Cellular Dynamics international

iCell® Endothelial Cells
Application Protocol

Assessing Barrier Function:
xCELLigence RTCA Cardio System

Introduction

Proper regulation of endothelium barrier integrity is a fundamental feature of angiogenesis and vascular homeostasis. Dysregulation of this barrier has been associated with pathological conditions, such as inflammation and vascular diseases, tumor metastasis, and early stages in the pathogenesis of atherosclerosis.

iCell® Endothelial Cells, derived from human induced pluripotent stem cells, exhibit morphological, biochemical, and pathophysiological characteristics of a native human endothelium. Due to their human origin, high purity, functional relevance, and ease of use, these cells represent an optimal in vitro test system for vascular biology interrogations in basic research and drug development.

The xCELLigence RTCA Cardio System (RTCA System) is a non-invasive, label-free platform that utilizes impedance changes across the cell monolayer to indirectly measure cell-cell interaction, transient contractions, and cell layer permeability. iCell Endothelial Cells can be cultured and maintained on an E-Plate to form a stable monolayer amenable to measuring drug-induced perturbation effects on endothelial barrier function. Together, iCell Endothelial Cells and the RTCA System enable in vitro screening of compound effects on human endothelium permeability.

This Application Protocol describes how to handle iCell Endothelial Cells for use on the RTCA System and provides basic instructions for compound treatment, data acquisition, and analysis.

Required Equipment, Consumables, and Software

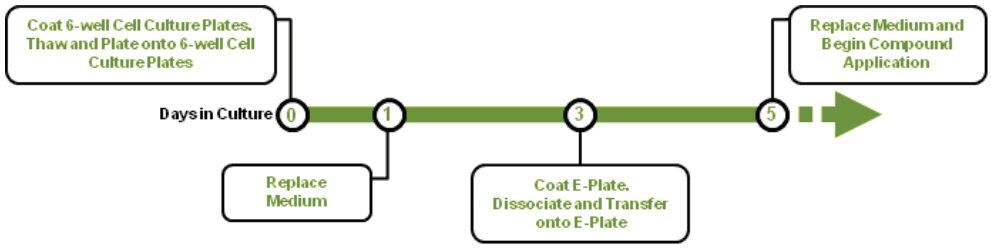
Notes

The following equipment and consumables are required in addition to the materials specified in the iCell Endothelial Cells User's Guide.

Item	Vendor	Catalog Number
Equipment		
12-channel Multichannel Pipettor, 20 and 200 µl	Multiple Vendors	
xCELLigence RTCA Cardio System	ACEA Biosciences	
Consumables		
iCell Endothelial Cells Kit	Cellular Dynamics International (CDI)	ECC-100-010-001
E-Plate Cardio 96 (E-Plate)	ACEA Biosciences	
Sterile Reagent Reservoir	Multiple Vendors	
Software		
RTCA Instrument Software	ACEA Biosciences	

Workflow

iCell Endothelial Cells are thawed and plated into a 6-well cell culture plate previously coated with fibronectin. On day 1 post-plating, spent medium is replaced with fresh Complete iCell Endothelial Cells Maintenance Medium. On day 3 post-plating, cells are dissociated and transferred to an E-Plate previously coated with fibronectin. 48 hrs post-plating onto the E-Plate, spent medium is replaced, cells are treated with compounds, and the barrier function recorded.



Methods

Culturing iCell Endothelial Cells

1. Prepare Complete iCell Endothelial Cells Maintenance Medium (Maintenance Medium) according to the iCell Endothelial Cells User's Guide.
2. Thaw and plate iCell Endothelial Cells into 6-well cell culture plates according to the User's Guide.
3. Incubate in a cell culture incubator at 37°C, 5% CO_2.
4. Maintain the endothelial cells for 3 days or until the cells reach confluency.

Notes

Preparing the E-Plate

1. Coat an E-Plate with fibronectin solution according to the iCell Endothelial Cells User's Guide.

Collecting iCell Endothelial Cells from the 6-well Cell Culture Plates

1. Passage iCell Endothelial Cells according to the iCell Endothelial Cells User's Guide.
2. Dilute the endothelial cell suspension in Maintenance Medium to a final concentration of 64,000 cells/ml.

Transferring iCell Endothelial Cells onto the E-Plate

1. Aspirate the fibronectin solution. Immediately add 150 µl/well of 37°C Maintenance Medium. Incubate in a cell culture incubator at 37°C, 5% CO_2 for 5 minutes.
2. Record a background measurement according to the RTCA Cardio Instrument Operator's Guide.
3. Invert the endothelial cell suspension 2 - 3 times. Transfer to a reagent reservoir.
4. Aspirate the Maintenance Medium from the E-Plate. Immediately add 150 µl/well of the endothelial cell suspension (30,000 cells/cm^2). Each well will have 9,600 viable endothelial cells.
5. Place the E-Plate in the biological safety cabinet at room temperature for 30 minutes to allow the endothelial cells to settle and ensure an even distribution.
6. Incubate in a cell culture incubator at 37°C, 5% CO_2.

   Note: *Place the E-Plate in a low traffic incubator and away from the door to minimize fluctuations in temperature and air movement. Minimize opening the incubator's door during the first 24 hours*.

Compound Application, Data Acquisition, and Data Analysis

The proliferation rate of iCell Endothelial Cells allows the cells to reach confluency in the E-Plate in 48 hours. After confluency occurs, inter-cell interactions within the monolayer can be measured and modulated by the addition of compounds.

The RTCA Cardio Instrument Software (RTCA software) offers various options for data acquisition and analysis. The instructions here are meant to provide general guidance. Refer to the RTCA Cardio Instrument Operator's Guide for specific instructions.

Compound Application

Notes

1. Monitor the endothelial cell growth on the E-Plate for the first 2 days post-plating to ensure cell confluency is reached.
2. Replace the Maintenance Medium at 48 hours post-plating. Tilt the E-Plate, remove 100 µl of Maintenance Medium, and add 130 µl of 37°C Maintenance Medium. Each well will have a final volume of 180 µl.

   Note: *A partial medium change with 37°C medium is recommended to minimize disturbing the endothelial cell layer and to facilitate the re-equilibration to the culture conditions.*
3. Incubate in a cell culture incubator at 37°C, 5% CO_2 for at least 1 hour.
4. Prepare test compounds in Maintenance Medium at 10X the final concentration in a regular 96-well cell culture plate

   Note: *Final DMSO concentrations above 0.1% should be used with caution. Therefore, if test compounds are dissolved in DMSO, the 10X compound solutions should not exceed 1% DMSO.*
5. Place the 96-well cell culture plate in a cell culture incubator at 37°C, 5% CO_2 to allow the compound solutions to equilibrate to the culture conditions.
6. Record a baseline measurement on the E-Plate immediately before compound application.
7. Quickly transfer 20 µl/well of the 10X compound solutions from the 96-well cell culture plate to the E-Plate. Each well will have a final volume of 200 µl.

   Note: *Minimize the time in which the E-Plate is kept outside of the incubator. A drop in Cell Index is usually observed when the E-Plate is removed from the incubator for a medium change or compound application.*
8. Record the compound effect immediately after compound application.

   Note: *The time needed to record the effect of a compound on the barrier function has to be determined empirically, depending on the drug, the concentration, and the treatment time.*

Data Acquisition and Analysis Using the RTCA Software

Refer to the RTCA Cardio Instrument Operator's Guide for specific instructions on using the RTCA software for data acquisition and analysis.

Example Data

The following figure illustrates the detection and analysis of barrier function in iCell Endothelial Cells using the RTCA software. Data are shown to exemplify the effects of modulating the barrier function by the application of compounds that have a direct effect on cytoskeletal remodeling.

Notes

The barrier function of iCell Endothelial Cells was challenged with thrombin, a potent edemagenic agent, which induced a rapid, dose-dependent disruption of the barrier integrity, as indicated by lower Cell Index values immediately after compound application. At a low concentration (0.5 U/ml), the disruption effect was partial and reversible, as the Cell Index values returned to baseline levels within 1 hour. At intermediate concentrations (1 U/ml and 1.5 U/ml), the disruption effect was complete, and the recovery phase lasted several hours without fully returning to baseline levels. At a high concentration (5 U/ml), the disruption effect was complete and irreversible.

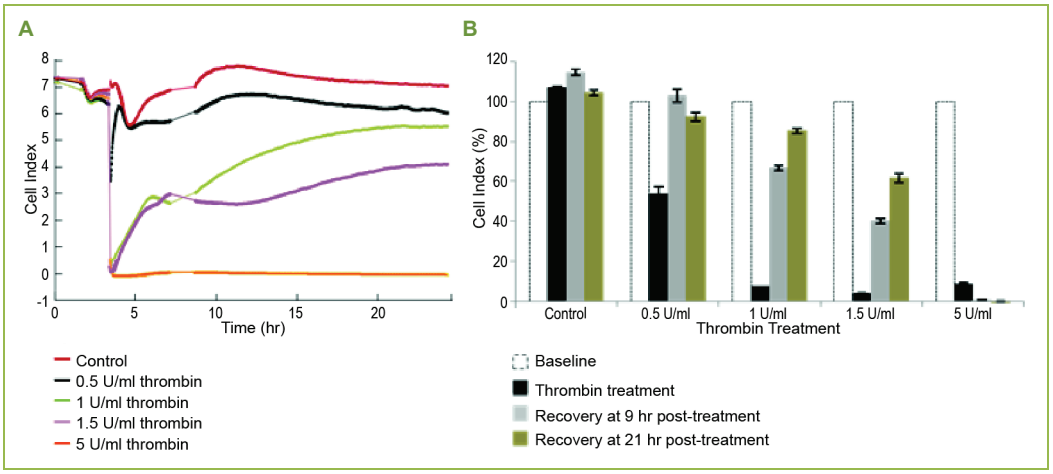


Figure 1: Barrier Disruption Effect of Thrombin on iCell Endothelial Cell
iCell Endothelial Cells were cultured for 48 hours on the E-Plate in Complete iCell Endothelial Cells Maintenance Medium before the addition of thrombin. (A) The representative Cell Index curves showed the dose-dependent disruption effect of thrombin on the barrier function and the recovery phase. (B) The percentage of Cell Index compared to pre-treatment baseline levels was calculated at the time of thrombin addition, at 9 hours post-treatment, or at 21 hours post-treatment (mean ± SD, n = 2 wells).

Summary

iCell Endothelial Cells provide an in vitro test system that recapitulates native human endothelium properties and functions while the xCELLigence RTCA Cardio System provides a label-free technology for non-invasive monitoring of cell behavior and viability. The methods and results presented here highlight how to gather relevant data on human endothelial viability and barrier function.

Version: May 2013
AP-ECBAR130508