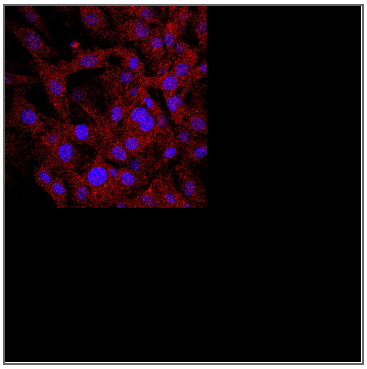

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We use this protocol and it's working

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Isolation and culture of primary mouse brain microvascular endothelial cells

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ABSTRACT

Immunofluorescent characterization of BMECs. The isolated BMECs were 95% positive for CD31(red), confirming the epithelial nature of these cells.

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2 Isolation and culture of primary mouse brain microvascular endothelial cells

Mouse brain microvascular endothelial cells (BMECs) were prepared from 2-week-old C57BL/6 mice as previously recommended [81]. Mice was soaked in 75% ethanol to disinfect 5 min after the cervical dislocation, then mice brains were removed and placed in a 10 cm petri dish containing precooled Hank's solution. The white matter, residual blood vessels and pia mater were removed and the cerebral cortex was retained. Brain tissue was minced and homogenized, and 10 ml Modified Eagle Medium (MEM) medium was added to the tissue suspension and centrifuge the cells at 1200 rpm for 10 min at 4°C. The supernatant was discarded and myelin was removed by centrifugation at 2600 r/min for 10 min in 22% bovine serum albumin (BSA, Sigma). The microvascular segments and cells were resuspended by using the Endothelial Cell Growth Media (ECGM) and centrifuge at 1200 r/min for 5 min. Then the BMECs were inoculated in 12-well culture dishes previously coated with type I collagen. BMECs were cultured in 5% CO2 incubator at 37℃ with medium containing 80% Dulbecco's Modified Eagle Medium (Gibco, cat# 10566016), 15% fatal bovine serum (Gibco, cat# 16010142), 0.05% endothelial cell growth supplements (Millipore, cat# 211F-GS), 0.1% heparin (Sigma, cat# H9267) and 1% antibiotic-antimycotic solution (100X, Invitrogen, cat# 15240096). On the second day, endothelial cell growth medium (ECGM) containing 4 μg/mL puromycin (Sigma, cat# P8833) was added to continue culture, and 72 h later it was replaced with conventional ECGM medium. The culture medium was changed every other day, and when the cells reached 90-95% fusion, 0.25% trypsin was used for passage. BMECs were seeded at a density of 1.0 × 104 cells/cm2 for subsequent experiments and passaged when BMECs reached 90% confluence. Serial passage of cells was performed by trypsinization with trypsin-EDTA (Gibco, cat# 25200072). The purity of BMECs was controlled by immunofluorescence staining of endothelium marker CD31 (1:500, CST, USA, cat# 3528S).