Video Article

Organotypic Culture of Full-thickness Adult Porcine Retina

Jianfeng Wang[1], Anton M. Kolomeyer[2], Marco A. Zarbin[2], Ellen Townes-Anderson[1]

[1]Neurology and Neurosciences, University of Medicine and Dentistry of New Jersey - UMDNJ
[2]Institute of Ophthalmology and Visual Science, University of Medicine and Dentistry of New Jersey - UMDNJ

Correspondence to: Ellen Townes-Anderson at andersel@umdnj.edu

URL: http://www.jove.com/index/Details.stp?ID=2655

DOI: 10.3791/2655

Citation: Wang J., Kolomeyer A.M., Zarbin M.A., Townes-Anderson E. (). Organotypic Culture of Full-thickness Adult Porcine Retina. JoVE. . http://www.jove.com/index/Details.stp?ID=2655, doi: 10.3791/2655

Abstract

There is a recognized demand for in vitro models that can replace or reduce animal experiments. Porcine retina has a similar neuronal structure to human retina and is therefore a valuable species for studying mechanisms of human retinal injury and degenerative disease. Here we describe a cost-effective technique for organotypic culture of adult porcine retina isolated from eyes obtained from an abattoir. After removing the anterior segment, a trephine blade was used to create multiple neural retina-Bruch's membrane-RPE-choroid-sclera explants from the posterior segment of adult porcine eyes. A piece of sterile filter paper was used to lift the neural retina off from each explant. The filter paper-retina complex was cultured (photoreceptor side up) atop an insert which was ~~lifted off~~ from the bottom of the culture dish by a custom-made stand. The stand allows for good circulation of the culture medium to both sides of the retina. Overall, this procedure is simple, reproducible, and permits preservation of native retinal structure for at least seven days, making it a useful model for a variety of morphological, pharmacological, and biochemical studies on mammalian retina.

Protocol

1. Making a custom-made stand for cell culture inserts

The base of a 5 ~~mL~~ pipette tip (ISC BioExpress, #P-3250-19) has an inner diameter of 13 mm, which perfectly matches the bottom size (12.6 mm, outer diameter) of a 10 mm (inner diameter) NUNC cell culture insert (8µm, Polycarbonate membrane, Thermal, #137443). To make the stand, the pipette was cut off near the base to produce a ~~6mm~~ long hollow cylinder. Then, pieces were removed from the side of the cylinder using a flame-heated blade to create 3 legs (4 mm in height) under a ring (2 mm in height), which raised the height of the inserts by approximately 5 mm.

2. Preparation of sterile filter paper for attachment and culture of porcine retina

Whatman 4M Filter paper (Cat No. 1004) was cut into a circular shape (6.3 mm in diameter) with a small, triangular handle that was used for moving the filter paper into a glass tube for sterilization or once the retina was attached (as shown in the video).

3. Preparation of retinal explants

Eyes from 5- to 9-month old, 150-230 lbs, American Yorkshire pigs were obtained from a local abattoir within three hours of enucleation (transported on ice). Upon arrival, eyes were cleaned of extraneous tissue, dipped in betadine solution (10% Povidone-iodine, The Purdue Frederique Company, Stamford, CT) and washed twice in Dulbecco's Modified Eagle Medium (DMEM with 1g/L glucose, L-glutamine, and sodium pyruvate, Cellgro-mediatech Inc., Manasses, VA) supplemented with ~~250µg/ml Fungizone~~ Amphotericin B (Gibco-Invitrogen, Carlsbad, CA). The anterior segment was removed, exposing the neural retina. Six-mm trephine blades (Storz Ophthalmic-Bausch and Lomb, Manchester, MO) were used to cut equatorial, full-thickness posterior segment explants. The retina was subsequently peeled off by gently applying a piece of dry sterile filter paper onto the ganglion cell layer, lifting off the neural retina, and placing the filter paper with attached retina onto the culture insert, photoreceptors facing up. The insert was then placed into culture medium, making sure to fully cover the retina, in a 12-well culture dish (Fisher). Multiple explants were obtained routinely and cultured from a single eye.

4. Tissue culture

The retinal tissue was cultured in Eagle's Minimum Essential Medium (MEM, Invitrogen-Gibco-Life Technologies Inc., Rockville, MD), pH 7.4, supplemented with 0.2 mg/~~mL~~ glutamine, 10 µg/ml porcine insulin, 1 mM pyruvate, 0.1 mM L-ascorbic acid, 100 U/ml penicillin, 100 µg/ml streptomycin, and 250 ng/ml amphotericin B (Antibiotic Antimycotic: Sigma, St. Louis, MO), and aerated with humidified 5% CO_2, balance air, at 37 °C. Medium was exchanged every two days.

Tissue can be used, treated with a variety of reagents or probes, or left untreated for subsequent biochemical or morphological analyses. The section below describes procedures to create consistent full-thickness retinal cross-sections.

5. Sectioning

1. Using a Cryostat
    1. Fix retinal tissue in 4% parafomaldehyde overnight at 4°C;
    2. Rinse tissue briefly with phosphate buffered saline (PBS);

3. Carefully remove the tissue from the filter paper while it is in PBS in a 35 mm dish using a dissection microscope;
4. Transfer tissue to 30% sucrose and keep overnight at 4°C;
5. Suction away the excess solution around the tissue, add sufficient Tissue-Tek medium (O.C.T. Compound 4583) to cover tissue, and let it soak at room temperature for 2 hours to permeate the tissue;
6. Build a square frame (10 mm x 10mm, 5 mm height) with dental wax and place it around the tissue; add more Tissue-Tek medium to fill the frame (make sure the tissue sample is flat) and place in a freezer (-20°C) for at least 1 hour;
7. Transfer the frozen sample block to a sample platform (make sure the sample block is vertical to the platform);
8. Mount the sample platform in a pre-cooled cryostat (-20°C, Leica, CM1900) and make sure the block is vertical to the blade;
9. Remove the wax and the frame block before sectioning the tissue at desired thickness;
10. Place the sections on gelatin-treated slides.

2. Using a Vibratome
    1. Fix and rinse the tissue as described above for the Cryostat;
    2. Place the tissue into pre-heated, melted 4% agar in a small container and keep at 4°C until the agar congeals completely;
    3. Use a sharp blade to trim the agar into a small block with the tissue sample inside;
    4. Glue the sample block on a vibratome sample holder with Krazy glue (make sure the piece of tissue sample is vertical to the platform of the sample holder, i.e., perpendicular to the blade) and mount the holder on a vibratome sectioning system (Leica, Series 1000) with the sample block completely submerged in icy water;
    5. Cut the sample block into 50 µm sections and place them on gelatin-treated slides with a soft brush.

6. Immunohistochemistry

Use any standard protocol. Immunolabeled thick sections can be viewed with a confocal microscope.

7. Representative Results:

Morphology was preserved during the seven-day period of culture. Images of retina before and after culturing are available in the video.

Disclosures

No conflicts of interest declared.

Discussion

Vision researchers have established a variety of retinal culture systems, including organotypic systems, to study a variety of issues, such as retinal stem cell transplantation[1], retinal regeneration[2], and exogenous gene expression[3-5]. However, adult mammalian retina is difficult to maintain *in vitro*, mainly due to the high-energy demands of the photoreceptors[6]. Koizumi et al. described a rabbit retinal organotypic culture system in which the oxygen supply for photoreceptors was ameliorated by raising the height of the culture insert and agitating the culture medium. They successfully maintained adult retinal tissue for up to six days[4,5]. Kobuch et al. described a perfusion culture system for adult porcine retina and retinal pigment epithelium (RPE) and were able to maintain the organotypic tissue for at least 10 days[7]. However, the procedure for preparing the retina-RPE-choroid sheets for laboratory manipulation was complicated and required special tools. In addition, each explant required its own perfusion system making in vitro culture of multiple tissue samples cumbersome. Kaempf et al. successfully created another organotypic culture model of adult mammalian neurosensory retina in co-culture with RPE but only showed results for a 3-day culture; the long-term survival of the tissue was not tested[8].

In this manuscript, we describe a simple and reproducible protocol for organotypic culture of adult porcine neural retina adopting an approach similar to that previously described for rabbit retina[4] with the following features:

1. Creation of a simple technique to make a custom stand to raise the height of the culture insert, ensuring good oxygen supply for the whole retina explant. Using a ~~5ml~~ pipette tip to create the stand assures that the stand can be autoclaved and that the height can be adjusted if desired.
2. Culture of the retina with photoreceptor layer facing upwards, as opposed to the ganglion cell layer. In this way the photoreceptors have increased access to oxygen, and agitation to further boost the oxygen supply is not necessary.
3. Minimization of mechanical trauma. The retinal explants are attached to sterile filter paper before peeling them from the RPE and then are cultured on the filter paper, thereby reducing mechanical stress on the tissue and preventing retinal curling.
4. Consistency in the size of retinal explants. Because a trephine is used to create the explants, each retinal explant is of the same size, thereby making it easy to compare the results of various treatments.
5. Preservation of morphology. Structure of the porcine retina in this culture system remains intact for at least seven days.

Given that porcine and human retina are very similar in terms of cell distribution and morphology, vascular pattern, layer thickness, and other physiologic characteristics[9,10], the organotypic culture of porcine retina provides an attractive animal model for studying manifestations of human retinal diseases, degenerations, and injuries. This system may be particularly important in cases where in vitro experiments on human retina are impossible to perform due, in part, to the lack of high-quality human tissue.

Acknowledgements

This work was supported by NIH grant EY 012031 (ET-A), an award from the F. M. Kirby Foundation (ET-A), Research to Prevent Blindness, Inc. (MAZ), The New Jersey Lions Eye Research Foundation (MAZ), The Eye Institute of New Jersey (MAZ), the Joseph J. and Marguerite DiSepio Retina Research Fund (MAZ), and the Janice Mitchell Vassar and Ashby Mitchell Fellowship (AMK).

References

1. Johnson, T.V. & Martin, K.R., Development and characterization of an adult retinal explant organotypic tissue culture system as an in vitro intraocular stem cell transplantation model. Invest Ophthalmol Vis Sci 49 (8), 3503-3512 (2008).
2. Kretz, A., Marticke, J.K., Happold, C.J., Schmeer, C., & Isenmann, S., A primary culture technique of adult retina for regeneration studies on adult CNS neurons. Nat Protoc 2 (1), 131-140 (2007).
3. Donovan, S.L. & Dyer, M.A., Preparation and square wave electroporation of retinal explant cultures. Nat Protoc 1 (6), 2710-2718 (2006).
4. Koizumi, A., Zeck, G., Ben, Y., Masland, R.H., & Jakobs, T.C., Organotypic culture of physiologically functional adult mammalian retinas. PLoS One 2 (2), e221 (2007).
5. Lye, M.H., Jakobs, T.C., Masland, R.H., & Koizumi, A., Organotypic culture of adult rabbit retina. J Vis Exp (3), 190 (2007).
6. Ames, A., 3rd, Li, Y.Y., Heher, E.C., & Kimble, C.R., Energy metabolism of rabbit retina as related to function: high cost of Na+ transport. J Neurosci 12 (3), 840-853 (1992).
7. Kobuch, K. et al., Maintenance of adult porcine retina and retinal pigment epithelium in perfusion culture: characterisation of an organotypic *in vitro* model. Exp Eye Res 86 (4), 661-668 (2008).
8. Kaempf, S., Walter, P., Salz, A.K., & Thumann, G., Novel organotypic culture model of adult mammalian neurosensory retina in co-culture with retinal pigment epithelium. J Neurosci Methods 173 (1), 47-58 (2008).
9. Guduric-Fuchs, J. et al., Immunohistochemical study of pig retinal development. Mol Vis 15, 1915-1928 (2009).
10. Chandler, M.J., Smith, P.J., Samuelson, D.A., & MacKay, E.O., Photoreceptor density of the domestic pig retina. Vet Ophthalmol 2 (3), 179-184 (1999).

