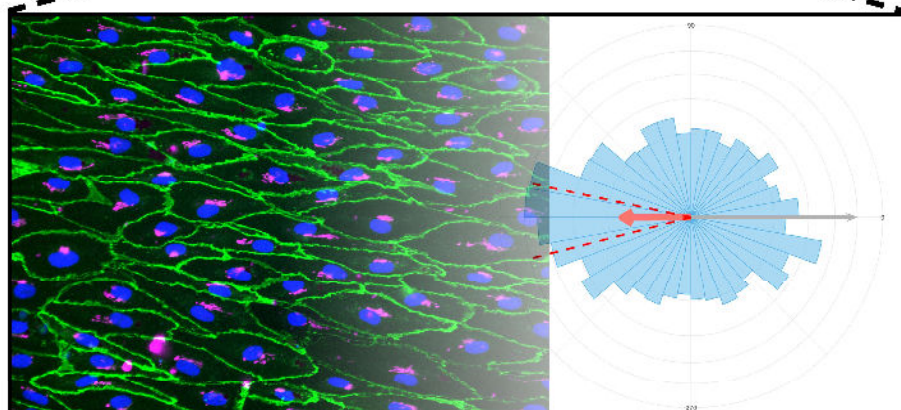
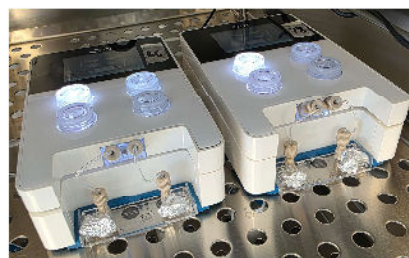


HUMAN IPSC-BASED VASCULAR *IN VITRO* MODELING: UNIDIRECTIONAL AND CONTINUOUS FLOW WITH RECIRCULATION USING FLUIGENT OMI™

This application note was made in collaboration with dr. Dhanesh Kasi, dr. Hanna Lammertse and dr. Valeria Orlova from the Leiden Organ-on-Chip Center and the Orlova group at the Leiden University Medical Center.

Unidirectional flow is essential for relevant and accurate **Vessel-on-Chip** and **other vascularized *in vitro*** models. However, conventional microfluidic setups can be complicated and labor-intensive to operate, whereas rocking platforms only support bidirectional flow. [Fluigent's Omi](#) is a compact and easy-to-use microfluidic system that allows researchers to connect their **Organ-on-Chip model** of interest and set up microfluidic unidirectional flow experiments with **recirculation**. Using Omi, we achieved unidirectional and continuous flow over the course of five days in microfluidic chips (Beonchip Be-Flow) that were seeded with *human induced pluripotent stem cell-derived endothelial cells (hiPSC-ECs)*. Alignment along the flow direction and collective polarization were achieved in chips that underwent **unidirectional flow** while these phenomena were absent in chips that were kept static or underwent bidirectional flow. The data indicates that the Omi platform can maintain **stable and unidirectional flow profiles over a prolonged period** of time and that a relevant endothelial phenotype can be induced, while continuously recirculating the small volume of culture medium. The user-friendly Omi platform is therefore suited for engineering physiologically relevant hiPSC-based vascular *in vitro* models.



1. INTRODUCTION

In vitro models have the potential to revolutionize biomedical research by complementing, reducing and potentially replacing animal experimentation. Combined with human induced pluripotent stem cells (hiPSCs) and differentiation protocols to derive organ-specific cells, advanced human *in vitro* models can be engineered to develop new drugs, to unravel human diseases, and to enable personalized medicine^{1,2}. Microfluidic Organ-on-Chip (OoC) models are especially suited for this because they contain living human cells in perfused, micrometer-sized chambers that mimic tissues and organs functions in a miniaturized format¹⁻³.

Controlled and physiological flow conditions are crucial for Vessel-on-Chip (VoC) and other vascularized *in vitro* models that aim to replicate stable vascular physiology, as continuous, laminar shear stress is a characteristic of healthy arteries and veins. Specifically, flow needs to be unidirectional for inducing and maintaining a quiescent, mature endothelial phenotype, including cell elongation, alignment parallel to the flow, polarization of golgi-nucleus orientation, robust barrier function, and anti-inflammatory gene expression^{4,5}.

In contrast, static (no flow) conditions, which are typical of traditional 2D cell culture, fail to provide this characteristic mechanical stimulation, leading to endothelial cells (ECs) with a non-aligned and often cobblestone morphology, reduced junctional integrity, and a generally non-physiological state. Similarly, bidirectional flow is suboptimal for vascular modeling, as it is also non-physiological and mimics disturbed flow patterns that are primarily associated with pathological conditions^{4,6}.

Fluigent's Omi OoC platform is a compact microfluidic pump system that allows researchers to connect their OoC model of interest and set up microfluidic flow experiments.

In this application note, we performed a 5-day unidirectional flow experiment using the Omi system connected to Beonchip Be-Flow chips that were seeded with hiPSC-derived ECs^{7,8}. This condition was compared to chips that were static and to chips that underwent bidirectional flow on a rocking platform. Unidirectional flow resulted in elongated and polarized ECs that aligned parallel to the flow direction, while static and bidirectional conditions resulted in no alignment. In addition, Omi was able to maintain the preconfigured flow profiles over 5 days. The data illustrate that Omi supports continuous perfusion and recirculation with the correct flowrates over a prolonged period of time, thereby providing opportunities for relevant vascular *in vitro* modeling.

2. MATERIALS & METHODS

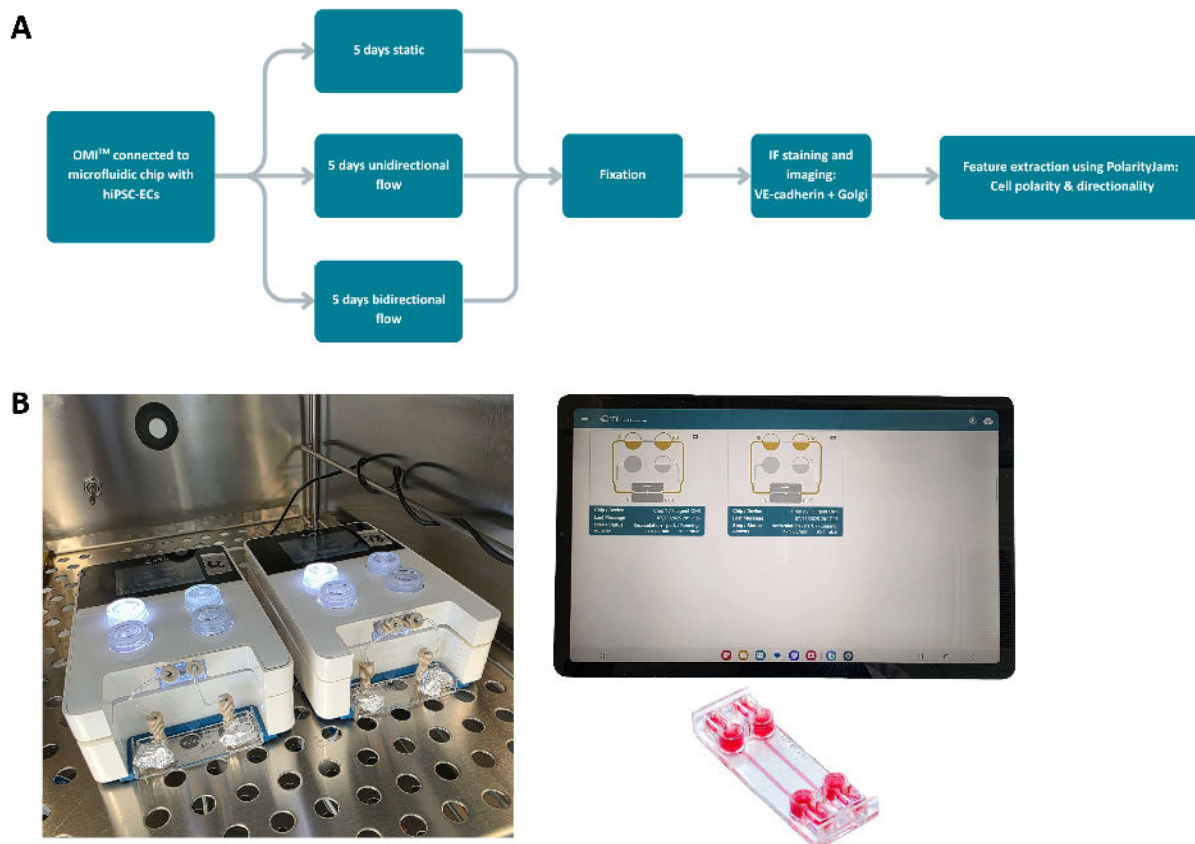


Figure 1. Overview of setup

(A) Workflow that was used in this application note.

(B) Left, two Fluigent Omi's with connected microfluidic chips running inside an incubator. Right, top, tablet with intuitive interface that controls Omi. Right, bottom, Beonchip Be-Flow chip that was used in this application note

2.1 Cell differentiation and culture + cell seeding

hiPSCs were maintained on recombinant vitronectin-coated plates in TeSR-E8 medium and passaged once a week. hiPSC-ECs were derived and maintained as previously described by the Orlova group^{7,8}. Beonchip Be-Flow microfluidic chips were coated with 50 µg/mL bovine fibronectin. hiPSC-ECs were dissociated and resuspended at a concentration of 5×10^6 cells/mL in EGM-2 supplemented with PenStrep. 50 µl of cell suspension was carefully pipetted into the chip inlets causing the cells to be passively pumped inside the chip. Cells were allowed to attach for 1 hour at 37 °C after which the chips were transferred to a rocking platform for 15 hours (overnight). The next day, experiments were started by putting the chips either static, keeping them on the rocking platform (bidirectional flow) or connecting them to Omi (unidirectional flow).

2.2 Omi Setup

An automated 5-day recirculation protocol was created using the Omi software and loaded onto the Omi device (Figure 2A). The software guides each step through built-in, step-by-step instructions, including:

1. Calibration: Setting the reservoir level sensors to ensure accurate flow measurements.
2. Sterilization: Flushing the Omi fluidic path and experimental tubing with water followed by 70% ethanol. Note: Prior to use, all connectors and tubing were sterilized either by autoclaving or by storage in a sterilizing solution.
3. Loading: Pre-filling the fluidic path with culture medium to remove air bubbles and ensure continuous flow.
4. Chip Connection: Omi was connected to the microfluidic chip (Beonchip Be-Flow) prior to recirculation (Figure 1B).

Microfluidic chips (Beonchip Be-Flow) containing the hiPSC-ECs were connected to Omi using standard luer fittings and microfluidic tubing for a leakproof connection (Figure 1B, left image). Multiple Omi's could simultaneously be controlled by an intuitive Android app that runs on a tablet (Figure 1B, right image). This app also enables users to configure flow profiles and to perform calibration and cleaning procedures. After configuring the desired flow profiles and timeframes (Figure 2A), a small amount of medium was added to Omi (3.3 mL). Finally, the experiment was started, and Omi was transferred to an incubator (Figure 1B, left image).

2.3 Immunofluorescence and imaging

Samples were fixed, permeabilized, blocked and incubated with primary antibodies overnight (VE-cadherin and GM130) after which they were stained with secondary antibodies and DAPI. Samples were imaged using an EVOS M7000 microscope (Thermo Fisher Scientific) with a 20X objective. Six images were taken at random locations in each channel. Four technical replicates per condition were used.

2.4 Quantification of hiPSC-EC alignment and polarization

Immunofluorescence images were analyzed using PolarityJam⁹ to quantify alignment and polarization of hiPSC-ECs. All images were analyzed in PolarityJam and the data were pooled to graph the orientation and polarization graphs.

3. RESULTS

3.1 Stable unidirectional flow profiles with recirculation

To support the hiPSC-ECs and gently promote alignment parallel to the flow direction, the flow profiles were configured as listed in Figure 2A. The flowrate was increased every 24 hours and was kept at a maximum rate of 200 $\mu\text{L}/\text{min}$, corresponding to a shear stress of $0.912 \text{ dyn}/\text{cm}^2$, for 48 hours. Omi was able to maintain these flowrates continuously and could switch to higher flowrates at the specified timepoints in a stable manner (Figure 2B).

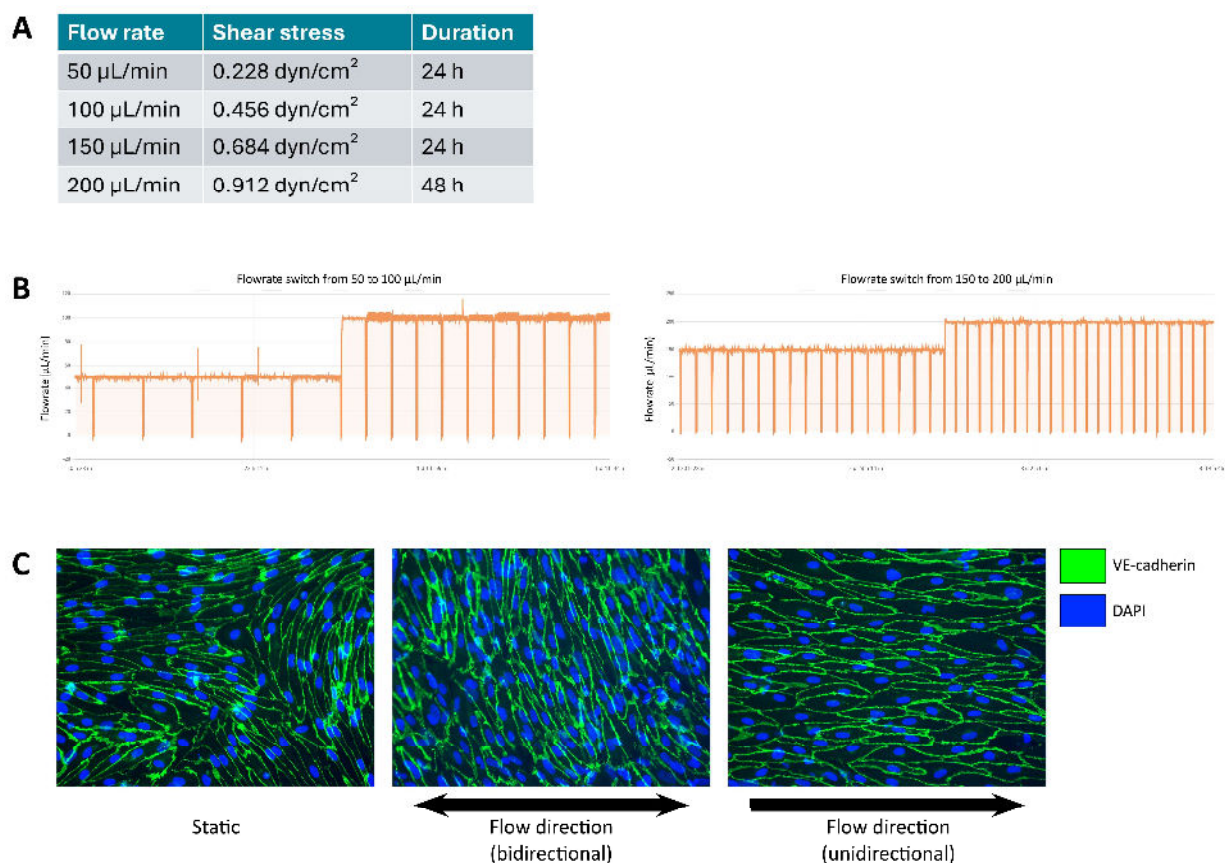


Figure 2. Flow control and endothelial response under different conditions

(A) Table of flowrates and time intervals used in the experiments.

(B) Representative flow traces demonstrating that Omi is able to switch between different flowrates and maintain them over prolonged periods of time, while providing recirculation.

(C) Immunofluorescence images of hiPSC-ECs cultured static, under bidirectional (using a rocking platform), and under unidirectional flow (using Omi).

It should be noted that each recirculation cycle is followed by a refill during which the flow is halted for up to a minute as can be seen by the drops to $0 \text{ }\mu\text{L}/\text{min}$ in the graph. Due to the recirculation feature, no additional medium was required during the experiment. The unidirectional flow resulted in strong alignment of hiPSC-ECs parallel to the flow as is evident by qualitative observation after immunostaining against VE-cadherin (Figure 2C).

3.2 Quantification of hiPSC-EC alignment and polarization

Using PolarityJam[®], hiPSC-EC alignment and polarization could be quantified (Figure 3A). After five days, hiPSC-ECs were strongly polarized and aligned parallel to the flow direction when unidirectional flow was applied using Omi (Figure 3A, left image and Figure 3B, top graphs).

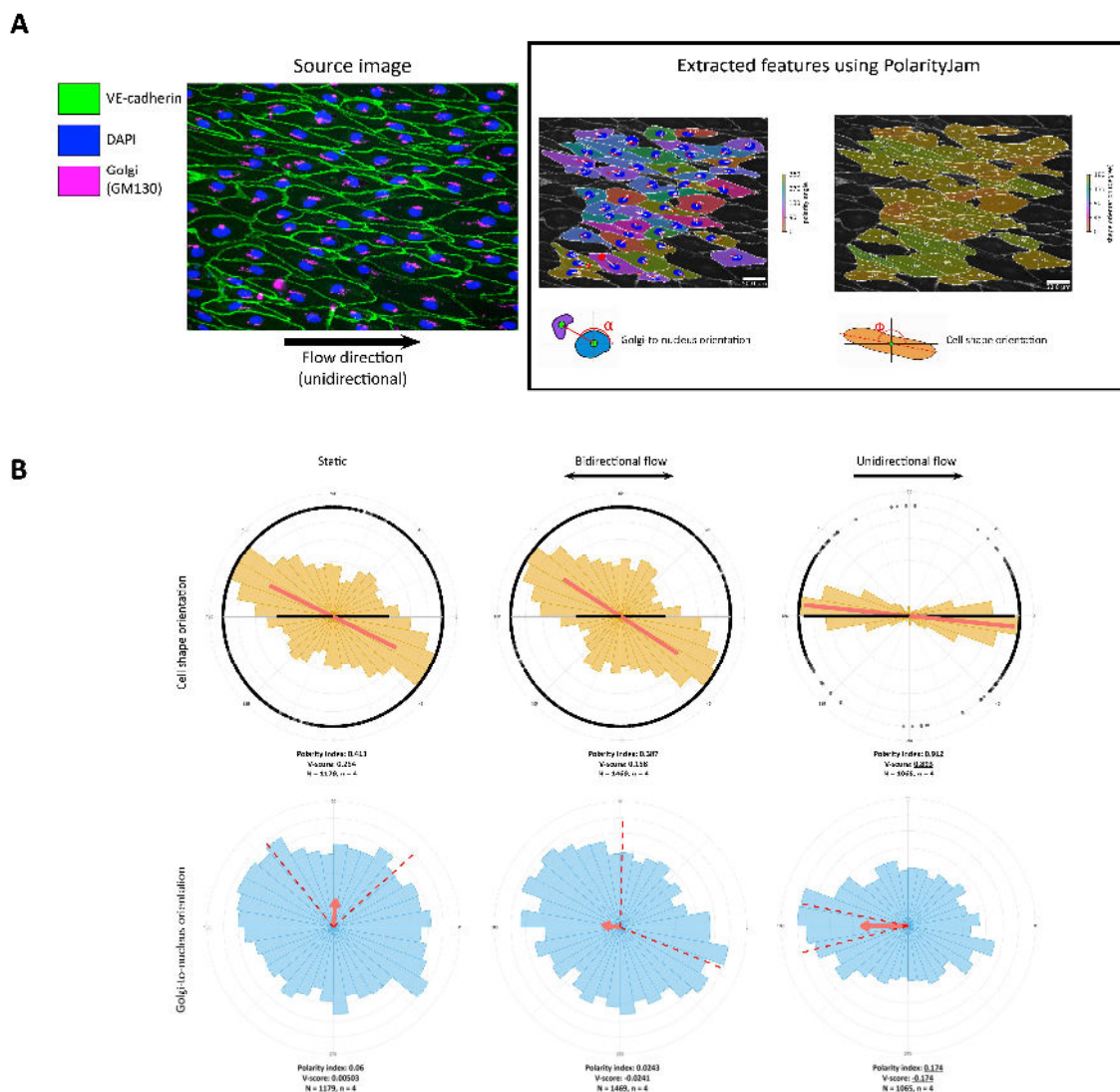


Figure 3. Flow-dependent endothelial alignment and polarity assessment

(A) Representative immunofluorescence image that was used for extraction of cell features (PolarityJam). hiPSC-ECs were immunostained for VE-cadherin (green color) and the golgi complex (GM130, pink color). The angle between the nucleus and golgi complex is defined as the golgi-nucleus orientation and is used for determining hiPSC-EC polarization. Cell shape orientation is used to indicate alignment of hiPSC-ECs parallel to the flow direction.

(B) The continuous unidirectional flow and shear forces provided by Omi resulted in cell shape orientation that strongly aligned parallel to the flow. Moreover, the unidirectional flow induced polarization of the golgi-nucleus orientation, as demonstrated by the polarity index that points exactly opposite to the flow direction, and the negative V-score. These effect were not present in static and bidirectional flow conditions. N (number of analyzed cells) is different for each condition. n (number of technical replicates) = 4.

Importantly, unidirectional flow resulted in golgi-nucleus polarization as is evident by the circular plot and polarity index (0.174, Figure 3B, bottom graphs). The mean direction of the golgi-nucleus polarization is opposite to the flow direction (red arrow), suggesting that the flow has induced the polarization.

The polarity index is visualized by the length of the red arrow and is a measure for the amount of polarization where a larger value indicates stronger polarization. The static and bidirectional conditions did not demonstrate this effect as their polarity indices were 0.06 and 0.0243, respectively. Moreover, this result is supported by the higher, negative V-score (-0.174, a measure of agreement with the flow direction) of the unidirectional flow samples, indicating polarization opposite to the flow direction.

Taken together, these data suggest that the unidirectional flow provided by Omi induces a relevant EC phenotype, even at a relatively low maximum shear stress of 0.912 dyn/cm². It is therefore plausible that when higher shear stresses are used, stronger golgi-nucleus polarization of hiPSC-ECs can be induced. The stable unidirectional flow demonstrated here is essential for accurate vascular *in vitro* modeling and can help hiPSC-ECs to attain a mature and relevant phenotype.

CONCLUSION

The unidirectional flow provided by Omi over the course of five days induced hiPSC-EC alignment parallel to the flow direction and golgi-nucleus polarization, indicating a relevant endothelial phenotype. In contrast, this effect was not observed under static and bidirectional flow conditions. Therefore, these results demonstrate that Omi combined with its unidirectional flow can contribute to physiological hiPSC-based vascular *in vitro* modeling. Moreover, Omi was able to maintain the preconfigured continuous flow profiles over the period of five days while also providing recirculation, thereby limiting the amount of cell culture medium required. It should be noted that any type of microfluidic chip can be connected to Omi using various types of connectors, thus enabling connection to many different OoCs. In conclusion, the Omi platform enables relevant vascular hiPSC-based *in vitro* modeling by enabling researchers to configure and maintain stable unidirectional flow profiles with recirculation over prolonged periods of time, using a simple single-device setup and minimal effort.

FUTURE DIRECTIONS

Can Omi be used for modeling **pathological flow environments**, such as those relevant for atherosclerosis or thrombosis?

Would Omi's recirculation feature **allow immune cell extravasation studies** in specialized Vessel-on-Chip models?

Using the **injection and sampling capabilities** that Omi offers, can vascular inflammatory responses be studied *in vitro*?

ACKNOWLEDGEMENTS

The work in Leiden OoC Center and Dr. Orlova is supported by the Novo Nordisk Foundation Center for Stem Cell Medicine that is supported by a Novo Nordisk Foundation grant (NNF21CC0073729) and the LymphChip project with project number NWA-ORC 2019 1292.19.019 of the NWA research program "Research on Routes by Consortia (ORC)," which is funded by the Netherlands Organisation for Scientific Research (NWO).

REFERENCES

1. Ingber, D. E. Human organs-on-chips for disease modelling, drug development and personalized medicine. *Nat Rev Genet*, (2022).
2. Low, L. A., Mummery, C., Berridge, B. R., Austin, C. P. & Tagle, D. A. Organs-on-chips: into the next decade. *Nat Rev Drug Discov* 20, 345-361 (2021).
3. Nahon, D. M. et al. Standardizing designed and emergent quantitative features in microphysiological systems. *Nat Biomed Eng* 8, 941-962 (2024).
4. Shakeri, A. et al. Engineering Organ-on-a-Chip Systems for Vascular Diseases. *Arteriosclerosis, Thrombosis, and Vascular Biology* vol. 43 2241-2255 Preprint at <https://doi.org/10.1161/ATVBAHA.123.318233> (2023).
5. Nguyen, J., Lin, Y. Y. & Gerecht, S. The next generation of endothelial differentiation: Tissue-specific ECs. *Cell Stem Cell* vol. 28 1188-1204 Preprint at <https://doi.org/10.1016/j.stem.2021.05.002> (2021).
6. Jang, S., Collin de l'Hortet, A. & Soto-Gutierrez, A. Induced Pluripotent Stem Cell-Derived Endothelial Cells: Overview, Current Advances, Applications, and Future Directions. *American Journal of Pathology* vol. 189 502-512 Preprint at <https://doi.org/10.1016/j.ajpath.2018.12.004> (2019).
7. Orlova, V. V. et al. Generation, expansion and functional analysis of endothelial cells and pericytes derived from human pluripotent stem cells. *Nat Protoc* 9, 1514-1531 (2014).
8. Orlova, V. V. et al. Functionality of endothelial cells and pericytes from human pluripotent stem cells demonstrated in cultured vascular plexus and zebrafish xenografts. *Arterioscler Thromb Vasc Biol* 34, 177-186 (2014).
9. Giese, W. et al. Polarity-JaM: an image analysis toolbox for cell polarity, junction and morphology quantification. *Nat Commun* 16, 1474 (2025).