APPLICATION NOTE

Modulation of Barrier in Primary Human Coronary Artery Endothelial Cells

INTRODUCTION

Endothelial cell barrier plays a crucial role in maintaining the integrity of blood vessels and regulating the exchange of substances between the bloodstream and surrounding tissues. Endothelial cells form a selective barrier that controls the passage of ions, nutrients, and immune cells, ensuring proper vascular function. Dysfunction in the endothelial barrier can lead to increased vascular permeability, contributing to various pathological conditions, including inflammation, edema, and vascular diseases. Understanding the mechanisms underlying endothelial barrier function is crucial for developing therapeutic strategies to address vascular disorders.

CytoTronics' technology enables measurement of endothelial barrier with high accuracy and sensitivity. Here, we show the development of an *in vitro* assay to model the human endothelial barrier using primary cells and show its transient modulation with Adenosine.

RESULTS

Primary human coronary aortic endothelial cells (HCAECs) are derived from the inner lining of coronary arteries and play a crucial role in regulation of coronary blood flow and cardiac function. A key player in this intricate regulatory system is Adenosine, which operates through specific receptors, notably Adenosine A2B, to enhance endothelial barrier integrity by promoting the formation of tight junctions between endothelial cells.[1] Additionally, Adenosine's anti-inflammatory properties contribute to its protective role in endothelial barrier function,[2] making it a potential target for therapeutic interventions aimed at mitigating vascular dysfunction in various pathological conditions.

In the context of biological barriers, the choice of substrate is key to promoting physiologically relevant phenotypes in cells. CytoTronics' microplates offer versatility by being compatible with a range of biological coatings. To model endothelial barrier in HCAEC cells, plates were pre-coated with fibronectin. Cells at low passages (<p5) were then seeded on the plates,[3] allowed to attach and grow. Figure 1 shows confluence and barrier measurements for HCAEC cells over the course of 72 hours. The cells have a low initial barrier signal, that slowly increases to reach steady state as the cells form junctions, and the cell sheet matures. This real-time assessment proves invaluable, particularly in dealing with primary cells where samples are limited, and variations between donors may exist. Ensuring a consistent starting state enables reliable and accurate assessment of the effect of modulations.

Once cells reached steady state confluency and barrier, they

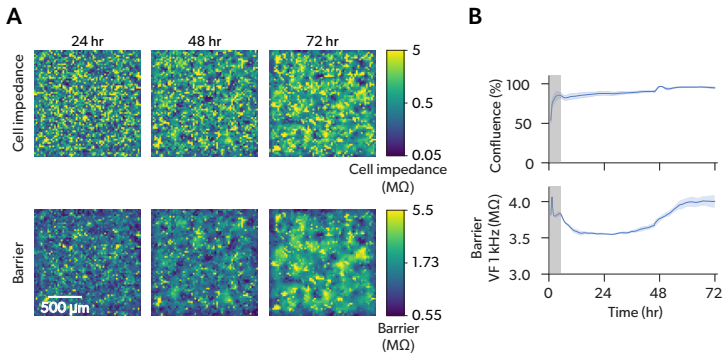

Figure 1. (A) Cell impedance (top) and barrier (bottom) images of HCAEC cells shown at 24, 48, and 72 hours after cell seeding. (B) Confluence and barrier for up to 72 hours post cell seeding. Shaded gray bar represents the time immediately post cell seeding during which the cells are settling and attaching to the microplate.

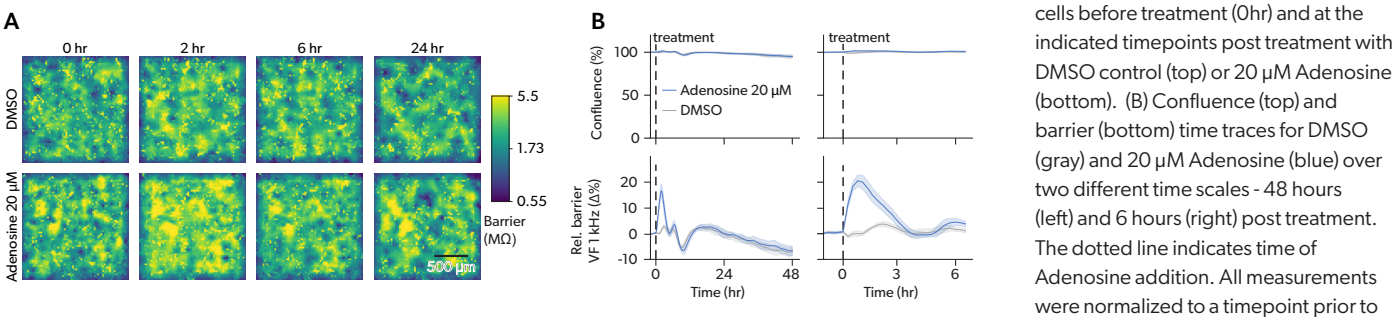

Figure 2. (A) Barrier images of HCAEC cells before treatment (0hr) and at the indicated timepoints post treatment with DMSO control (top) or 20 µM Adenosine (bottom). (B) Confluence (top) and barrier (bottom) time traces for DMSO (gray) and 20 µM Adenosine (blue) over two different time scales - 48 hours (left) and 6 hours (right) post treatment. The dotted line indicates time of Adenosine addition. All measurements were normalized to a timepoint prior to compound addition.

were treated with either 20 µM Adenosine or DMSO (control). Figure 2 shows the effect of Adenosine on barrier and cell confluence. Adenosine causes an increase in barrier that peaks at approximately 2-3 hours and returns to baseline by 6 hours post treatment. Of note, Adenosine does not affect confluence of the cell sheet over the entire experiment duration of 48 hours.

CONCLUSION

CytoTronics' 96-well microplates can measure a variety of impedance-based parameters in real-time at high-resolution. In this note, we highlight the ability of our platform to establish an *in vitro* assay of tissue barrier using primary endothelial cells. This approach can be extended to other endothelial cell types and may be used to model endothelial or vascular dysfunction. The scalability of the platform facilitates high-throughput screening, enabling the development of novel therapeutic strategies.

METHODS

Cell Lines and Culture

Primary human coronary artery endothelial cells (HCAEC) were obtained from Lonza (#CC-2585), maintained in a humidified incubator at 37°C and 5% CO_2, and cultured in EGM™-2 MV Microvascular Endothelial Cell Growth Medium-2 on fibronectin-coated tissue culture flasks (25 µg/mL).

Treatment and Measurement

Impedance measurements were taken inside a humidified incubator at 37°C and 5% CO_2 every 15 minutes throughout the experiment.

Cytotronics' microplates were initially coated with Fibronectin (25 µg/mL) prior to cell seeding. Cells were then seeded at 40k cells per well and grown for 72 hours until measurable barrier was detected. Adenosine (SelleckChem S1647) was dissolved in DMSO and added at 20 µM with a 0.1% (v/v) DMSO control using a half media exchange method. Briefly, half of the cell culture media was removed and a 2x solution of adenosine in media was added to the plate. Compound in media was temperature and CO_2 equilibrated prior to addition to cells.

Data Analysis

The well median of each measurement (barrier and confluence) was plotted over time, with the standard error calculated across 3 technical replicates. Confluence was calculated as a percentage of electrodes occupied by cells. To determine occupancy, the impedance response of electrodes without cells was measured. When impedance response increases above the bare electrode due to attachment of cells, the electrode is considered occupied. When applicable, data is normalized to a timepoint one hour before compound addition.

REFERENCES

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2. Xu, Y. et al. Regulation of endothelial intracellular adenosine via adenosine kinase epigenetically modulates vascular inflammation. *Nat Commun* **8**, 943 (2017).
3. Chitale, S. et al. A semiconductor 96-microplate platform for electrical-imaging based high-throughput phenotypic screening. *Nat Commun* **14**, 7576 (2023).

38 Wareham St, Boston, MA 02118

contact@cytotronics.com

cytotronics.com