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PermaCell Inserts - 8mm Clear PTFE Inserts | Application Note

UTILITY OF CLEAR PERMACELL INSERTS FOR CELL CULTURE

Objective

To demonstrate the ability to visualize cells growing on the clear PTFE PermaCell inserts (Part #: CCI24-PTFE-0.4) using brightfield and fluorescence microscopy.

Methods

Brightfield microscopy: A mixture of Normal Human Epidermal Keratinocytes (NHEK, MatTek NHEK-CRY-AD) and Normal Human Melanocytes (NHM, MatTek NHM-CRY-AD) were seeded onto the apical membrane surface of the clear PermaCell inserts (part #: CCI24-PTFE-0.4). The co-cultures were fed submerged for 72 hours with 0.5 mL of medium in the basolateral compartment and 0.4 mL medium on the apical surface. After 72 hrs, the apical medium was carefully aspirated, and the inserts were placed into a 12-well plate and suspended using a hang-top plate (part #: CCI12-HANGTOP). Tissues were cultured at the air-liquid interface (ALI) by adding 5.0 mL of medium in the basolateral compartment only. The cultures were fed again on Day 5, 7, and 8 with 5.0 mL of medium in the basolateral compartment only. On Day 10, the cultures were imaged using a brightfield 10X objective on an ECHO Rebel microscope.

Fluorescence microscopy: 2 x 10^5 human coronary artery endothelial cells (hCAECs) were encapsulated in 75 µL of fibrin hydrogel and cultured on the clear PermaCell inserts (part #: CCI24-PTFE-0.4). On day 2, a live/dead assay was performed by staining cells with the commonly used fluorescent dyes, calcein (live cells) and propidium iodide (dead cells). Images were captured and overlaid using an ECHO Revolve microscope outfitted with a 10X objective and standard green fluorescent protein (GFP) and red fluorescent protein (RFP) filter cubes.

Results

As shown in Figure 1, the clear, high pore density PermaCell inserts support the culture and imaging of NHEK together with NHM. The NHM spontaneously pigment and adopt their in-vivo like dendritic morphology.

As shown in Figure 2, coronary artery endothelial cells were successfully cultured and imaged on the clear, high pore density PermaCell inserts. A live/dead assay showed that the vast majority of the cells were viable while very few dead cells were observed.

Figure 1. Top, trans-membrane view of pigmented, dendritic melanocytes in the NHM/NHEK co-culture grown on clear PermaCell inserts (CCI24-PTFE-0.4).

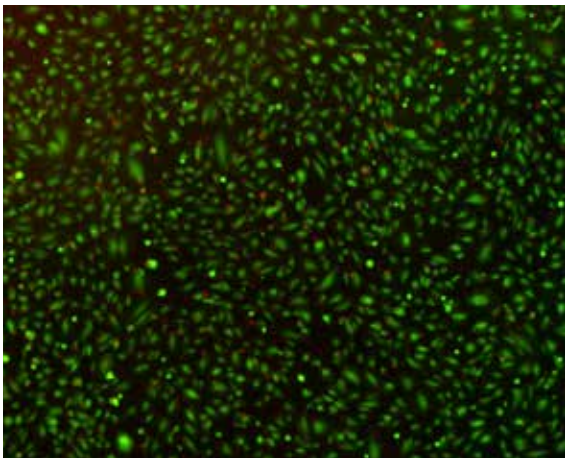

Figure 2. Top, trans-membrane view of hCAECs growing in a fibrin gel cultured on clear PermaCell inserts (CCI24-PTFE-0.4). Live cells were stained with calcein (green) and dead cells with propidium iodide (red).

Conclusion

MatTek clear PTFE PermaCell inserts provide researchers with a unique platform for culturing cells or 3-dimensional tissues. The inserts utilize a high pore density, microporous membrane that allows for a high flux of nutrients, proteins, and other factors to cells growing on the membrane. In addition, under aqueous conditions, the membrane is optically clear allowing for visualization of cells using both brightfield and fluorescence microscopy.