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Title: Characterization of a Novel Human Organotypic Retinal Culture Technique

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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. **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. **This will depend on the situation regarding Covid-19 in New Zealand and precautions advised by the government. Currently New Zealand is in level one Covid-19 alert thus no personal protective equipment is required but physical distancing is still recommended.**

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

Current Protocol Length

Number of Steps: 10
Number of Shots: 19

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Odunayo Mugisho:** This technique preserves all retinal layers and cell types in situ, making it clinically more relevant compared to animal and in vitro models.

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

1.2. **Odunayo Mugisho:** The main advantage of this protocol is that it minimizes retinal integrity disruption during tissue handling, which is essential when comparing healthy and diseased retina.

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

OPTIONAL:

1.3. **Odunayo Mugisho:** Retinal diseases can be mimicked by culturing the tissue under specific conditions. This also allows for testing of new drug therapies.

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

1.4. **Odunayo Mugisho:** The dissection and sample collection procedures are complex, therefore visual demonstration is critical to help the readers understand the written protocol and conduct the procedures successfully.

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

Introduction of Demonstrator on Camera

1.5. **Odunayo Mugisho:** Demonstrating the procedure will be Charisse Kuo, a second year PhD student from my laboratory.

1.5.1. INTERVIEW: Author saying the above.

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

Ethics Title Card

1.6. Human donor eye cups were obtained from the New Zealand National Eye Bank following corneal excision for transplantation and as approved by the Northern B Health and Disability Ethics Committee (NTX/06/19/CPD/AM07).

Protocol

2. Media Preparation and Extraction of the Sandwich Retinal Explants from the Eyecup

2.1. Prepare the culture medium containing Dulbecco's Modified Eagle Medium nutrient mixture F-12 *(F-twelve)* and a 1x antibiotics and antimycotics mixture **[1]**.

2.1.1. ~~Talent preparing the culture medium~~. NOTE: This shot was changed to Talent taking the culture medium out of the fridge.

2.2. Prior to explant extraction, place 500 microliters of medium in a 24-well plate **[1]** and equilibrate it in a humidified incubator at 37 degrees Celsius and 5% carbon dioxide to avoid dislodging of the retina with the subsequent addition of medium **[2]**. *Videographer: This step is important!*

2.2.1. Talent adding medium to 24-well plate.

2.2.2. Talent placing the plate into the incubator.

2.3. For extracting retinal explants, begin by placing the eye cup on a Petri dish, with the iris and lens facing upwards and the ONH contacting the Petri dish **[1]**.

2.3.1. Talent placing the eye cup on petri dish. **TEXT: ONH - optic nerve head**

2.4. Hold the eye cup steady at the limbus using forceps and detach the iris and lens by making small cuts circumferentially along the outer edge of the limbus **[1]**. Remove the iris and lens carefully, making sure to avoid disturbing the retina **[2]**. *Videographer: This step is important!*

2.4.1. Talent cutting along the margin of the iris (white band).

2.4.2. Talent taking the lens out of the eye globe.

NOTE: For 2.4.1 and 2.4.2, please use cross fade for the entire procedure to flow well.

2.5. Identify the ONH using a bright white light source and incise at the four quadrants towards the ONH, rotating the Petri dish for easier handling **[1]**. Spread and flatten the eye cup carefully **[2]**. Apply the forceps to the sclera instead of the retina to avoid disrupting the retinal integrity **[3]**. *Videographer: This step is important!*

2.5.1. Talent incising towards the ONH.

2.5.2. Talent flattening the eye cup. NOTE: This step occurs right after 2.5.1.

2.5.3. ~~Talent applying forceps on the sclera.~~ NOTE: This is not a separate shot and occurs in 2.5.1 and 2.5.2. Thus, step 2.5 includes only 2.5.1 and 2.5.2

2.6. Remove the plate containing the prepared media from the incubator **[1]**.

2.6.1. Talent removing the plate from incubator.

2.7. Place a surgical trephine on the retina in a region without retinal folds **[1]** and press hard to penetrate the sclera, which should generate a cracking sound as you break through the Petri dish **[2]**. *Videographer: This step is difficult and important!*

2.7.1. Talent placing the trephine on retina.

NOTE: 2.7.1, 2.7.2, 2.8.1 occur one after another.

2.7.2. Talent pressing the retina to penetrate sclera.

2.8. Rotate the trephine by 180-degrees to ensure the sclera has been penetrated fully such that the retinal explant is now separated from the rest of the sample **[1]**. Apply the forceps at the sclera **[2]** and transfer the sandwich retinal explant to the culture medium **[3-TXT]**. *Videographer: This step is important!*

2.8.1. Talent twisting the trephine to penetrate the sclera.

2.8.2. Talent applying forceps at the sclera.

2.8.3. Talent transferring the sandwich retinal explant to the culture medium. **TEXT: Obtain 2-3 explants from the peripheral retina of each quadrant.**

NOTE: Use last take as it shows the tissue in the correct orientation when being placed into the media.

2.9. Culture the collected sandwich retinal explants at 27 degrees Celsius for up to 72 hours in a humidified 5% carbon dioxide incubator **[1]**.

2.9.1. Place plate in the incubator.

~~**3. Culture sandwich retinal explants under diabetic retinopathy environment**~~

~~3.1. To induce diabetic retinopathy-like changes, culture retinal explants in medium containing a combination of 32.5 millimolar high glucose with pro-inflammatory cytokines, 10 nanograms per milliliter tumor necrosis factor-alpha, and interleukin-1 beta **[1]**.~~

~~3.1.1. Talent adding the reagents to the medium.~~

~~3.2. Analyze the cell supernatant for interleukin-18, interleukin-6, interleukin-8 and vascular endothelial growth factors after 24 and 72 hours using a magnetic assay, following instructions from the supplier **[1]**.~~

~~3.2.1. Talent performing magnetic assay.~~

Results

4. Observation of Cellular Integrity of Retinal Explants Using H&E and IHC and Analysis of Cytokine Release Using Magnetic Assay

4.1. Hematoxylin and Eosin staining showed that the sandwich retinal explants preserved integrity and a distinct lamellae structure from the GCL to the ONL with compact nuclei in the inner and the outer nuclear layers **[1-TXT]**.

4.1.1. LAB MEDIA: Figure 4A. *Video editor focus on the compact purple cells in INL and ONL.* **TEXT: GCL-ganglion cell layer, ONL-outer nuclear layer, INL-inner nuclear layer.**

4.2. Disrupted retinal integrity was found at the edges of the same sample, where the retina had detached from the underlying RPE-choroid and sclera, showing reduced retinal thickness and loss of nuclei in the INL and ONL **[1]**.

4.2.1. LAB MEDIA: Figure 4B. *Video editor focus on the distorted purple cells in INL and ONL.*

4.3. No TUNEL-positive cell nuclei, which mark cellular apoptosis, were found in retinal explants cultured in basal conditions, demonstrating sustained cellular vitality even after 72 hours **[1-TXT]**.

4.3.1. LAB MEDIA: Figure 5A-5C. *Video editor focus on the fewer green cells stained in Figure 5A, 5B, and 5C.* **TEXT: TUNEL-transferase dUTP nick end labeling**

4.4. GFAP labelling was restricted to the GCL and the IPL without glial fibrosis, a sign of pathology which would be identified as extended expression of GFAP into the ONL **[1-TXT]**.

4.4.1. LAB MEDIA: Figure 5D-5F. *Video editor focus on the red cells restricted in GCL and IPL.* **TEXT: GFAP-glial fibrillary acidic protein, IPL-inner plexiform layer.**

4.5. Vimentin labelling was highly expressed from the ganglion cell layer to the outer nuclear layer, showing preserved Müller cell integrity, as seen in normal retinal tissues **[1]**.

4.5.1. LAB MEDIA: Figure 5G-5I. *Video editor focus on the red stained region.*

4.6. Luminex magnetic assay showed that the IL-18, VEGF *(pronounce 'veg-F')*, IL-6, and IL-8 levels increased significantly above baseline in the sandwich retinal explant cultures in high glucose and pro-inflammatory cytokines after 24 hours **[1-TXT]**.

4.6.1. LAB MEDIA: Figure 6A. *Video editor focus on the points above red dotted lines and label the line "Baseline Level".* **TEXT: IL-Interleukin, VEGF-Vascular endothelial growth factor.**

4.7. After 72 hours, IL-18 and VEGF levels remained significantly increased, but no significant difference was found in IL-6 and IL-8 levels **[2]**.

4.7.1. LAB MEDIA: Figure 6B. *Video editor focus on the points of IL-18 and VEGF above red dotted lines*

Conclusion

5. Conclusion Interview Statements

5.1. **Charisse Kuo:** When performing this procedure, leave sufficient residual vitreous which will weigh the retina down, so that it does not detach from the RPE-choroid and float in the culture medium.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.5.2, 2.5.3.*

5.2. **Charisse Kuo:** The cultured retina can be further characterized using immunohistochemistry and histology, while the inflammatory cytokines released into the cultured medium can be measured using a Luminex magnetic assay.

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

5.3. **Charisse Kuo:** This model is more clinically translatable compared to animal and in vitro models, which renders it more suitable for testing the efficacy of novel therapeutic drugs for retinal diseases.

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