Video Article

Organotypic Culture of Adult Rabbit Retina

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Abstract

Organotypic culture systems of functional neural tissues are important tools in neurobiological research. Ideally, such a system should be compatible with imaging techniques, genetic manipulation, and electrophysiological recording. Here we present a simple interphase tissue culture system for adult rabbit retina that requires no specialized equipment and very little maintenance. We demonstrate the dissection and incubation of rabbit retina and particle-mediated gene transfer of plasmids encoding GFP or a variety of subcellular markers into retinal ganglion cells. Rabbit retinas cultured this way can be kept alive for up to 6 days with very little changes of the overall anatomical structure or the morphology of individual ganglion- and amacrine cells.

Protocol

Making gene gun bullets (GOLD)

1. Make 100X PVP (0.5mg/ml) in 100% EtOH.
2. Weigh out 30mg of BioRad 1.6 Micron microcarrier gold and place it into a 1.5 ml eppendorf tube.
3. Calculate the amount of plasmid needed to give a concentration of 0.5-3 µg plasmid /mg gold, and place the plasmid into the tube with the gold microcarriers. Combine several plasmids if needed.
4. Bring volume of plasmid to 100 µl with distilled water.
5. Add 100 µl of 0.05M spermidine into the tube. Vortex tube 30 seconds.
6. Vortex 1 minute at full speed. Sonicate for 3-5 minutes..
7. Dry an appropriate length of Tefzel tubing in a Biorad PDS-1000/He apparatus for 15 minutes.
8. After sonication, vortex for 1-2 minutes at full speed. Reduce speed slowly to allow the tube to be opened while vortexing.
9. Add 100 µl of 1 M $CaCl_2$ slowly to the tube drop wise while vortexing.
10. Leave tube at room temperature for 10 minutes.
11. Make 5 Micron filter.
    1. Take two 5ml syringes without needles.
    2. Remove the plunger, and detach the black caps. Taking small scissors, cut off a part of the black cap to give a ring. Repeat on the other plunger to net two rings.
    3. Sandwich a 5 Micron (Small Parts, Inc. nylon filter, ½ inch diameter) filter between the two rings, and push the sandwich into the tip of syringe.
    4. Dangle syringe over a 50ml conical flask.
12. After 10 minutes, vortex the tube for 30 seconds.
13. Centrifuge the tube for 30 seconds and save the pellet.
14. Add 1ml of 100% EtOH to pellet. Use pipette tip to break up the pellet, and vortex 30 seconds. Spin 30 seconds and remove EtOH. Repeat 2 more times.
15. Add 1ml of 100% EtOH to the pellet (break up with tip). Place this into the 5 Micron filter and let it flow through by gravity into the 50ml conical tube. Use more EtOH as needed to wash out tube or help with flow through.
16. Spin the 50ml tube at 2000rpm in centrifuge for 5-10 minutes. Remove supernatant, save pellet.
17. Depending on the desired density of microcarriers needed, add 800µl-1.5ml 100% EtOH and corresponding 8 µl-15 µl PVP solution.
18. Swirl and immediately go to the Biorad PDS-1000/He apparatus.
19. Biorad PDS-1000/He apparatus
    1. Disconnect the tube connecting the N2 gas tank to the drying tube
    2. Take out drying Tefzel tubing from machine
    3. Insert one end into the 50ml conical tube, and attach a syringe to the other end of the tubing. Suck brown slurry into the middle of the tube.
    4. Insert tubing back into machine.
    5. Wait (without rotation) for 4 minutes
    6. After 4 minutes, suck off EtOH slowly and carefully, making sure not to disturb microcarriers.
    7. Rotate the tubing for 1 minute.
    8. After 1 minute, connect the tube connecting the N_2 gas tank to the drying tube and let tubing dry for 15 minutes.
20. After 15 minutes, cut the tubing to bullet size to be used immediately or stored at 4°C in an exsiccator. Bullets can be stored up to 3 months.

Preparation of gene-gun

1. Place bullets into a cartridge. Each piece of retina can be shot with 1-3 bullets.
2. Insert the cartridge into the gene-gun and attach the gun to a helium source. Set the pressure to 110 Psi.

Preparation of media

1. *Dissection medium*

1 bottle Ames solution (Sigma, 8.8g)
1.9 gm sodium bicarbonate
10 ml 1% pen/strep/L-Glutamine (1:100) (Gibco/Invitrogen)
990 ml distilled water to 1L
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1 Liter

2. Filter the dissection medium using a 0.22 µm filter.
3. *Incubation medium (500ml)*

485 ml of filtered dissection media
5 ml of 1% N2 supplement (Gibco/Invitrogen)
10ml 1% horse serum (Sigma)
500 µl of phenol red

0.5 L

4. Filter the incubation medium using a 0.22 µm filter.
5. Bubble O_2 into the dissection medium for 15 minutes before beginning the retina harvesting protocol.

Preparation of incubation chambers for retina

1. Under a hood, fill several 60 mm deep dishes (incubation chambers, Nunc) with 25 ml of incubation medium. The number of dishes depends on the number of retinas needed.
2. Place a filter stand into each deep dish. Only the very tips of the stand should not be submerged.
3. Place all deep dishes into the incubator until the retinas are ready.
4. Fill two 100 mm petri dishes and bring to the dissection bench.

Preparation of rabbit retina

1. Sacrifice a rabbit according to established protocols. The following does not have to be performed under a hood.
2. With angled scissors, insert it behind the eye into the eye socket of the rabbit, cutting the membranes and optical nerve that are attached to the eye.
3. Gently lift out the eye and wash it with oxygenated dissection media.
4. Place the eye (cornea facing up) into the well of an eye slicer and close the lid.
5. Place the blade horizontally right under the lid. In one smooth motion, draw the blade across the eye to cut off the cornea, leaving an eye cup.
6. Using forceps, remove the retina from the eye slicer well and place it onto a filter paper.
7. Remove the vitreous and lens by clamping an area near the severed optic nerve and pulling backwards on a filter paper. Repeat several times until almost all the vitreous is removed and the eye cup looks deflated.
8. Place eye cup into a petri dish with dissection media to wash.
9. Cut the eye cup into the desired number of pieces (5 pieces maximum).
10. Take one piece of eye cup and place it under the microscope.
11. Clamp the sclera and choroid lightly together on the edge of retina using forceps. With the other hand, use a fine brush to brush right between the choroid and retina.
12. Using short, gentle motions, tease the retina off the choroid until it becomes detached.
13. Cut 1 inch off a sterile transfer pipette. Use this to suck up the retina and place into a petri dish of incubation medium to wash.
14. Using another sterile transfer pipette with 1 inch cut off, carefully transfer the retina onto a 0.4 µm Millicell filter (Millipore, Cat No: PICMORG50).
15. Use a brush to orient the retina with the ganglion cell side facing upwards.
16. Flatten out the retina by brushing lightly on the edges of the retina. Minimize touching the ganglion cell layer with the brush as much as possible.
17. Remove excess medium on filter with a transfer pipette.
18. Using forceps, transfer the filter onto a modified suctioner. Draw on the suctioner 2-3 times to remove all the incubation medium from the filter.

Gene gunning and incubation of retina

1. Under a hood, place each of the retina-containing-filters onto the filter stands in the 60 mm deep dishes (incubation chambers).
2. Ensure that the medium is in contact with the underside of the filter and that the medium does not spill over the sides of the filter onto the retina. The filter should act as an interface between the retina and medium.
3. Shoot each piece of retina with two bullets. Place gene gun directly above tissue, about 3-4 cm away.
4. Place all incubation chambers onto a shaker in the 35 - 37°C incubator.
5. Replace with incubation medium every day. The retina can be incubated for a maximum of 6 days.

Discussion

1. What can I do with this method?

- See cell morphology clearly.
- Label cells to record from them.
- Overexpress proteins, like PSD95, but also other proteins that modulate the cell's response, e.g. ion channels, receptors.
- Express inhibitory RNAs.

2. How long can I keep adult retina?

- 4 days is no problem for adult rabbit. After six days the cells look "sick", i.e. the axons retract, you see swelling of dendrites and the recording of light responses becomes unreliable (only ~50% of the cells were found to be light-responsive after that time.
- We kept mouse retinas for 2 days. Mice are more difficult, because the retinas are vascularized, and also thicker than rabbit retina.

3. How do I make sure your retina is healthy after this time?

- The gold standard is recording from them. Ganglion cells should still react to light. You should protect your tissue from light during the incubation to avoid bleaching the photopigment, because you have to dissect the retina off the pigment epithelium for incubation.
- We occasionally also used microelectrode array recording to test our retinas.

4. Why do you want to manipulate only a few cells in the retina at a time?

- Some questions cannot be addressed otherwise. Consider for instance GABA receptors on a ganglion cell. You could use GABA blockers, but you would inhibit all GABAergic transmission in the retina, not just the input to the cell you are interested in. It can be very difficult to distinguish between an effect on that cell in particular, and overall "network effects" on the whole retina.
- If you want to see the morphology clearly, you cannot label all the cells, otherwise you just get a mess.

5. Is this method suitable as a population stain?

- No. Gene gunning is a random procedure. You'll get many ganglion cells, many displaced amacrine cells, some Muller cells, and much less of all the other cell types.

6. Are there any "tricks" I have to know to make it work?

- Not really. But it is important that you do the dissection of the retina relatively quickly, so you have your pieces on the filter in less than 30 minutes. The pieces should not be too small, about one square centimeter is ok. Expect 3-4 pieces from one rabbit eye.
- Keep your dissection tools strictly away from anything that comes into contact with fixatives and other harsh chemicals.
- Try setting your incubator to 35 C rather than 37, so the retina never gets too hot.
- You don't need to add growth factors like BDNF to your medium, at least we don't do it routinely, but you should use N2 supplement, 1% horse serum, and antibiotics. We provide a list of all the things you&'ll need as a supplementary file to this documentation.

7. Could you gene-gun other stuff, rather than plasmids?

- Yes, we have used dye-conjugated dextrans or DiI and DiO. But you could use calcium-indicator dyes, or pretty much anything you can coat onto gold or tungsten bullets.

8. What are the limitations?

- First of all, time. You have to cut the optic nerve, which means that the ganglion cells will eventually degenerate. This is not a problem for up to 6 days, but after that we would not trust the retinas any more. Now, if you just want to express GFP or some other cell-filling marker, an incubation of 2-3 days is enough, but in some cases, like when you want to express inhibitory RNAs, the effect can take 4 or more days to show. Here, you have to keep the timeline in mind.

9. What other researcher's work should one be aware of?

- In the retina field, certainly Rachel Wong's lab at Washington University. There's also a lot of work people have done in other systems, like hippocampus slice preparation. We don't pretend to be able to give a full survey of the literature, but check out the papers in the references list.

References

1. Landreth GE, Agranoff BW. Explant culture of adult goldfish retina: a model for the study of CNS regeneration. Brain Res 1979;161(1):39-55.

2. Smalheiser NR, Crain SM, Bornstein MB. Development of ganglion cells and their axons in organized cultures of fetal mouse retinal explants. Brain Res 1981;204(1):159-78.

3. Adler R, Magistretti PJ, Hyndman AG, Shoemaker WJ. Purification and cytochemical identification of neuronal and non-neuronal cells in chick embryo retina cultures. Dev Neurosci 1982;5(1):27-39.

4. Rehm H, Schafer T, Betz H. Beta-bungarotoxin-induced cell-death of neurons in chick retina. Brain Res 1982;250(2):309-19.

5. Kim SU, Takahashi H. Tissue culture study of adult human retina neurons. Invest Ophthalmol Vis Sci 1988;29(9):1372-9.

6. Caffe AR, Soderpalm A, van Veen T. Photoreceptor-specific protein expression of mouse retina in organ culture and retardation of rd degeneration in vitro by a combination of basic fibroblast and nerve growth factors. Curr Eye Res 1993;12(8):719-26.

7. Wong HC, Thompson S, Yu DY, Cringle SJ, Alder VA, Taylor SJ. Comparison of growth rates of bovine retinal and brain microvascular pericytes in different oxygen concentrations in vitro. Aust N Z J Ophthalmol 1995;23(4):299-308.

8. Seigel GM. The golden age of retinal cell culture. Mol Vis 1999;5:4.

9. Caffe AR, Ahuja P, Holmqvist B, Azadi S, Forsell J, Holmqvist I, et al. Mouse retina explants after long-term culture in serum free medium. J Chem Neuroanat 2001;22(4):263-73.

10. Hatakeyama J, Kageyama R. Retrovirus-mediated gene transfer to retinal explants. Methods 2002;28(4):387-95.

11. Zhang SS, Fu XY, Barnstable CJ. Tissue culture studies of retinal development. Methods 2002;28(4):439-47.

12. Wang SW, Mu X, Bowers WJ, Klein WH. Retinal ganglion cell differentiation in cultured mouse retinal explants. Methods 2002;28(4):448-56.

13. Inoue T, Hojo M, Bessho Y, Tano Y, Lee J, Kageyama R. Math3 and NeuroD regulate amacrine cell fate specification in the retina. Development 2002;129(4):831-842.

14. Kretz A, Hermening SH, Isenmann S. A novel primary culture technique for adult retina allows for evaluation of CNS axon regeneration in rodents. J Neurosci Methods 2004;136(2):207-19.

15. Liljekvist-Larsson I, Johansson K. Retinal neurospheres prepared as tissue for transplantation. Brain Res Dev Brain Res 2005;160(2):194-202.

16. Reidel B, Orisme W, Goldmann T, Smith WC, Wolfrum U. Photoreceptor vitality in organotypic cultures of mature vertebrate retinas validated by light-dependent molecular movements. Vision Res 2006;46(27):4464-71.

17. Xin H, Yannazzo JA, Duncan RS, Gregg EV, Singh M, Koulen P. A novel organotypic culture model of the postnatal mouse retina allows the study of glutamate-mediated excitotoxicity. J Neurosci Methods 2007;159(1):35-42. About gene gunning (and “dye gunning”). These are general references to the method, though slanted towards the use in neuronal tissues:

18. Johnston SA, Tang DC. The use of microparticle injection to introduce genes into animal cells in vitro and in vivo. Genet Eng (N Y) 1993;15:225-36.

19. Lo DC, McAllister AK, Katz LC. Neuronal transfection in brain slices using particle-mediated gene transfer. Neuron 1994;13(6):1263-8.

20. Rajagopalan LE, Malter JS. Turnover and translation of in vitro synthesized messenger RNAs in transfected, normal cells. J Biol Chem 1996;271(33):19871-19876.

21. McAllister AK. Biolistic transfection of neurons. Sci STKE 2000;2000(51):PL1.

22. Gan W-B, Grutzendler J, Wong W, Wong R, Lichtman J. Multicolor "DiOlistic" labeling of the nervous system using lipophilic dye combinations. Neuron 2000;27(Aug):219-225.

23. Sato H, Hattori S, Kawamoto S, Kudoh I, Hayashi A, Yamamoto I, et al. In vivo gene gun-mediated DNA delivery into rodent brain tissue. Biochem Biophys Res Commun 2000;270(1):163-70.

24. Zhang G, Selzer ME. In vivo transfection of lamprey brain neurons by gene gun delivery of DNA. Exp Neurol 2001;167(2):304-11.

25. Sohn RL, Murray MT, Schwarz K, Nyitray J, Purray P, Franko AP, et al. In-vivo particle mediated delivery of mRNA to mammalian tissues: ballistic and biologic effects. Wound Repair Regeneration 2001;9(4):287-296.

26. O'Brien JA, Holt M, Whiteside G, Lummis SC, Hastings MH. Modifications to the hand-held Gene Gun: improvements for in vitro biolistic transfection of organotypic neuronal tissue. J Neurosci Methods 2001;112(1):57-64.

27. Klimaschewski L, Nindl W, Pimpl M, Waltinger P, Pfaller K. Biolistic transfection and morphological analysis of cultured sympathetic neurons. J Neurosci Methods 2002;113(1):63-71.

28. Kettunen P, Demas J, Lohmann C, Kasthuri N, Gong Y, Wong RO, et al. Imaging calcium dynamics in the nervous system by means of ballistic delivery of indicators. J Neurosci Methods 2002;119(1):37-43.

29. Gamper N, Shapiro MS. Calmodulin mediates Ca2+-dependent modulation of M-type K+ channels. J Gen Physiol 2003;122(1):17-31.

30. Wirth MJ, Wahle P. Biolistic transfection of organotypic cultures of rat visual cortex using a handheld device. J Neurosci Methods 2003;125(1-2):45-54.

31. O'Brien J, Lummis SC. Biolistic and diolistic transfection: using the gene gun to deliver DNA and lipophilic dyes into mammalian cells. Methods 2004;33(2):121-5.

32. Benediktsson AM, Schachtele SJ, Green SH, Dailey ME. Ballistic labeling and dynamic imaging of astrocytes in organotypic hippocampal slice cultures. J Neurosci Methods 2005;141(1):41-53. About gene gunning (and dye gunning) in the retina:

33. Chow L, Levine EM, Reh TA. The nuclear receptor transcription factor, retinoid-related orphan receptor beta, regulates retinal progenitor proliferation. Mech Dev 1998;77(2):149-64.

34. Rockhill RL, Daly FJ, MacNeil MA, Brown SP, Masland RH. The diversity of ganglion cells in a mammalian retina. J Neurosci 2002;22(9):3831-43.

35. Sun W, Li N, He S. Large-scale morphological survey of mouse retinal ganglion cells. J Comp Neurol 2002;451(2):115-126.

36. Sun W, Li N, He S. Large-scale morophological survey of rat retinal ganglion cells. Vis Neurosci 2002;19(4):483-93.

37. Stacy RC, Wong RO. Developmental relationship between cholinergic amacrine cell processes and ganglion cell dendrites of the mouse retina. J Comp Neurol 2003;456(2):154-66.

38. Strettoi E, Pignatelli V, Rossi C, Porciatti V, Falsini B. Remodeling of second-order neurons in the retina of rd/rd mutant mice. Vision Res 2003;43(8):867-77.

39. Pignatelli V, Strettoi E. Bipolar cells of the mouse retina: a gene gun, morphological study. J Comp Neurol 2004;476(3):254-66.

40. Connaughton VP, Graham D, Nelson R. Identification and morphological classification of horizontal, bipolar, and amacrine cells within the zebrafish retina. J Comp Neurol 2004;477(4):371-85.

41. Edqvist PH, Hallbook F. Newborn horizontal cells migrate bi-directionally across the neuroepithelium during retinal development. Development 2004;131(6):1343-51.

42. Jakobs TC, Libby RT, Ben Y, John SW, Masland RH. Retinal ganglion cell degeneration is topological but not cell type specific in DBA/2J mice. J Cell Biol 2005;171(2):313-25.

43. Qin Y, Xu G, Wang W. Dendritic abnormalities in retinal ganglion cells of three-month diabetic rats. Curr Eye Res 2006;31(11):967-74.

44. Roizenblatt R, Weiland JD, Carcieri S, Qiu G, Behrend M, Humayun MS, et al. Nanobiolistic delivery of indicators to the living mouse retina. J Neurosci Methods 2006;153(1):154-61.

45. Koizumi A, Zeck G, Ben Y, Masland RH, Jakobs TC. Organotypic culture of physiologically functional adult Mammalian retinas. PLoS ONE 2007;2:e221.

