>> Development and Functional Assessment of iPSC-derived Endothelial Cells using a Novel Non-Invasive Workflow

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Kinetic and Dynamic Cell Tracking

Omni: Automated whole-vessel imaging

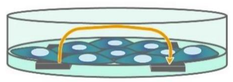
In vitro models are essential for studying diseases and development. While traditional 2D cell culture models have provided valuable insights, they often fail to replicate in vivo complexity. This has led to increased interest in 3D models such as spheroids and organoids, which better mimic in vivo conditions.

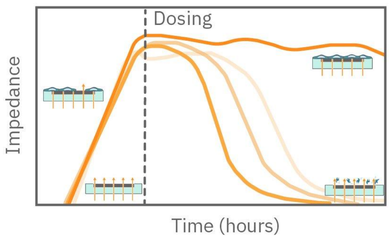
Live-cell provides a powerful technique for studying these 3D models, enabling real-time visualization and analysis at defined time intervals.

Maestro Z: Impedance Technology

Cell-based in vitro trans-endothelial electrical resistance (TEER) assays are a valuable tool for monitoring barrier integrity across endothelial cell layers. Many in vitro TEER methods require low-throughput, electrode-by-electrode measurements. Maestro Z automates TEER acquisition across 96 wells simultaneously.

Axion BioSystems' Maestro Z platform offers impedance-based cell analysis for label-free, noninvasive impedance recording for long-term barrier monitoring. Here, we used the Maestro Z to characterize a TEER assay for high-throughput screening and dose response analysis.

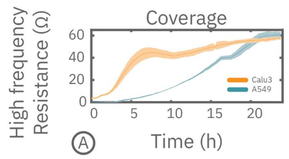


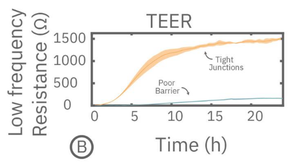


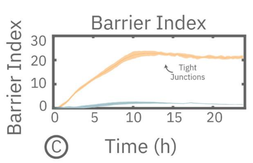
TEER assays measure the electrical resistance across a cell layer. As epithelial or endothelial cells form tight junctions, they restrict ion movement, increasing resistance and indicating stronger barrier integrity. Maestro Z simultaneously measures cell coverage and barrier resistance using multi-frequency impedance, enabling accurate normalization of TEER signals.

TEER on the Maestro Z

Maestro Z TEER assays provide kinetic insight into barrier biology by capturing both gradual barrier maturation and rapid permeability changes. By measuring TEER continuously, researchers can distinguish true barrier formation from simple cell coverage and detect transient disruptions, recovery dynamics, and mechanism-specific responses that are missed by endpoint measurements.







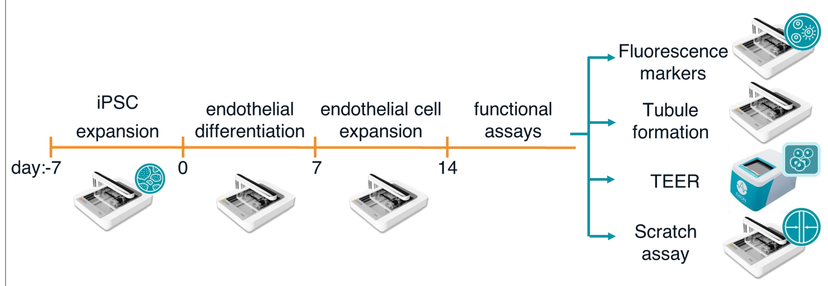
Real-time Monitoring of iPSCs

Background

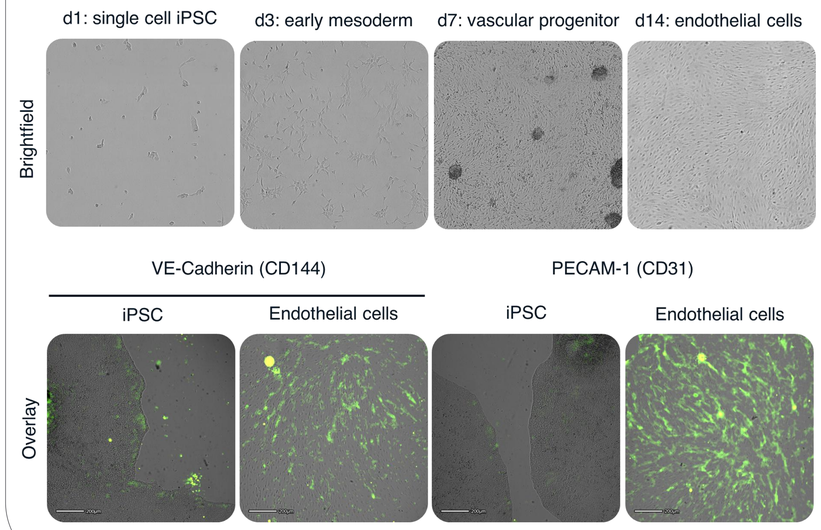
Induced pluripotent stem cells (iPSCs) are widely used in studies of embryonic development, disease modelling, and tissue engineering. A promising application for iPSCs is the generation of endothelial cells, critical for forming blood vessels within larger tissue. Effective differentiation of iPSCs into mature, functional endothelial cells presents several challenges, requiring monitoring of the differentiation process and recapitulating complex vascular networks. This study presents a novel non-invasive workflow for analysis and functional testing of iPSC-derived endothelial cells (id-ECs).

iPSC Differentiation

iPSCs (STEMCELL Technologies) were differentiated into endothelial cells using the suppliers' 14-day protocol. Functional assessment of id-ECs was performed to validate important endothelial behavior like migration (scratch assay), tubule formation and barrier integrity (TEER). The actin polymerization inhibitor Cytochalasin D (CytoD) was added at various concentrations to validate functionality.



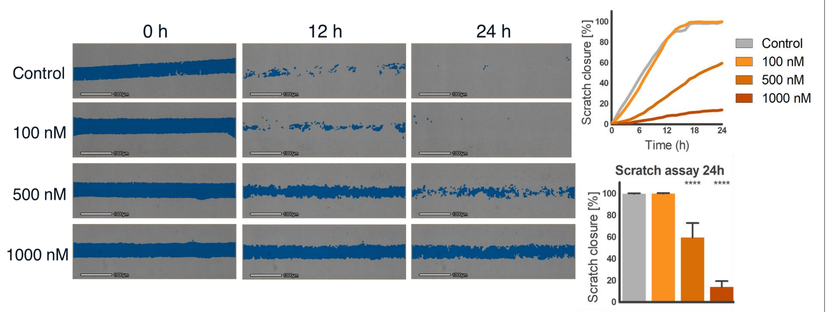
Morphological changes (e.g. size and shape) were monitored using the Omni live-cell imager. Endothelial differentiation was verified via fluorescence staining of the endothelial marker CD31 and the absence of stem cell markers SSEA-4 and TRA-1-60.



iPSC-Endothelial Workflow

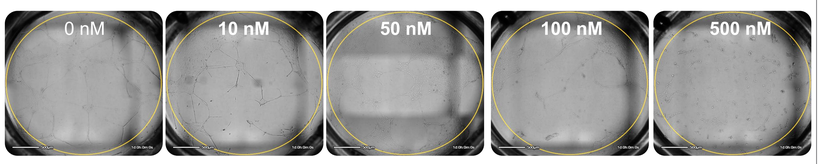
Cell Migration

Brightfield imaging of the scratch assay using the Omni showed 100% wound closure by id-ECs within 24 hours. Scratch closure was reduced 59% and 86% by 500 nM and 1000 nM CytoD, respectively.



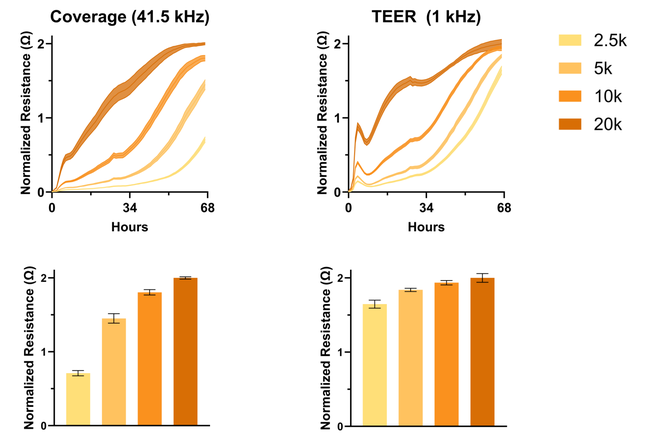
Tubule Formation Assay

Interconnected tubular networks, characterized by long tubules and large mesh sizes were formed in the tubule formation assay. The addition of CytoD disrupted this in a dose-dependent manner, reducing tubule length and mesh integrity from CytoD concentrations as low as 50 nM.



Barrier Formation

Coverage measurements using the Maestro Z indicate a density-dependent barrier formation, with values increasing by 11.7% (5k cells), 17.5% (10k cells), and 21.4% (20k cells) compared to 2.5k cells.



Conclusion

This non-invasive workflow combines live-cell imaging and real-time impedance measurements for efficient monitoring and functional validation of id-ECs, supporting advancements in vascularized tissue engineering and iPSC-based regenerative medicine.