolateral compartments, suggesting potential physical interactions alongside soluble factor exchange [1].

6.3.1. LAB MEDIA: Figure 6A. *Video editor: Show the insert image and highlight the yellow thread-like things passing extending through the small micropillar gaps between the two separate channels .*

6.4. A cross-sectional z-stack image of the apical channel showed the endothelial cell layer developing a lumen-like morphology around the channel's perimeter [1].

6.4.1. LAB MEDIA: Figure 6B.

1. Tygon

Pronunciation link: No confirmed link found

IPA: /'tɑɪɡɑ:n/

Phonetic Spelling: TY·gon

2. Microfluidic

Pronunciation link: <https://www.merriam-webster.com/dictionary/microfluidic>

IPA: /,maɪkroʊflu'ɪdɪk/

Phonetic Spelling: my·kroh·floo·ID·ik

3. Electrode

Pronunciation link: <https://www.merriam-webster.com/dictionary/electrode>

- IPA: /ɪˈlektroʊd/
Phonetic Spelling: ih·LEK·trohd
4. Perfusion
Pronunciation link: <https://www.merriam-webster.com/dictionary/perfusion>
IPA: /pərˈfjuːʒən/
Phonetic Spelling: per·FYOO·zhuhn
 5. Apical
Pronunciation link: <https://www.merriam-webster.com/dictionary/apical>
IPA: /ˈeɪpɪkəl/
Phonetic Spelling: AY·pih·kuhl
 6. Basolateral
Pronunciation link: No confirmed link found
IPA: /ˌbeɪzəʊˈlætərəl/
Phonetic Spelling: bay·zoh·LAT·er·uhl
 7. Pneumatic
Pronunciation link: <https://www.merriam-webster.com/dictionary/pneumatic>
IPA: /nuːˈmætɪk/
Phonetic Spelling: noo·MAT·ik
 8. Multiport
Pronunciation link: No confirmed link found
IPA: /ˈmʌltiˌpɔːrt/
Phonetic Spelling: MUL·tee·port
 9. Manifold
Pronunciation link: <https://www.merriam-webster.com/dictionary/manifold>
IPA: /ˈmænəˌfoʊld/
Phonetic Spelling: MAN·uh·fohld
 10. Luer-lock
Pronunciation link: No confirmed link found
IPA: /ˈluːər lɑːk/
Phonetic Spelling: LOO·er·lock
 11. Transendothelial
Pronunciation link: No confirmed link found
IPA: /ˌtrænzˌendəʊˈθiːliəl/
Phonetic Spelling: tranz·en·doh·THEE·lee·uhl
 12. Laminin
Pronunciation link: <https://www.merriam-webster.com/dictionary/laminin>
IPA: /ˈlæmɪnɪn/
Phonetic Spelling: LAM·ih·nin
 13. Matrigel
Pronunciation link: No confirmed link found
IPA: /ˈmertriˌdʒel/
Phonetic Spelling: MAY·trih·jel
 14. Fibronectin
Pronunciation link: <https://www.merriam-webster.com/dictionary/fibronectin>

- IPA: /ˌfaɪbroʊˈnektɪn/
Phonetic Spelling: fy·broh·NEK·tin
15. Biosafety
Pronunciation link: No confirmed link found
IPA: /ˌbaɪoʊˈseɪfti/
Phonetic Spelling: by·oh·SAYF·tee
16. Kilohertz
Pronunciation link: <https://www.merriam-webster.com/dictionary/kilohertz>
IPA: /ˈkɪləˌhɜːts/
Phonetic Spelling: KIL·uh·hurts
17. Impedance
Pronunciation link: <https://www.merriam-webster.com/dictionary/impedance>
IPA: /ɪmˈpiːdəns/
Phonetic Spelling: im·PEE·duhns
18. Cerebral
Pronunciation link: <https://www.merriam-webster.com/dictionary/cerebral>
IPA: /səˈriːbrəl/, /ˈserəbrəl/
Phonetic Spelling: suh·REE·bruhl / SER·uh·bruhl
19. Microvascular
Pronunciation link: No confirmed link found
IPA: /ˌmaɪkroʊˈvæskjələr/
Phonetic Spelling: my·kroh·VAS·kyuh·ler
20. Endothelial
Pronunciation link: <https://www.merriam-webster.com/dictionary/endothelial>
IPA: /ˌɛndoʊˈθiːliəl/
Phonetic Spelling: en·doh·THEE·lee·uhl
21. Microcentrifuge
Pronunciation link: No confirmed link found
IPA: /ˌmaɪkroʊˈsentriˌfjuːdʒ/
Phonetic Spelling: my·kroh·SEN·trih·fyooj
22. Meniscus
Pronunciation link: <https://www.merriam-webster.com/dictionary/meniscus>
IPA: /məˈnɪskəs/
Phonetic Spelling: muh·NIS·kuhs
23. PDMS
Pronunciation link: No confirmed link found
IPA: /ˌpiː diː ɛm ˈɛs/
Phonetic Spelling: P·D·M·S
24. Astrocyte
Pronunciation link: <https://www.merriam-webster.com/dictionary/astrocyte>
IPA: /ˈæstrəˌsaɪt/
Phonetic Spelling: AS·truh·site
25. Immunofluorescence
Pronunciation link: No confirmed link found

- IPA: /ˌɪmjənoʊfləʊˈresəns/
Phonetic Spelling: im·yuh·noh·floor·ESS·uhns
26. Astrocytic
Pronunciation link: No confirmed link found
IPA: /ˌæstrəˈsɪtɪk/
Phonetic Spelling: as·truh·SIT·ik
27. Micropillar
Pronunciation link: No confirmed link found
IPA: /ˈmaɪkroʊˌpɪlər/
Phonetic Spelling: MY·kroh·pil·er
28. Cross-sectional
Pronunciation link: <https://www.merriam-webster.com/dictionary/cross-sectional>
IPA: /ˈkrɒs ˈsɛkʃənəl/
Phonetic Spelling: KRAWS·SEK·shuh·nuhl
29. Z-stack
Pronunciation link: No confirmed link found
IPA: /ˈziː stæk/
Phonetic Spelling: ZEE·stack
30. Lumen
Pronunciation link: <https://www.merriam-webster.com/dictionary/lumen>
IPA: /ˈluːmən/
Phonetic Spelling: LOO·muhn