Development of an image-based HCS compatible method for endothelial barrier function assessment

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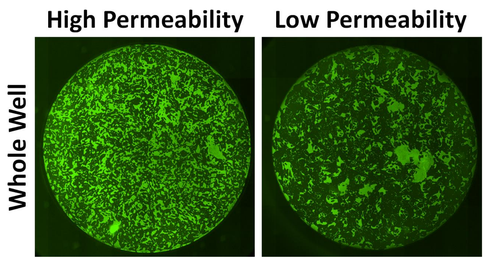
1. ABSTRACT

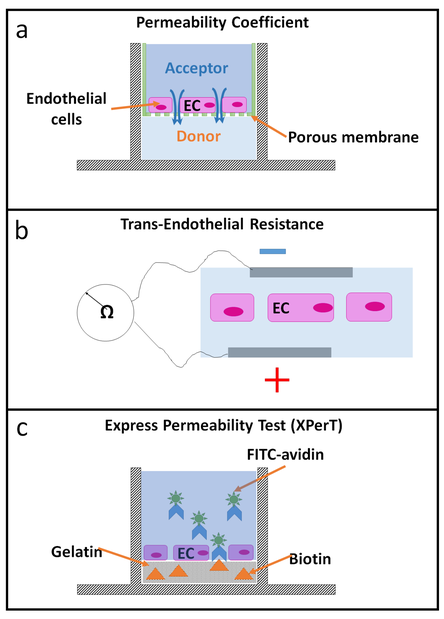
Acute respiratory distress syndrome (ARDS) is a serious condition with high mortality rate that has increased in the recent years due to the COVID-19 pandemic. Currently, effective pharmacological therapies have yet been discovered for ARDS, despite decades of laboratory and clinical studies. ARDS onset typically generates an increase in the endothelial permeability causing the development of pulmonary edema that leads to respiratory failure during the primary event. In this work, we propose the use of phenotypic drug discovery (PDD) approach in the search for effective ARDS treatment due to its ability to identify first-in-class drugs and deliver results when the exact molecular mechanism is partially obscure. However, the PDD approach requires novel cell-based assays compatible with high-throughput and high-content screening (HTS/HCS) capability. Here we demonstrate a novel fluorescence-based image cytometry method for directly determining endothelial barrier function. The image cytometry method simultaneously allows for rapid measurement of cell monolayer permeability and cell-based analysis. The time-dependent cell permeability showed an increase in human pulmonary artery endothelial cells (HPAEC) in response to the thrombin and TNF-α treatment, which correlates with previously published data obtained by trans-endothelial resistance measurements (TER). The incorporation of image cytometry in combination with digital image analysis can substantially reduce assay variability and improves the signal window. Furthermore, the proposed image cytometry method can be easily adapted for HTS/HCS applications, which may be extended to assay permeability of brain endothelium, gut epithelium, and other cell barriers for potential therapeutic discovery.

2. CELIGO IMAGING CYTOMETRY FOR CELL PERMEABILITY DETECTION



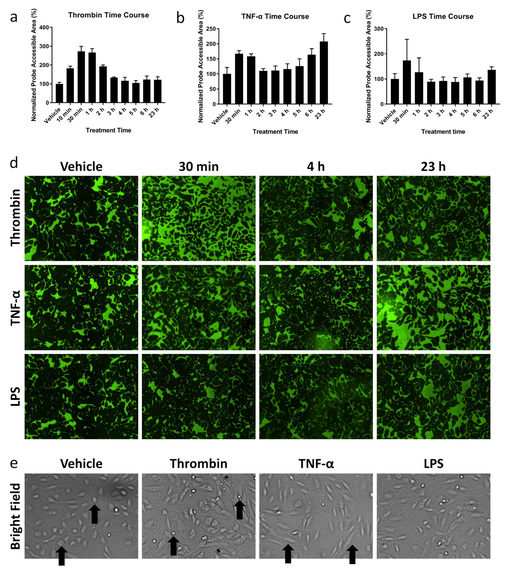
1. Celigo Imaging Cytometer is a plate-based cytometer that can scan the entire well of standard microplates and capture bright-field and fluorescent images
2. The captured images are analyzed with the Celigo software to measure size, morphology, cell count, confluence, and fluorescent intensity
3. Applications: Cell proliferation kinetic data, GFP/RFP expression, tumor spheroid size change, DNA cell cycle analysis, apoptosis, ADCC cytotoxicity, cell permeability and transwell assays.

3. ENDOTHELIAL BARRIER FUNCTION ASSESSMENT METHODS



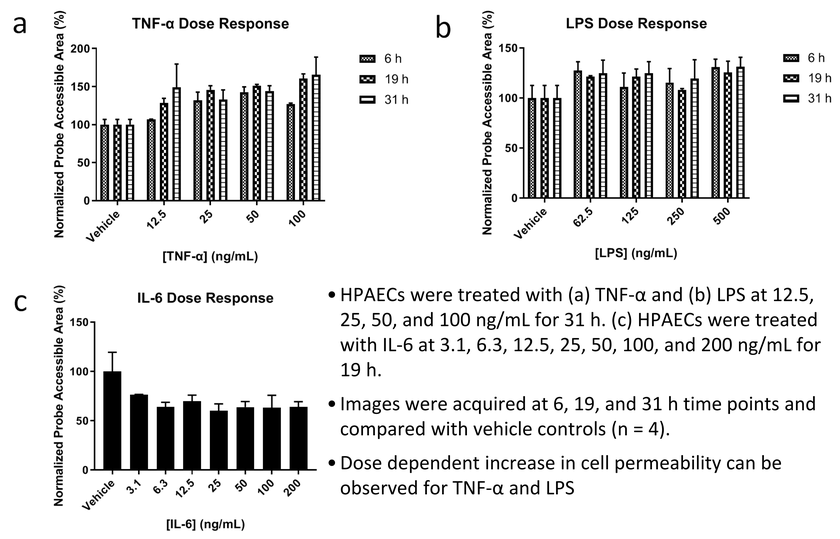
- (a) The permeability coefficient is measured by using a Transwell cell culture plate insert
  - A tracer dye is deposited in the acceptor compartment.
  - As it diffuses into the donor compartment, the permeability coefficient is calculated as a time-dependent function of the tracer concentration in the donor compartment.
- (b) The TER is used to measure the barrier function by determining the electrical resistance or impedance between the two electrodes.
- (c) The lateral diffusion is measured by using biotinylated gelatin-coated cell culture plates.
  - A fluorescein-labeled biotin is allowed to flow over the cells and bind to the biotinylated gelatin coated surface.
  - Unbound FITC–avidin is washed out and cells are fixed by a formaldehyde solution. The permeability is visualized and quantified by the FITC fluorescent area.

4. TIME-COURSE ASSAY WITH THROMBIN, TNF-α, AND LPS TREATED CELLS



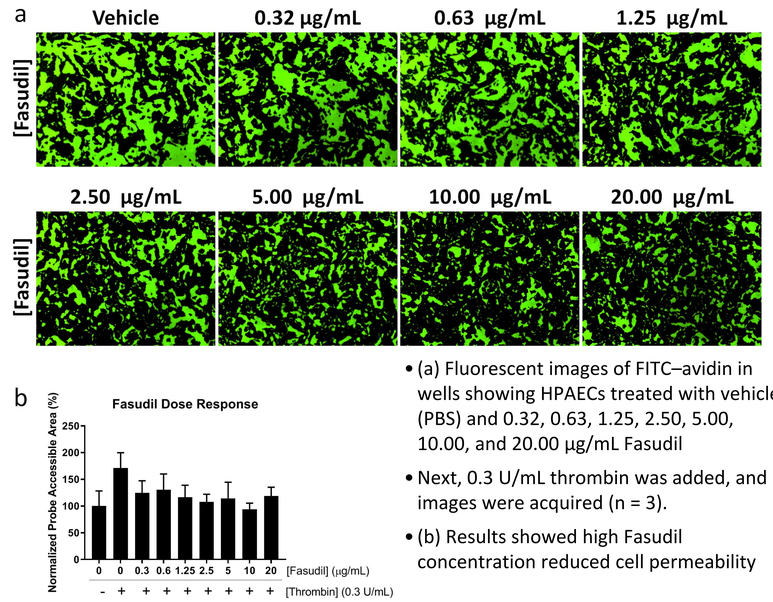
- The XPerT assay was performed with (a) thrombin, (b) TNF-α, and (c) LPS-treated HPAECs for 23 h and were imaged with Celigo at 0.5, 1, 2, 3, 4, 6, and 23 h.
- (d) Time-dependent green fluorescent images showing an increase or decrease in cell permeability
  - Thrombin showed a large fluorescent area between 0.5 and 2 h, and both TNF-α and LPS showed a larger area at 23 h.
- (e) Bright-field images showing morphological changes due to the soluble factors
  - Vehicle, round;
  - Thrombin, smaller;
  - TNF-α, elongated;
  - LPS, both morphologies

5. DOSE-DEPENDENT ASSAY WITH TNF-α, LPS, and IL-6 TREATED CELLS



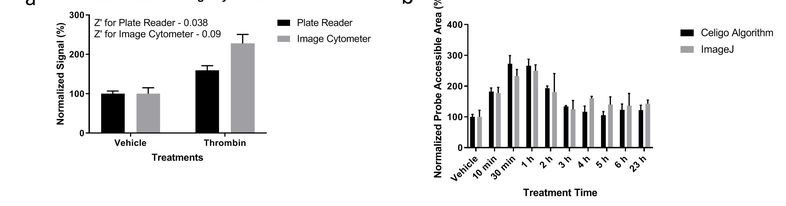
- HPAECs were treated with (a) TNF-α and (b) LPS at 12.5, 25, 50, and 100 ng/mL for 31 h. (c) HPAECs were treated with IL-6 at 3.1, 6.3, 12.5, 25, 50, 100, and 200 ng/mL for 19 h.
- Images were acquired at 6, 19, and 31 h time points and compared with vehicle controls (n = 4).
- Dose dependent increase in cell permeability can be observed for TNF-α and LPS

6. DETECTION OF CELL PERMEABILITY INHIBITION USING FASUDIL



- (a) Fluorescent images of FITC–avidin in wells showing HPAECs treated with vehicle (PBS) and 0.32, 0.63, 1.25, 2.50, 5.00, 10.00, and 20.00 µg/mL Fasudil
- Next, 0.3 U/mL thrombin was added, and images were acquired (n = 3).
- (b) Results showed high Fasudil concentration reduced cell permeability

7. CELL PERMEABILITY MEASUREMENT IS COMPARABLE WITH CELIGO



- (a) Comparison results between the image cytometer and plate reader. Celigo showed improved assay quality Z' value
- (b) Comparison results between Celigo software and ImageJ analysis for cells treated with thrombin. Celigo showed lower standard deviation.

8. SUMMARY AND CONCLUSION

- The Celigo was able to rapidly image and determine cell permeability using the XperT assay
- The image cytometry method showed improved assay quality
- The Image cytometry method showed more precise results compared to traditional method
- Future work is to design the assay for 384-well plates to further increase the throughput