Submission ID #: 68482
Scriptwriter Name: Poornima G
Project Page Link: https://review.jove.com/account/file-uploader?src=20887058

Title: An Ex Vivo Explant Model for Studying Glial Interactions in the Mouse Retina

Authors and Affiliations:
Paul F Cullen, Yixi Xue, Milica A. Margeta

Department of Ophthalmology, Schepens Eye Research Institute of Massachusetts Eye and Ear, Harvard Medical School

Corresponding Authors:
Milica A. Margeta milica_margeta@meei.harvard.edu

Email Addresses for All Authors:
Paul F Cullen pfcullen@meei.harvard.edu
Yixi Xue yxue5@meei.harvard.edu
Milica A. Margeta milica_margeta@meei.harvard.edu

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? **Yes, DONE**

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? **No**

Current Protocol Length
Number of Steps: 20
Number of Shots: 51 (26 Scope)

Introduction

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

1.1. **Milica Margeta:** We want to understand neuroinflammation in glaucoma, the leading cause of irreversible blindness worldwide. Specifically, we're studying how glial support cells in the retina influence neuronal loss during disease progression.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What are the current experimental challenges?

1.2. **Paul Cullen:** Glia – such as astrocytes and microglia – are thought to influence glaucoma progression, but many tools used to study neuronal function in live animal models are ill-suited for these cells.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.5.1*

What research gap are you addressing with your protocol?

1.3. **Paul Cullen:** Retinal explants are used to study neuroinflammation and glial function, but the learning curve is steep. Our protocol makes it more approachable and hopefully enables wider adoption of the technique.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.1.1*

What advantage does your protocol offer compared to other techniques?

1.4. **Yixi Xue:** Compared to traditional in vitro cell culture, our explant approach better preserves the natural environment for cells in the inner retina, enabling more accurate investigation into their physiological functions.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.3.4*

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

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the Schepens Eye Research Institute

NOTE: Videographer may also have shot wide angle scenes for many scope shots, but don't use them. Use the scope shots provided on the project page whenever a shot is labeled SCOPE.

2. Retinal Isolation from Mouse Eye and Mounting

Demonstrator: Paul Cullen

2.1. To begin, place the extracted mouse eye in a dissection dish filled with sterile room temperature PBS **[1].** Identify an appropriate holding point and grasp it with angled forceps **[2]**, then gently position the eye on the submerged lab wipe while ensuring the anterior-posterior axis, from the cornea to the optic nerve, is positioned horizontally **[3]**.

2.1.1. WIDE: Talent placing the mouse eye in the dissection dish.

2.1.2. Talent using angled forceps to grasp the eye at a holding point. **NOTE**: Shot 2.1.2 and 2.1.3 were filmed together

2.1.3. Talent rotating the eye and holding it in the correct horizontal orientation.

2.2. While maintaining a firm hold with angled forceps, use the tip of a number 11 scalpel to make an incision parallel and approximately 0.5-millimeters posterior to the limbus, where the cornea transitions to the sclera **[1]**. Insert one blade of the spring scissors inside the globe **[2]** and cut circumlimbally around the eye, repositioning as needed with forceps **[3]**.

~~2.2.1. Talent submerging the eye in buffer while holding it with angled forceps.~~ **NOTE**: not filmed, VO merged with the next shot

2.2.2. SCOPE: PFC 221 to 222 – 0:20-00:34. SCOPE: PFC 223.

2.2.3. SCOPE: PFC 224. 00:28-00:32 and 02:05-02:10

2.3. After completing the circumlimbal cut, remove the anterior segment and lens with forceps **[1]**. If a long piece of optic nerve remains, trim it to a length of 1 to 2 millimeters using fine scissors **[2]**. Then rotate the eyecup so that it faces upward to enable visual inspection and facilitate vitreous removal **[3]**.

2.3.1. SCOPE: PFC 231 to 233, 00:05-00:10 and 00:20-00:25.

2.3.2. SCOPE: PFC 231 to 233. 00:50-00:55.

2.3.3. SCOPE: PFC 231 to 233. 01:00-01:08.

2.4. Continue using angled forceps to immobilize the eyecup and inspect the retina for visible damage and examine the vitreous chamber for pigmented cell debris from the retinal pigment epithelium or choroid **[1]**. ~~Use angled forceps to immobilize the eyecup in position **[2]**~~. Using a modified transfer pipette, flush the vitreous chamber with PBS, keeping the pipette tip submerged to prevent air bubbles **[3]**.

2.4.1. SCOPE: PFC 241 to 243. 00:00-00:06

~~2.4.2. Talent stabilizing the eyecup using angled forceps.~~ **NOTE**: Delete this shot

2.4.3. SCOPE: PFC 241 to 243. 00:50-00:59.

2.5. Then, with a fine watercolor brush, gently remove larger debris while minimizing contact with the retina **[1]**. For persistent debris, use fine-tipped forceps carefully, avoiding direct metal contact with the retina **[2]**.

2.5.1. SCOPE: PFC 251 00:00-00:20.

2.5.2. SCOPE: PFC 252. 00:05-00:25

2.6. After clearing visible debris, flush the vitreous chamber with PBS from the transfer pipette 3 to 5 times **[1]** and use the fine brush to probe near the periphery for residual ciliary body elements, detecting vitreous by the drag on brush fibers **[2]**. If pockets of vitreous remain, sweep outward toward the periphery with the brush, keeping fibers trailing at a shallow angle to prevent retinal damage **[3-TXT]**.

2.6.1. SCOPE: PFC 261 to 263, 00:14 to 00:34.

2.6.2. SCOPE: PFC 261 to 263. 0:48-00:58

2.6.3. SCOPE: PFC 261 to 263. 00:59-01:15 **TXT: Separate the outer retina; Preserve the optic nerve head**

~~2.7. Once only minimal vitreous remains, stabilize the sample using angled forceps and gently begin to separate the outer retina from the eyecup **[1]**. Carefully preserve the optic nerve head to serve as an anchor point for the retina **[2]**.~~ **NOTE**: Delete this step; VO moved as on screen text

~~2.7.1. Talent steadying the eyecup and peeling the retina outward.~~

2.7.2. ~~Close-up of optic nerve head being left intact~~.

2.8. Hold a pair of forceps in a closed position with the tips touching and gently insert them between the retina and choroid using any natural gaps formed during handling **[1]**. Use the flat arms of the forceps to gradually enlarge the space between the retina and choroid until full separation is achieved **[2]**.

2.8.1. SCOPE: PFC 282 to 292. 00:00-00:10

2.8.2. SCOPE: PFC 282 to 292. 00:11-00:30.

2.9. Continue stabilizing the sample with one pair of forceps while using a second pair to gently pull the eyecup, including the sclera and the choroid, downward **[1]**. If the retina descends with the eyecup, use the forceps to gently probe and detach any remaining points of connection, avoiding the optic nerve head **[2]**.

2.9.1. Talent holding the retina stable with one pair of forceps and pulling the eyecup downward with the second pair.

2.9.2. SCOPE: PFC 282 to 292. 00:32-00:50.

2.10. Then, with the retina still anchored at the optic nerve head, continue holding the tissue steady and use the second pair of forceps to bunch up the eyecup below the optic nerve head **[1]**. Inspect to confirm that the retinal periphery is not folded due to residual vitreous, particularly at sites where ciliary body remains. If needed, flush the chamber with PBS using a transfer pipette **[2]** and use the brush to gently uncurl any folded retina and remove excess vitreous **[3]**.

2.10.1. Talent gathering the eyecup below the optic nerve head using forceps.

2.10.2. SCOPE: PFC 2102 to 2103 .

2.10.3. SCOPE: PFC 2104 00:20-00:32.

2.11. After the retina is exposed from both sides, use spring scissors to make a series of relieving cuts approximately 90 degrees apart, from the retinal periphery toward the optic nerve head **[1-TXT]**. While holding the submerged tissue, use forceps to

laterally pull the lab wipe away from the sample and remove it from the dish without touching the retina **[2]**.

2.11.1. SCOPE: PFC 2111 00:10-00:18 . **TXT: Stop cutting about 1 mm from the center**

2.11.2. Talent removing the lab wipe laterally using forceps, avoiding contact with the retina.

2.12. While holding the retina in place, use the spring scissors to sever the optic nerve just beneath the retina **[1]**. Then, carefully lift and remove the remaining eyecup tissue from the dish **[2]**.

2.12.1. SCOPE: PFC 2111 01:40-01:53.

2.12.2. Talent removing the eyecup tissue from the dish.

3. Mounting the Isolated Retina on a Filter Square

3.1. Now, fill a 35-millimeter Petri dish with PBS **[1]** and use forceps to place a filter square at the bottom with the rough, matte side facing upward, avoiding any creases **[2]**.

3.1.1. Talent filling a 35 millimeter Petri dish with room temperature PBS. **NOTE**: 3.1.1 AND 3.1.2 were shot together

3.1.2. Talent placing an uncreased filter square at the bottom of the dish with the matte side up.

3.2. Then, using the transfer pipette, gently aspirate the retina and transfer it into the Petri dish **[1]**. Use the brush to orient the retina with the inner surface facing upward and position it directly above the filter square **[2]**. Slowly aspirate PBS to lower the retina onto the filter **[3-TXT]**.

3.2.1. SCOPE: PFC 321 to 323 00:00 to 00:08 .

3.2.2. SCOPE: PFC 321 to 323 01:00 to 01:10 .

3.2.3. SCOPE: PFC 321 to 323 01:28 to 01:32 and 01:36-01:40 **TXT: Pause and**

reposition the tissue when necessary

3.3. Once the retina is seated on the filter, use the brush to gently unroll any peripheral folds **[1]**. Adjust the PBS level to balance retinal stability and hydration, allowing smooth brush movement without drying the tissue **[2]**. Now, use the transfer pipette to drip PBS from about 1 centimeter above to rinse the surface and inspect the retina again for debris **[3-TXT]**.

3.3.1. SCOPE: PFC 321 to 323 01:42 to 01:47.

3.3.2. SCOPE: PFC 321 to 323 01:55 to 02:05..

3.3.3. SCOPE: PFC 321 to 323 02:15 to 02:20.. **TXT: Do not let the retina float in PBS**

4. Transferring Retina to the Culture Media

4.1. Close the lid of the 35-millimeter dish and carry it to the biosafety cabinet **[1]**.

4.1.1. Talent closing the lid on the 35 millimeter dish.

4.1.2. ~~Talent takin the dish towards the biosafety cabinet~~. **NOTE**: Not filmed, VO merged

4.2. Place the closed dish inside the biosafety cabinet without contacting any interior surfaces or equipment **[1]**.

4.2.1. Talent carefully placing the sealed dish into the biosafety cabinet without contact with other surfaces.

4.3. Sterilize or replace gloves when transitioning to aseptic work **[1]** and transfer the 6-well plate preloaded with explant media from the incubator into the biosafety cabinet **[2]**. Within the cabinet, remove the lid of the 35-millimeter dish and use angled forceps to lift the filter square without touching the retina **[3]**. Then, open the 6-well plate and gently lower the filter onto the center of the insert in one well, immersing it slowly into the media **[4]**.

4.3.1. Talent spraying and wiping gloves with ethanol or switching to new gloves.

4.3.2. Talent carrying the 6-well plate from the incubator to the biosafety cabinet.

4.3.3. Talent removing the lid of the 35 millimeter dish and lifting the filter square using angled forceps.

4.3.4. Talent positioning and lowering the filter into the well insert containing media.

4.4. Once the retina separates from the filter, slowly move the filter aside and remove it from the well using angled forceps **[1]**. Use a 1-milliliter pipette to aspirate 500 microliters of media from the insert, trapping the retina between the insert and the air-liquid interface **[2]**.

4.4.1. Talent gently withdrawing the filter from the media using forceps.

4.4.2. Talent removing 500 microliters of media from the well insert using a 1 milliliter pipette.

4.5. Finally, replace the lid on the 6-well plate **[1]** and return it to the incubator, ensuring the retina remains centered within the well **[2-TXT]**.

4.5.1. Talent placing the lid on the 6-well plate.

4.5.2. Talent placing the plate in the incubator. **TXT: After incubation, perform immunostaining to assess the retina**

Results

5. **Results**

5.1. To examine large-scale changes in retinal glia, 3-day explanted retinas were compared with sham explants fixed immediately after isolation instead of being cultured **[1]**. Microglia in sham retinas showed a regular, non-overlapping distribution pattern **[2]**, whereas by day-3, the explanted retina's organization became irregular with cells appearing clustered, implying migration **[3]**.

5.1.1. LAB MEDIA: Figure 2

5.1.2. LAB MEDIA: Figure 2. *Video editor: Highlight image A*.

5.1.3. LAB MEDIA: Figure 2. *Video editor: Highlight image D*.

5.2. Retinal astrocytes in sham retinas displayed close alignment with the vasculature **[1]**, which diminished significantly after 3 days in vitro **[2]**.

5.2.1. LAB MEDIA: Figure 2. *Video editor: Highlight B*.

5.2.2. LAB MEDIA: Figure 2. *Video editor: Highlight E*.

5.3. GFAP expression in Müller cells was faint or absent in sham retinas **[1]**, but became distinctly visible by day 3 in vitro, especially near tissue edges **[2]**.

5.3.1. LAB MEDIA: Figure 2B. *Video editor: Highlight C*.

5.3.2. LAB MEDIA: Figure 2E. *Video editor: Highlight F*.

5.4. After 1 day in vitro, microglia exhibited process retraction and early signs of activation **[1]**, which progressed to a compact, amoeboid morphology by day 3 **[2]**. At the 24 hour mark, retinal ganglion cell density quantified using Brn3a *(B-R-N-3-A)* showed a modest but marked decline in culture explants over sham ones **[3]**.

5.4.1. LAB MEDIA: Figure 3. *Video editor: Highlight F*.

5.4.2. LAB MEDIA: Figure 3. *Video editor: Highlight J*.

5.4.3. LAB MEDIA: Figure 3 Show only A E and M. *Video editor: Highlight the bar for "1DIV" in M.*

5.5. TMEM119 *(T-mem-1-nineteen)*, a homeostatic microglial marker, was highly expressed in sham retinas **[1]** but was nearly undetectable after 3 days in vitro **[2]**.

5.5.1. LAB MEDIA: Figure 4. *Video editor: Highlight C*.

5.5.2. LAB MEDIA: Figure 4. *Video editor: Highlight G*.

5.6. CD206 expression, marking hyalocytes, remained stable after 3 days of in vitro culturing **[1]**.

5.6.1. LAB MEDIA: Figure 4B and F. *Video editor: Highlight F*.

5.7. GFAP staining revealed astrocyte and Müller cell reactivity around sites of mechanical injury **[1]** sustained during dissection and handling **[2]**.

5.7.1. LAB MEDIA: Figure 4J. *Video editor: Highlight the area pointed by the white arrowheads.*

5.7.2. LAB MEDIA: Figure 4K. *Video editor: Highlight the area pointed by the white arrowheads*.

1. Limbus

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/limbus
- **IPA**: /ˈlɪm.bəs/
- **Phonetic Spelling**: LIM-buhsmerriam-webster.com+3merriam-webster.com+3merriam-webster.com+3merriam-webster.com+4merriam-webster.com+4merriam-webster.com+4

2. Sclera

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/sclera
- **IPA**: /ˈsklɪə.rə/
- **Phonetic Spelling**: SKLEER-uhmerriam-webster.com+3merriam-webster.com+3merriam-webster.com+3

3. Vitreous

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/vitreous
- **IPA**: /ˈvɪ.tri.əs/
- **Phonetic Spelling**: VIT-ree-uhsmerriam-webster.com+8merriam-webster.com+8merriam-webster.com+8

4. Choroid

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/choroid

- **IPA**: /ˈkɔːr.ɔɪd/
- **Phonetic Spelling**: KOR-oydmerriam-webster.com+2merriam-webster.com+2merriam-webster.com+2

5. Astrocytes

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/astrocyte
- **IPA**: /ˈæs.trəˌsaɪts/
- **Phonetic Spelling**: ASS-troh-sytsmerriam-webster.com+3merriam-webster.com+3merriam-webster.com+3merriam-webster.com

6. Müller Cells

- **Pronunciation link**: https://www.merriam-webster.com/medical/M%C3%BCller%20cell
- **IPA**: /ˈmjuː.lər sɛlz/
- **Phonetic Spelling**: MYOO-lur selzmerriam-webster.com+2merriam-webster.com+2merriam-webster.com+2