Isolation of primary bovine retinal endothelial cells and pericytes using the gentleMACS™ Dissociator

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Background
Homeostasis of the retina is maintained by the inner blood retinal barrier (iBRB), formed by endothelial cells of retinal capillaries. Intact BRB function is essential for proper vision and its breakdown greatly contributes to the pathology and vision loss in retinal disorders such as diabetic retinopathy (DR), age-related macular degeneration and uveitis. The major cause of loss of vision in patients with DR is diabetic macular edema, which is characterized by vascular leakage and the deposition of hard exudates in the central retina. Up to date cellular mechanisms underlying BRB dysfunction are poorly understood. Therefore, Wisniewska-Kruk et al. developed and characterized a novel in vitro BRB model, based on primary bovine retinal endothelial cells (BRECs), bovine retinal pericytes (BRPCs) and rat glial cells. The morphological and functional integrity of the BRB and the presence of influx and efflux transporters as well as the effects of VEGF on BRB breakdown were assessed. This protocol describes the standard procedure used by Wisniewska-Kruk et al. to isolate bovine retinal endothelial cells as well as primary bovine retinal pericytes using the gentleMACS™ Dissociator.

Materials and methods

Materials
- gentleMACS Dissociator or gentleMACS Octo Dissociator
- gentleMACS C Tubes
- Incubator (37 °C, 10% CO₂)
- Centrifuge
- Surgical scissors
- Nylon net filter (60 µm and 70 µm)
- Fine-tipped paint brush
- 75 cm² cell culture flask, coated with collagen IV (0.01 mg/mL in 0.01% acetic acid) and fibronectin (0.01 mg/mL stock solution in phosphate-buffered saline (PBS))
- DMEM
- Digestion mix (DMEM including 10% fetal bovine serum (FBS), 210 U/mL collagenase IV, and 91 U/mL pronase E)
- Initial culture medium (DMEM including 25 mM HEPES and 4.5 g/L glucose, supplemented with 10% FBS, 1× MEM non-essential amino acids, 1% penicillin/streptomycin, gentamicin sulfate amphotericin B, 2 mM L-glutamine, 0.1 mg/mL hydrocortisone)
- PBS
- Puromycin
- Collagen IV
- Fibronectin

Methods
1. Keep fresh cow eyes on ice until isolation of cells.
3. Separate retina from the retinal pigment epithelium using a fine-tipped paint brush and cut loose retina from the optic nerve.
4. Wash isolated retinas with 10 mL DMEM thoroughly.
5. Transfer three retinas into a gentleMACS C Tube containing 5 mL PBS.
6. Tightly close the C Tube and attach it upside down onto the sleeve of the gentleMACS Dissociator.
7. Run the gentleMACS Program _m_brain_ 01 twice.
8. Add 10 mL DMEM and centrifuge the homogenate for 10 minutes at 900xg.
9. Remove supernatant and resuspend cell pellet in 10 mL DMEM.
10. Filter suspension through a 60 µm nylon net filter and wash with additional 10 mL DMEM.
11. Discard the filtrate and transfer the homogenate remaining on the filter into 10 mL DMEM.
12. Centrifuge for 10 minutes at 200xg.
13. Discard supernatant and resuspend cell pellet in 6 mL digestion mix.
14. Incubate for 30 minutes at 37 °C with gently shaking every 5 minutes.
15. Filter cell suspension through a 70 µm nylon net filter and centrifuge flow-through for 10 minutes at 200×g.
16. Collect isolates of the three retinas and resuspend cells in pre-heated culture medium.
17. Seed cells in a 75 cm² cell culture flask.
18. Wash cells after adherence with PBS twice and replace initial culture medium by 15 mL DMEM supplemented with 4 µg/mL puromycin.
19. Culture cells at 37 °C at 10% CO₂.
20. After two days, medium was replaced with DMEM.
21. Pericytes were isolated in the same way, but cultured in collagen IV–coated flasks without puromycin treatment. Endothelial cells were removed after short trypsinization with 0.05% trypsine EDTA.

Results

Wisniewska-Kruk et al. present a reliable and reproducible protocol for assembling an in vitro model of the BRB of the healthy normal retina, as well as of BRB loss as occurs in diabetic macular edema (DME). The described BRB model reflects the in vivo situation, thus may contribute to future studies of BRB physiology, pathology, and pharmacology.

Conclusion

Isolation of primary bovine retinal endothelial cells and pericytes can be accomplished with ease using the gentleMACS Dissociator.

Reference