

A Real-Time Multiplexed Assay for Evaluating Compound-Induced Disruption of Endothelial Barrier Integrity

Jing Zhang and Xiaoyu Zhang
Agilent Technologies, La Jolla, CA, USA

Introduction

Cellular barriers, including epithelial and endothelial layers, serve as protective interfaces that tightly regulate the passage of ions, molecules, and pathogens. When compromised, increased permeability contributes to inflammation, vascular injury, and compound-induced toxicity. Accurate assessment of barrier integrity is therefore critical for evaluating cytotoxicity and permeability-related effects. However, conventional assays primarily provide end point measurements and lack real-time kinetic resolution.

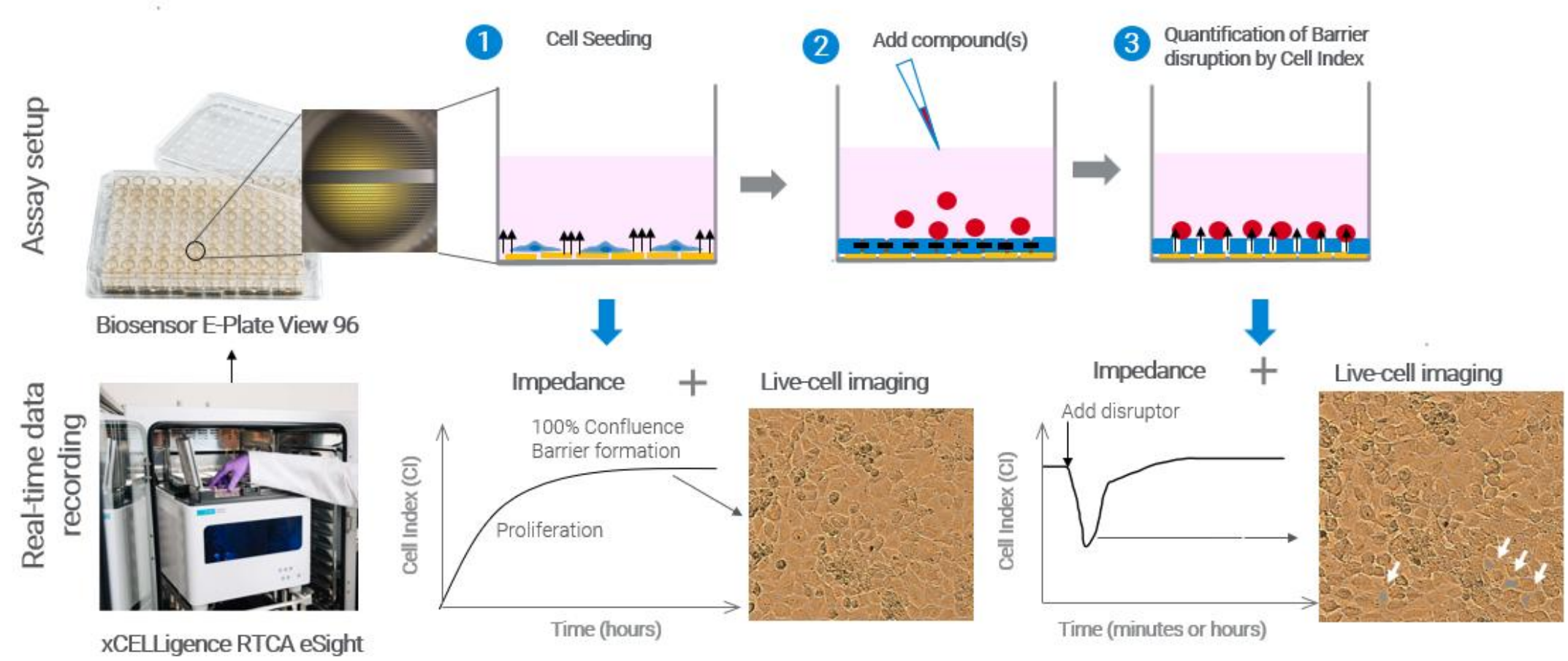
In this study, we introduce a real-time, multiplexed assay that combines impedance monitoring and live-cell imaging using the Agilent xCELLigence Real Time Cell Analysis (RTCA) eSight system. EA.hy926 endothelial cells were seeded at 5,000 cells per well and monitored continuously to model barrier formation and disruption. Barrier integrity was quantified by impedance measurements reported as Cell Index.

Thrombin, a well-established barrier disruptor, induced a dose-dependent and reversible decline in Cell Index. Live-cell imaging confirmed morphological changes with increased intercellular gaps, indicating elevated permeability rather than reduced viability. Pretreatment with Argatroban, a direct thrombin inhibitor, prevented the thrombin-induced impedance decline and preserved barrier integrity.

The impedance- and imaging-based platform was further validated by FITC-dextran permeability assays and ZO-1 immunofluorescence staining. Together, these data demonstrate a robust and integrated approach for real-time assessment of endothelial barrier dysfunction with the xCELLigence RTCA eSight system.

Materials and Methods

Workflow for the xCELLigence real-time barrier function assay



EA.hy926, an immortalized human vascular endothelial cell line purchased from ATCC (part number CRL-2922), was seeded at 5,000 cells per well on a fibronectin-coated Agilent E-Plate VIEW 96 (Agilent, part number 300601030). Cells were cultured until a tight and confluent cell monolayer was formed, as indicated by a plateau in Cell Index and 100% confluency in the cell image. Following the addition of barrier modulator(s), the barrier integrity was immediately monitored using the Agilent xCELLigence RTCA eSight system and automatically quantified by the system software.

Results and Discussions

Real-time monitoring of endothelial barrier disruption on the xCELLigence RTCA eSight system through impedance and live-cell imaging

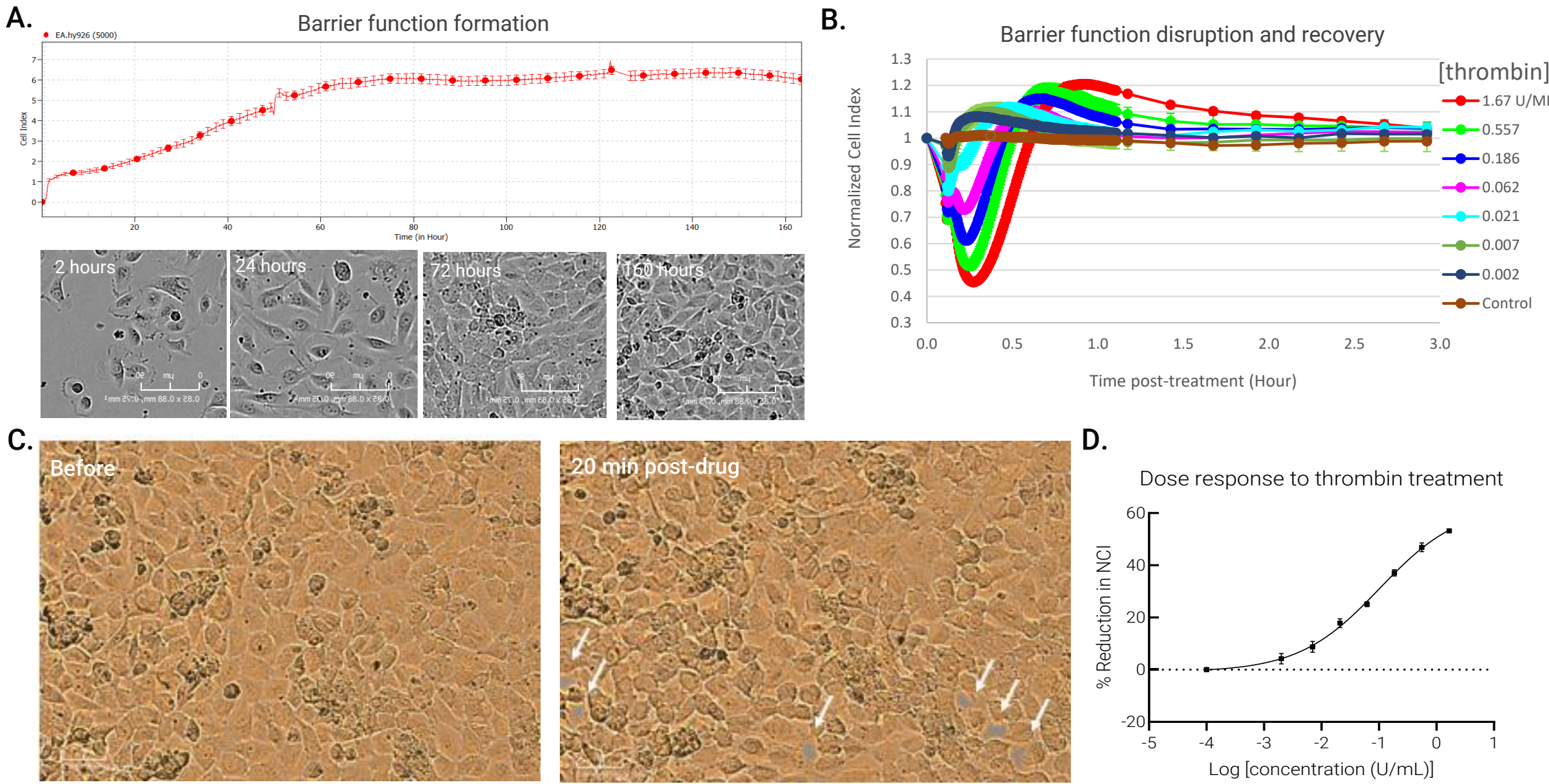


Figure 1. Disruption of EA.hy926 barrier integrity was monitored in real time on the Agilent xCELLigence RTCA eSight system. **A)** EA.hy926 cells formed a stable endothelial monolayer, as indicated by progressive Cell Index increase and plateau formation. Representative brightfield images at 2, 24, 72, and 160 hours illustrate cell attachment, proliferation, and development of a confluent endothelial barrier. **B)** Real-time impedance measurements revealed rapid, dose-dependent reductions in normalized Cell Index after thrombin treatment compared with vehicle control. **C)** Brightfield imaging with segmentation mask confirmed intact morphology prior to treatment and visible junctional disruption, evidenced by grey color areas indicated with the arrows, at the lowest Cell Index following thrombin exposure. **D)** Dose-response analysis demonstrated a reproducible concentration-dependent decrease in barrier integrity (mean $\pm$ SEM, n = 3).

Argatroban preserves endothelial barrier integrity following thrombin challenge

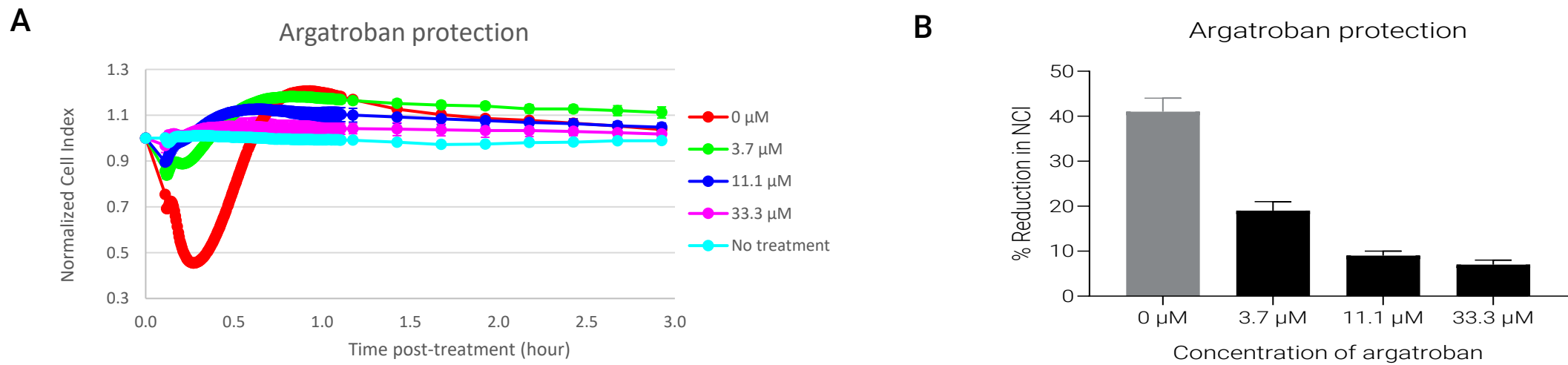


Figure 2. Argatroban preserves endothelial barrier integrity following thrombin challenge as monitored in real-time on the Agilent xCELLigence RTCA eSight system. **A)** EA.hy926 monolayers were pretreated with Argatroban prior to thrombin exposure. Real-time impedance monitoring demonstrated dose-dependent attenuation of thrombin-induced reductions in normalized Cell Index. **B)** Quantitative analysis derived from impedance curves showed that Argatroban significantly attenuated thrombin-induced barrier disruption in a concentration-dependent manner (mean $\pm$ SEM, n = 3).

Results and Discussion

Independent confirmation of barrier function disruption

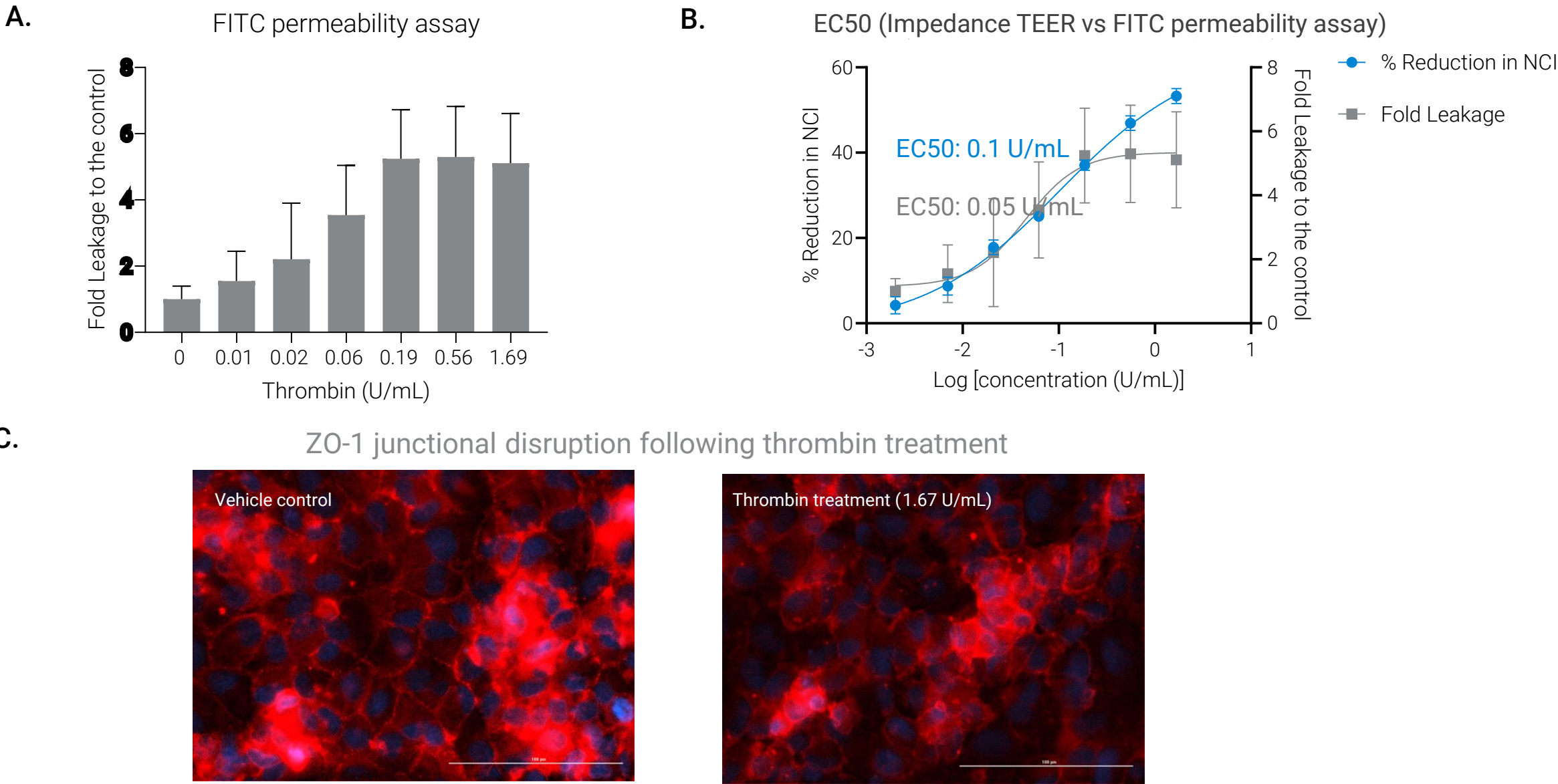


Figure 3. Conventional permeability and immunofluorescence assays validate thrombin-induced endothelial barrier disruption measured by impedance using the Agilent xCELLigence RTCA eSight system. **A)** EA.hy926 cells were seeded at a density of 2,500 cells per well on fibronectin-coated transwell inserts and cultured for seven days. Dose-dependent thrombin-induced barrier leakage was quantified by fluorescence reading using an Agilent BioTek Synergy H1 multimode plate reader after a 30-to-40-minute incubation with thrombin and FITC-dextran. **B)** EC₅₀ values for thrombin-mediated barrier disruption were comparable between the impedance-based readout on the xCELLigence RTCA eSight system and the FITC-dextran permeability assay. **C)** The cells were immediately fixed and stained with ZO-1 antibodies when the Cell Index showed maximal reduction after thrombin treatment. The fluorescent images captured with a 40x objective on an Agilent BioTek Lionheart FX automated microscope revealed tight junction disruption following thrombin treatment.

Conclusions

- Thrombin induces a rapid, dose-dependent disruption of the endothelial barrier, evidenced by a real-time decline in impedance and the appearance of sparse gaps in the cell monolayer captured by live-cell imaging on the Agilent xCELLigence RTCA eSight system.
- Argatroban prevents thrombin-mediated tight junction remodeling, as shown by the attenuated decline in normalized Cell Index following thrombin treatment, demonstrating pharmacological specificity.
- Assessment of barrier integrity by impedance correlates strongly with increased permeability measured by the FITC-dextran assay and with ZO-1 fragmentation, providing both functional and molecular validation.
- Continuous monitoring barrier function using impedance measurement resolves the full kinetics of barrier disruption and recovery.
- The xCELLigence RTCA eSight advances a dynamic, multiparametric workflow for investigating barrier function and supporting therapeutic discovery.