Cellular Dynamics international

Application Note

iCell® Endothelial Cells:

Assaying Barrier Function

iCell® Endothelial Cells, derived from human induced pluripotent stem cells, exhibit morphological, biochemical, and pathophysiological characteristics of a native human endothelium. These attributes make iCell Endothelial Cells an optimal in vitro test system for vascular biology interrogations in life science research and drug development.

Endothelial cells are involved in many aspects of vascular biology including the formation of a selective barrier between the blood and surrounding tissue. Dysregulation of this barrier function is a hallmark for numerous disease processes, such as atherosclerosis, inflammation, and tumor cell metastasis.

To test their barrier function, iCell Endothelial Cells were cultured, treated with thrombin, and analyzed using two impedance-based systems: ECIS System (Applied BioPhysics) and xCELLigence RTCA Cardio System (ACEA Biosciences). These non-invasive, label-free platforms allow for the real-time detection of cell-cell interaction, transient contractions, and cell layer permeability by monitoring changes in electrical impedance across the cell monolayer.

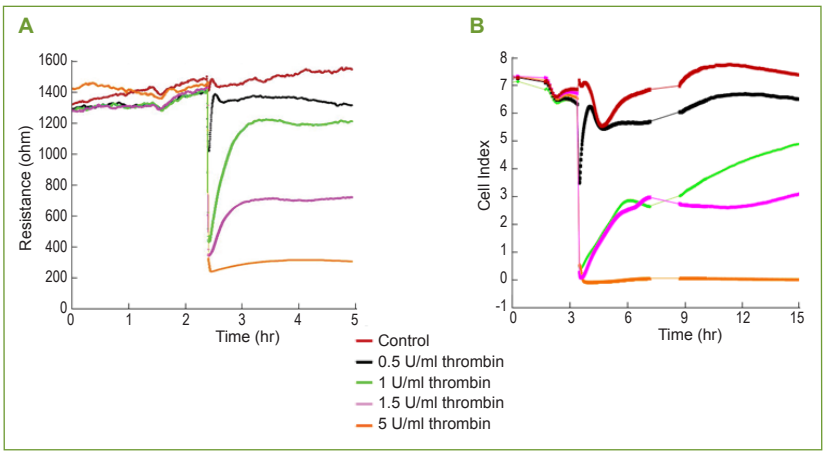
Methods

ECIS System. Two 8W10E electrode arrays (Applied BioPhysics #70010) were pre-coated with fibronectin (15 µg/cm^2, Invitrogen #33016-015) for 1 hour at room temperature. iCell Endothelial Cells were seeded in Complete iCell Endothelial Cells Maintenance Medium (Maintenance Medium) at 62,500 cells/cm^2 to provide ~50,000 cells/well in a final volume of 220 µl/well. The electrode arrays were transferred to an incubator at 37°C, 5% CO_2. At day 2 post-plating, 220 µl/well of spent medium was exchanged with 200 µl/well of fresh Maintenance Medium. A baseline measurement was recorded on the ECIS software at a single frequency of 4000 Hz until a resistance of 1100 Ω was detected. Barrier function was tested by the addition of 22 µl of a 10X solution of thrombin in each well to obtain a final concentration of 0 (control), 0.5, 1, 1.5, or 5 U/ml. The cellular response to thrombin was monitored for 2.5 hours.

xCELLigence RTCA System. iCell Endothelial Cells were cultured on an E-Plate (ACEA Biosciences) pre-coated with fibronectin (1). The cells were seeded in Maintenance Medium at 30,000 cells/cm^2 to provide ~9,600 cells/well in a final volume of 150 µl/well. The E-Plate was transferred to an incubator at 37°C, 5% CO_2, and Cell Index values were recorded. At day 2 post-plating, 100 µl of spent medium from each well was exchanged with 130 µl of fresh Maintenance Medium. A baseline measurement was recorded 1 hour after medium exchange. Barrier function was tested by the addition of 20 µl of a 10X solution of thrombin in each well to obtain final concentrations of 0 (control), 0.5, 1, 1.5, or 5 U/ml. The cellular response to thrombin was monitored for 12 hours.

Results & Discussion

The potent edemagenic agent thrombin has been previously shown to disrupt endothelial barrier function by electrical impedance measurement using human umbilical vein endothelial cells (HUVEC) (2). In the barrier function assays using iCell Endothelial Cells, the addition of thrombin induced a rapid, dose-dependent disruption of the barrier integrity (Figure 1). At low concentrations of thrombin, the barrier function recovered to baseline levels within 1 hour post-treatment. Increasing concentrations of thrombin resulted in a progressively more significant and irreversible barrier disruption effect.



▲ **Figure 1: Data for Barrier Function Assays Using iCell Endothelial Cells**
iCell Endothelial Cells were cultured for 2 days in iCell Endothelial Cells Maintenance Medium before the addition of thrombin. Panels A and B show the cellular response to thrombin-induced barrier disruption monitored on the ECIS System and xCELLigence RTCA Cardio System, respectively.

Conclusion

iCell Endothelial Cells exhibit barrier function activity that can be disrupted by treatment with thrombin and reliably assessed using impedance-based platforms. Together, iCell Endothelial Cells and impedance-based technologies offer a valuable cell model system for understanding the endothelial barrier characteristics, mechanisms of endothelial barrier dysfunction, and dynamic modulation of the endothelium permeability, enabling a wide range of applications in academic and pharmaceutical research.

References

1. Cellular Dynamics International, Inc. Assaying iCell Endothelial Cells Barrier Function Using xCELLigence RTCA System Application Protocol. www.cellulardynamics.com/lit
2. Moy AB, Blackwell K, Kamath A. Differential Effects of Histamine and Thrombin on Endothelial Barrier Function through Actin-myosin Tension. Am J Physiol Heart Circ Physiol 282, H21-H29 (2002).

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