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Title: Long-Term, Serum-Free Cultivation of Organotypic Mouse Retina Explants with Intact Retinal Pigment Epithelium

Authors and Affiliations:

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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? **Yes**

   If **Yes**, can you record movies/images using your own microscope camera?

   Yes, but send scope kit just in case

   If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit (through a camera port or one of the oculars). Please list the make and model of your microscope.

   Stemi 305, Zeiss

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

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 13
Number of Shots: 21

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Soumaya Belhadj:** We describe a technique for the isolation of the mouse neuroretina together with its retinal pigment epithelium. The retina can be cultured for at least two weeks with normal development, as it would *in vivo*, retaining the typical tissue architecture and morphology.

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

1.2. **Arianna Tolone:** Organotypic retinal explant cultures make it possible to investigate and manipulate the retina *in vitro*, under entirely controlled conditions, avoiding the use of serum and antibiotics.

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

1.3. **Francois Paquet-Durand:** Organotypic retinal explant cultures can be employed to model a variety of retinal diseases, including retinitis pigmentosa and diabetic retinopathy. Explant cultures are also useful for pre-clinical development of novel treatments for retinal diseases.

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

Introduction of Demonstrator on Camera

1.4. **Francois Paquet-Durand:** Demonstrating the procedure will be **Soumaya Belhadj and Arianna Tolone**, two PhD students from my laboratory.

1.4.1. INTERVIEW: Author saying the above.

1.4.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Ethics Title Card

1.5. Procedures involving animal subjects have been approved by the Tübingen University committee on animal protection.

Protocol

2. Explant Preparation

2.1. Begin by incubating the enucleated eyes in BM for 5 minutes at room temperature **[1-TXT]**. Then, transfer the eyes into preheated BM with 0.2% proteinase K and incubate them at 37 degrees Celsius for 15 minutes **[2]**.

2.1.1. Talent incubating the eye in BM. **TEXT: BM-Basal R16 medium**

2.1.2. Talent incubating the eye in BM with proteinase K.

2.2. To inactivate proteinase K, transfer the eyes to BM containing 20% fetal calf serum and incubate for 5 minutes at room temperature on a cool pack **[1-TXT]**.

2.2.1. Talent incubating the eye in BM containing FCS. **TEXT: Work in a laminar air flow hood**

2.3. Transfer the eyes to a Petri dish containing fresh BM **[1]** and aseptically dissect the eyes under the stereoscope as soon as possible **[2]**.

2.3.1. Talent transferring the eyes to a Petri dish with fresh BM.

2.3.2. Talent placing the Petri dish under the stereoscope.

2.4. Using forceps, hold the eye from the optic nerve and make a small incision in the cornea with fine scissors, creating two edges. Peel the cornea, choroid, and the sclera gently from these edges using 2 pairs of fine forceps or by inserting one of the scissor blades into the edge opening **[1]**. *Videographer: This step is difficult and important!*

2.4.1. SCOPE: retina explant video1.mp4. 0:15 – 0:25, then 1:45 – 2:00.

2.5. Grasp the lens with fine forceps and pull to extract it from the eye cup using a second pair of forceps placed perpendicularly to the first **[1]**. Remove the vitreous and the ciliary body attached to the retina **[1]**. *Videographer: This step is important!*

2.5.1. SCOPE: retina explant video1.mp4. 2:00 - 2:25.

2.6. Cut the retina perpendicular to its edges at four points, creating a four-leaf clover shape **[1]**. *Videographer: This step is important!*

2.6.1. SCOPE: retina explant video1.mp4. 2:00 - 2:45. Talent cutting the retina.

2.7. Hold the retina in a hanging drop of medium using a 1-milliliter pipette tip with a broadly cut base **[1]**. Transfer it to a culture dish insert placed in a 6-well culture plate with the RPE layer facing the membrane **[2-TXT]**. *Videographer: This step is difficult and important!*

2.7.1. SCOPE: retina explant video1.mp4. 2:45 - 2:53. Talent holding the retina with the tip.

2.7.2. Talent placing the retina in a culture dish. **TEXT: RPE-Retinal pigment epithelium**

2.8. Remove the excess medium from the insert **[1]** and add 1 milliliter of CM per well from the sides **[2-TXT]**. Incubate the dish in a sterile incubator at 37 degrees Celsius with 5% carbon dioxide **[3]**. *Videographer: This step is important!*

2.8.1. Talent removing excess medium from the insert.

2.8.2. Talent adding CM to the well. **TEXT: CM-Complete R16 medium with supplements**

2.8.3. Talent placing the dish in the incubator.

2.9. Avoid submerging the retina in the medium as it will reduce oxygenation and cause tissue degeneration. Keep it at the air-liquid interface **[1]**. Change the medium every second day by discarding 700 microliters of medium and replacing it with 900 microliters of fresh medium **[2]**.

2.9.1. Retina at the air-liquid interface of liquid and air.

2.9.2. Talent changing the medium every second day.

2.10. After culturing the explants, fix them with 4% paraformaldehyde for 45 minutes **[1]**. Then, perform gradual sucrose cryoprotection **[2-TXT]**.

2.10.1. Talent adding paraformaldehyde to the well.

2.10.2. Talent adding sucrose to a well. **TEXT: 10% sucrose for 10 min, 20% for 20 min and 30% for 2 h at RT or ON at 4 °C**

2.11. When finished, cut the membrane around the retinal explants **[1]**. Embed both the membrane and retinal tissue in the medium for frozen tissue **[2]** and freeze using liquid nitrogen **[3]**. *Videographer: This step is important!*

2.11.1. SCOPE: retina explant video1.mp4. 3:03 – end. Talent cutting the membrane around the explant.

2.11.2. Talent embedding the specimen in the medium.

2.11.3. Added shot: Talent freezing the block containing the tissue embedded in medium with liquid nitrogen

Results

3. Analysis of cell types found in retinal explant

3.1. Dissected and cultured retinal explants preserve their normal tissue architecture, with distinct layers shown in *rd1* mutant and wild type animals by nuclear staining with DAPI in blue, rod photoreceptors in red, and Müller cells in green **[1]**.

3.1.1. LAB MEDIA: Figure 2.

3.2. Retina and RPE derived from *rd1* or wild type animals were fixed with 4% PFA at post-natal day 11 and prepared for cryosectioning **[1]**.

3.2.1. LAB MEDIA: Figure 3A.

3.3. To assess cell death of histological sections from treated, non-treated, and wild type specimens, the analysis of terminal deoxynucleotidyl transferase dUTP nick end labeling assay was performed **[1]**.

3.3.1. LAB MEDIA: Figure 3B.

3.4. Simulation of type-2 diabetes mellitus on wild type retina using 2-deoxy-glucose treatment lead to extensive increase in neuronal cell death of the retina **[1]**.

3.4.1. LAB MEDIA: Figure 3C. *Video editor focus on the T2DM bar graph.*

Conclusion

4. Conclusion Interview Statements

4.1. **Soumaya Belhadj:** The retina is highly sensitive to touch, so any direct contact between instruments and retina should be avoided. Moreover, it is important to work under aseptic conditions to avoid contamination.

4.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.4*

4.2. **Arianna Tolone:** The preservation of retinal tissue architecture and neuronal circuitries enables a variety of investigations, including functional analysis using multi-electrode-array recording. The tissue can be processed further for histological, metabolomic, transcriptomic, or proteomic analysis.

4.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

4.3. **Francois Paquet-Durand:** Complete control over experimental conditions makes it possible to simulate and study retinal diseases in unprecedented ways. Diseases that can be difficult to model, such as hereditary retinal degeneration or diabetic retinopathy, can be investigated using this method.

4.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.