Imaging of Cellular Fluorescence on Corning's Transwell® Permeable Supports

Application Note

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Introduction

Corning's Transwell permeable supports can be used for a variety of cell culture applications to better mimic the *in vivo* environment. Transwell inserts with larger pore sizes, such as 3.0, 5.0, or 8.0 µm, are typically used for migration, chemotaxis, and invasion assays, while Transwell inserts with smaller pore sizes, such as 0.4, 1.0, or 3.0 µm, can be used for co-culture applications and cell polarization studies. In co-culture and cell polarization applications, fluorescent imaging of cells cultured on the permeable membrane may be required to confirm cell membrane integrity or the presence of differentiated cell types. Traditionally, fluorescent imaging of cells cultured on Transwell inserts requires the membranes to be cut out of the Transwell insert and placed onto a slide or onto the bottom of a multiwell plate. However, with improvements in the capabilities and resolution of current imaging systems, especially confocal imagers, this may no longer be necessary. Here, we demonstrate fluorescent imaging of MDCKII/MDR1 cells directly on Transwell permeable supports in their cell culture receiver plate. This study utilized Transwell permeable supports with both polyester (PET) and polycarbonate (PC) membranes with pore sizes ranging from 0.4 to 3.0 µm.

Materials/Methods

MDCKII/MDR1 cells were obtained from Dr. Piet Borst (Netherlands Cancer Institute) and seeded into HTS 96-well Transwell permeable supports and 6.5 mm Transwell permeable supports at 1 x 10^5 cells/cm^2 in Dulbecco's Modification of Eagle's Medium (DMEM; Corning Cat. No. 10-013-CMR) supplemented with 10% Fetal Bovine Serum (FBS; Corning Cat. No. 35-015-CV). Transwell permeable support membranes tested included 0.4, 1.0, and 3.0 µm PET as well as 0.4 and 3.0 µm PC. The cells were cultured overnight in a 37°C, 5% CO_2 humidified incubator.

The next day, cells were stained live with 5 µM Calcein AM (Corning Cat. No. 354216), 350 nM MitoTracker® Orange CMTMRos (Thermo Fisher Cat. No. M7510), and 10 µg/mL Hoechst 33342 (Thermo Fisher Cat. No. 62249) in Hank's Balanced Salt Solution with calcium and magnesium (HBSS; Corning Cat. No. 21-023-CMR) for 45 minutes in a 37°C, 5% CO_2 humidified incubator. Cells were rinsed with HBSS prior to imaging with a Thermo Fisher CellInsight™ CX7 High-Content Screening (HCS) platform with a 20X objective in confocal mode, with 5 z-stack images of 2 µm steps taken for each representative composite image.

Results

Fluorescently labeled cells were imaged directly on each membrane type. Live cell stain Calcein AM (green), mitochondrial health stain MitoTracker Orange CMTMRos (red), and nuclear stain Hoechst 33342 (blue) were imaged in confocal mode and representative composite images are shown in Figure 1. The data demonstrates that removal of the PET and PC membranes from their supports is unnecessary for imaging, thus facilitating high throughput fluorescence microscopy of cells grown in Transwell permeable supports.

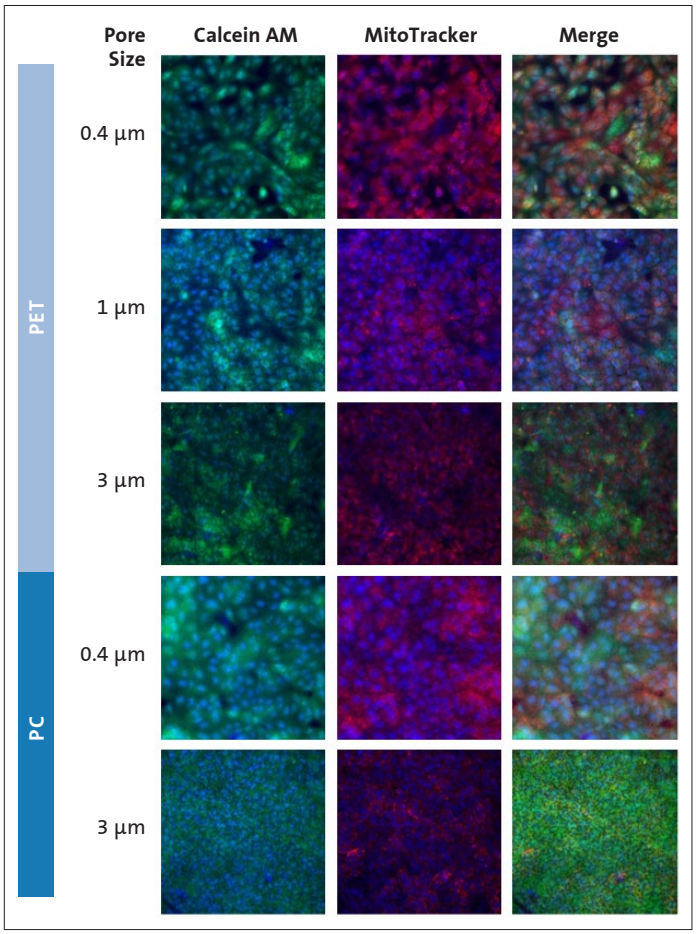

Figure 1. Representative composite confocal images of MDCKII/MDR1 cells stained live with Calcein AM (green), MitoTracker Orange CMTMROs (red), and Hoechst 33342 (blue). Cells were stained and imaged directly in Transwells on a variety of membrane types.

Recommendations for Protocol Optimization

- Follow fluorescent staining protocols typically used for live or fixed cell staining.
- Create a new form factor in your imager specifically for Transwell® permeable supports. Start with a Corning® 24-well or 96-well form factor and adjust the Z-height. The height may be outside of the range of auto-focus and need to be manually focused. To focus and find the correct Z-height for Transwell permeable supports, we recommend staining cells cultured on a Transwell permeable support with crystal violet or staining the cell nuclei.
- To accommodate for the increased distance between the cells and the imager, the exposure time may need to be increased compared with imaging of cells cultured on a microplate.

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