APPLICATION NOTE

ECLIPSE LV100ND Upright Microscope (Episcopic/diascopic illumination model)
ECLIPSE Ti2 Series Inverted Microscope

Observation of Cell Culture Inserts Using Episcopic Brightfield Method

The blood-brain barrier (BBB) is a mechanism that limits the exchange of substances between blood vessels and cells in the brain, and is responsible for maintaining the optimal environment for neurological function. A BBB kit consisting of vascular endothelial cells, pericytes and astrocytes, the component cells of the BBB, is an *in vitro* model utilizing cell culture inserts. The kit is widely used for drug development to treat brain diseases, such as the evaluation of drug delivery to the brain, the study of compound effects on the BBB, the study of cell membrane polarity inhibition by receptors and disease model analysis. The BBB kit has visualization issues with cells under culture at the top and bottom of translucent membranes in the culture inserts, and inside wells. For example, since it is difficult to confirm cell growth states using living cells in the preparatory stage of drug evaluation, whether those cells do not meet the criteria for use due to irregular growth can only be known after confirmation by immunostaining etc. after evaluation. This application note introduces an observation method for cells cultured on cell culture inserts used for the BBB kit, etc.

Keywords: episcopic brightfield illumination, drug discovery, blood-brain barrier, live cell, cell culture inserts

Experiments

Sample: Rat BBB model kit™ (RBT-24H) (PharmaCo-Cell Co., Ltd.), consisting of rat primary cell cultures, cellQART® 9320402 cell culture inserts (SABEU) and a medium
Culture: After thawing the rat BBB model kit and adding the heated medium, a culture was started at 37°C in a CO_2 incubator. The first change of medium was performed after 2-3 hours of incubation, the second on the next day, and the experiment was initiated from the 4th day onwards.

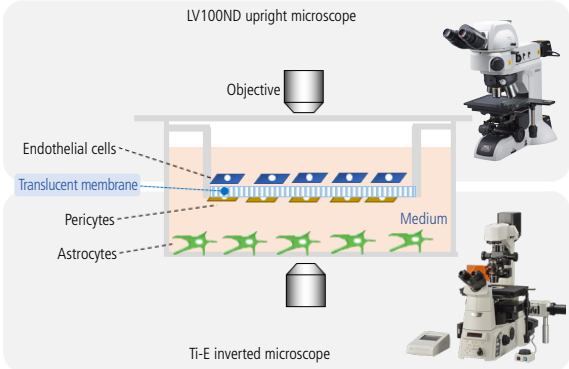

Fig. 1 Overview of observation of cell culture inserts using episcopic brightfield method

1. Observation of Endothelial Cells with an Upright Microscope

Methods

①Live cell observation of endothelial cells cultured for 5 days was performed with an upright microscope. An episcopic brightfield image was captured after performing reversible image enhancement using the Look-up Tables (LUTs) function of NIS-Elements software.
②After fixation, the cells were dyed with the anti-GAPDH antibody and Hoechst.
③An episcopic brightfield image and fluorescence images of fixed endothelial cells were captured and compared with the episcopic brightfield image of live cells.

Equipment

- Upright microscope: LV100ND
- Objective: TU Plan Fluor EPI 10x (NA 0.30)
- Software: NIS-Elements
- Aperture diaphragm: Adjusted for sample (around minimum aperture)
- Camera: Hamamatsu Photonics ORCA-Flash 4.0

Results

- The episcopic brightfield image of living endothelial cells revealed many dark areas outlined in white throughout the field of view. The episcopic brightfield image of fixed cells had slightly less contrast than the live-cell image, but looked similar.
- Comparison of the episcopic brightfield image of fixed cells with the anti-GAPDH antibody staining image revealed that the dark areas in the episcopic brightfield image corresponded to cell nuclei, and the individual regions outlined in white corresponded to cells.
- The state of living endothelial cells could be confirmed without staining by episcopic brightfield observation with an upright microscope.

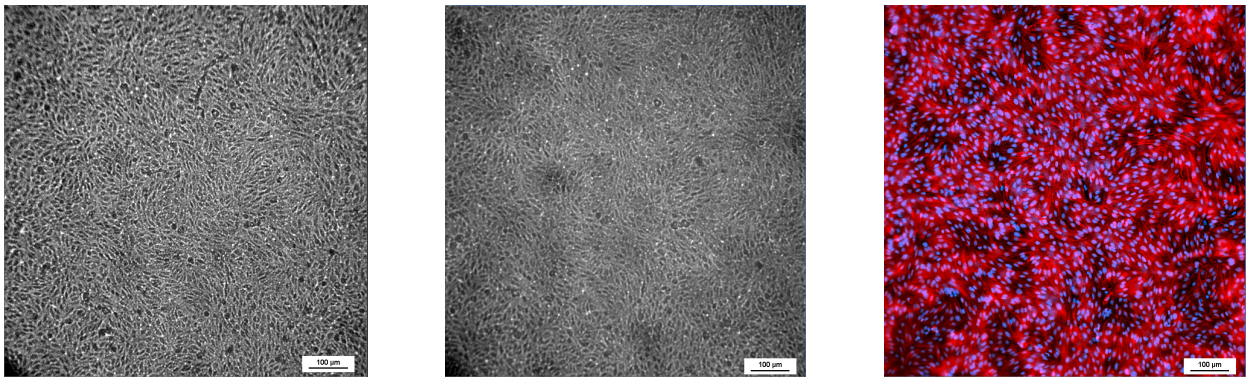

Fig. 2 Left: An episcopic brightfield image of living primary endothelial cells derived from a rat, cultured on a translucent membrane; Center: An episcopic brightfield image of fixed primary endothelial cells derived from a rat; Right: A merged image of anti-GAPDH antibody staining (cytoplasm and cell membrane: red) and Hoechst staining (cell nucleus: blue).

2. Observation of Pericytes and Astrocytes via an Inverted Microscope

Methods

①Pericytes were stained with Hoechst and Calcein, and astrocytes were stained with Hoechst and CellMask™ Deep Red.

②Live cell observation of pericytes and astrocytes cultured for 4 days was performed with an inverted microscope. An episcopic brightfield image was captured using the LUTs function of NIS-Elements. Fluorescence images of the same field of view were subsequently captured and compared with the episcopic brightfield image.

Equipment

- Inverted microscope: Ti-E
- Objective: TU Plan Fluor EPI 10x (NA 0.30)
- Software: NIS-Elements
- Aperture diaphragm: Adjusted for sample (around minimum aperture)
- Camera: Hamamatsu Photonics ORCA-Flash4.0

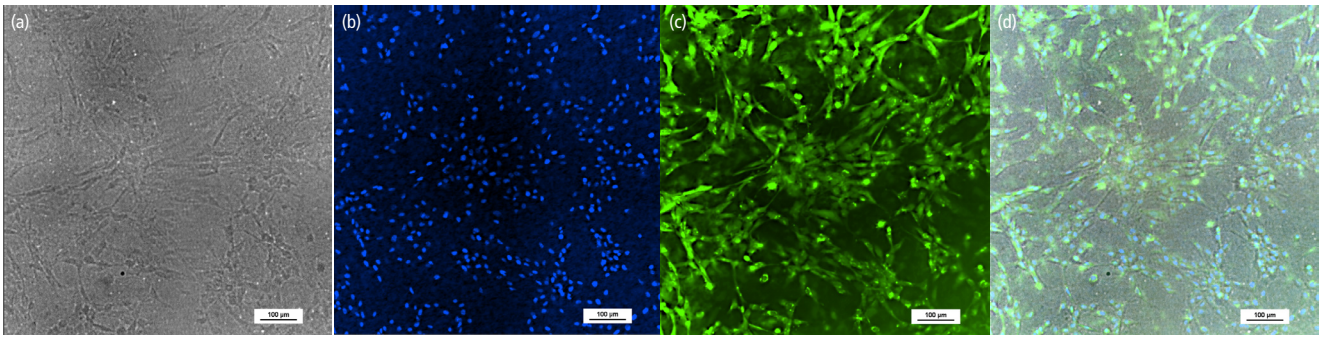

Fig. 3 (a) An episcopic brightfield image of the pericytes of living rat-derived primary cells, cultured under a translucent membrane; (b) Hoechst-stained cell nuclei; (c) calcein-stained cytoplasm; (d) a merged image of episcopic brightfield image (a) and images (b) and (c).

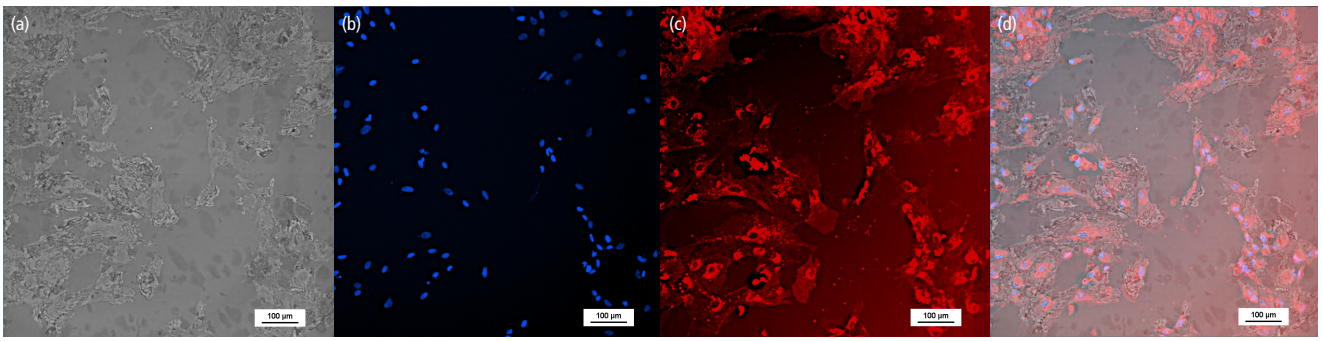

Fig. 4 (a) An episcopic brightfield image of the astrocytes of living rat-derived primary cells, cultured in the bottom of a well; (b) Hoechst-stained cell nuclei; (c) CellMask deep red-stained cytoplasm; (d) a merged image of episcopic brightfield image (a) and images (b) and (c).

Results

- Figure 3 confirms that in episcopic brightfield images of living pericytes cultured under a translucent membrane, areas that appear darker than surrounding areas correspond to calcein signals.
- Figure 4 confirms that in episcopic brightfield images of living astrocytes cultured in the bottom of a well, areas that appear brighter than surrounding areas mostly correspond to CellMask Deep Red signals.
- It was concluded that the status of pericytes and astrocytes can be confirmed in their living states without staining, by episcopic brightfield observation using an inverted microscope.

Summary

Episcopic brightfield observation allows live cell imaging of cells under culture at the top and bottom of a translucent membrane in cell culture inserts and within wells. Cells can be used for drug response tests using a BBB model after confirmation of growth status in real time, eliminating the need for cell fixation and staining for post-test cell condition assessment, thereby improving work efficiency.

Product Information

ECLIPSE LV100ND upright microscope (Episcopic/diascopic illumination model)

Supports brightfield, fluorescence, DIC and polarizing observation using an episcopic illuminator with a built-in high-intensity halogen lamp, in addition to bright-field, phase contrast, DIC and polarizing observation with diascopic illumination.

TU Plan Fluor EPI 10X objective

This CFI60-2 objective, with a high NA (0.3) and long working distance (17.5mm), is optimized for episcopic observation. Sharp and high-contrast images can be obtained with excellent chromatic aberration correction.

ECLIPSE Ti2 series inverted microscope

A large FOV of 25 allows efficient acquisition of samples over a wide area. A manual model, a model with operation assist guide features, and a motorized model that supports multi-dimensional imaging are available.