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

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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.

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The Omni platform software modules simplify assay setup, offer real-time cellular visualization, and enable fast analysis.

- Cell Confluence
- Clonogenic Assay
- Scratch Assay
- Organoid Analysis
- Fluorescence Analysis
- iPSC Monitoring

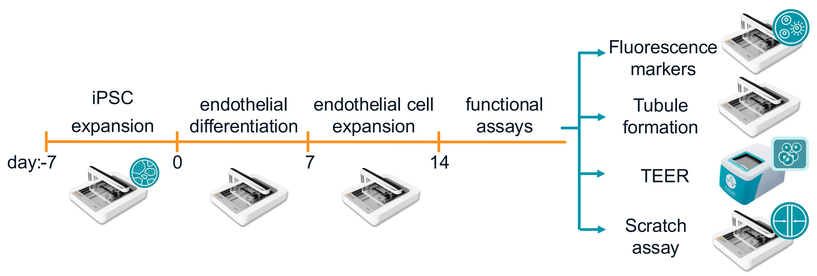
Real-time Monitoring of iPSCs

Background

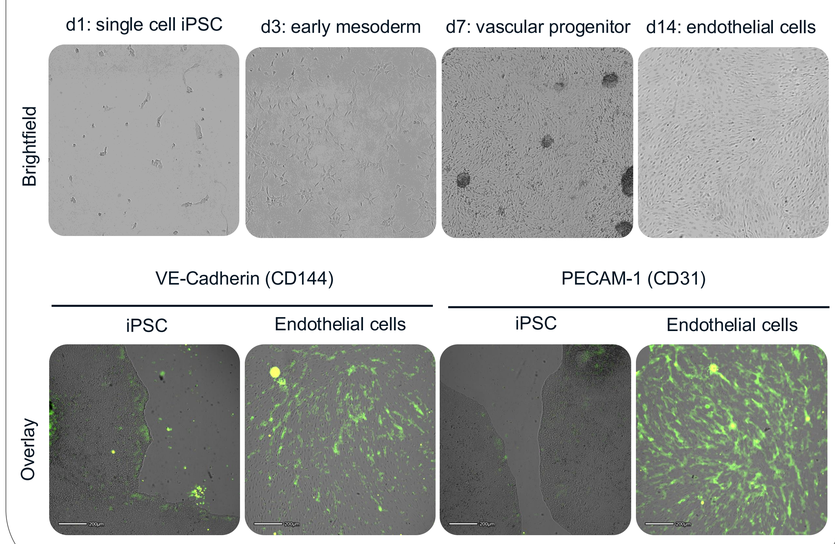
Induced pluripotent stem cells (iPSCs) are widely used in studies of embryonic development, disease modelling, and tissue engineering. A promising application for iPSCs is the generation of endothelial cells, critical for forming blood vessels within larger tissue. Effective differentiation of iPSCs 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.



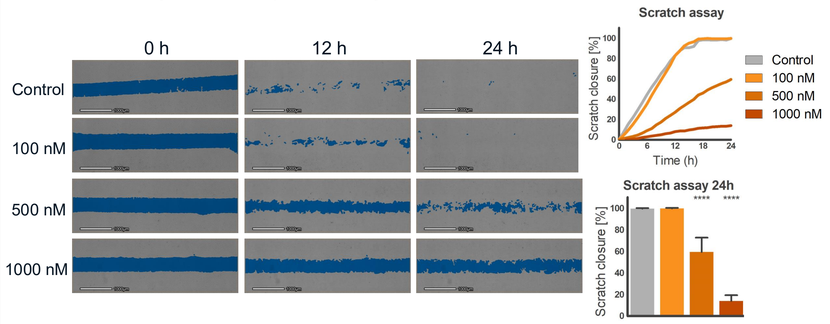
Morphological changes (e.g. size and shape) were monitored using the Omni live-cell imager. Endothelial differentiation was verified via fluorescence staining of the endothelial marker CD31 and the absence of stem cell markers SSEA-4 and TRA-1-60.



iPSC-Endothelial Workflow

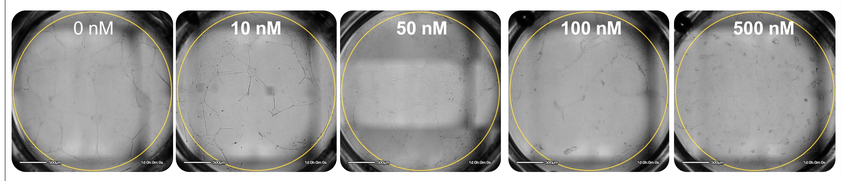
Cell Migration

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.



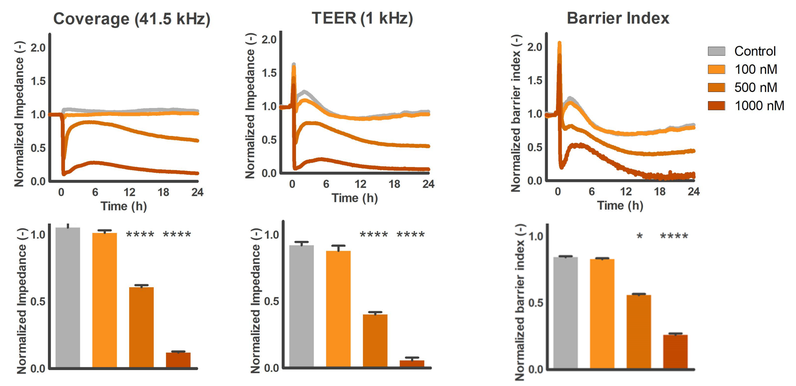
Tubule Formation Assay

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.



TEER

TEER measurements with the Maestro Z revealed that CytoD reduced barrier integrity, with barrier index values (TEER normalized to confluency) dropping by 34% (500 nM) and 69% (1000 nM), correlating with tight junction disruption.



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.