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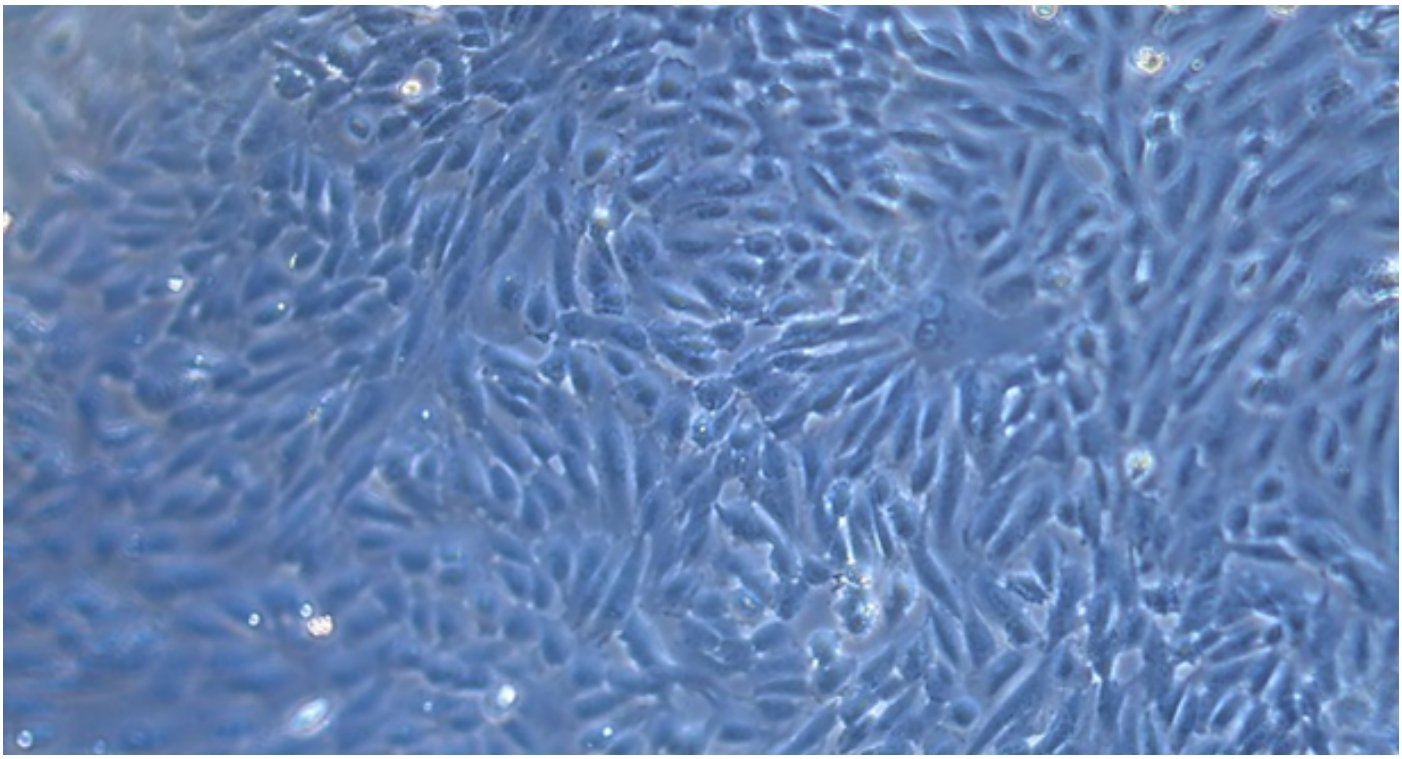
ENDOTHELIAL CELL CULTURE UNDER PERFUSION USING THE OMI.

This Application Note contains protocol for how to create a monolayer of human umbilical vein endothelial cells (HUVEC) inside the BeonChip BeFlow microfluidic chip. After coating and cell seeding, the endothelial monolayer is exposed to unidirectional, laminar shear stress using the Fluigent organ-on-chip automated platform, Omi.



1. INTRODUCTION

Materials Endothelial Cells for Organ-on-Chip Applications

Human umbilical vein endothelial cells (HUVECs) are a widely used model system in vascular biology and cardiovascular research. Isolated from the endothelium of veins in human umbilical cords, HUVECs exhibit characteristics of endothelial cells found in the vascular system, including the ability to form a monolayer, produce extracellular matrix components, and respond to angiogenic factors. Their use in vitro provides a valuable tool for studying complex interactions within the vascular endothelium.

These cells are widely used in organ-on-chip (OoC) studies due to their ability to mimic human vascular endothelium. Organ-on-chip technology aims to recreate the complex microenvironments of human organs in vitro, using microfluidic devices to control and manipulate the cellular and biochemical conditions.

Endothelial Cell Culture Under Perfusion

Culturing endothelial cells under perfusion in microfluidic systems is essential to replicate the dynamic environment of blood vessels and to study cell functions under physiological conditions. HUVECs are crucial for simulating blood vessel formation and function within OoC systems. By lining the microchannels of these chips with cells, researchers can study the dynamics of blood flow, shear stress, and the barrier properties of endothelial cells, which are essential for understanding various vascular-related phenomena.

HUVECs are also used to study the effects of mechanical forces, such as shear stress, on endothelial function. By subjecting them to fluid flow within the microchannels, researchers can investigate how these forces influence cell morphology, gene expression, and functional responses, providing insights into the role of hemodynamics in vascular health and disease. Perfusion culture offers several advantages, including improved cell viability, differentiation, and functionality due to continuous nutrient supply and waste removal.

Application examples

Vascularized Kidney-on-Chip: HUVECs are used to create a vascular network within kidney-on-chip models, enabling the study of renal diseases and drug nephrotoxicity.

Blood-Brain Barrier Models: HUVECs are used to form part of the endothelial layer in blood-brain barrier models on chips, allowing researchers to study the transport of drugs across the barrier and the effects of neurological diseases.

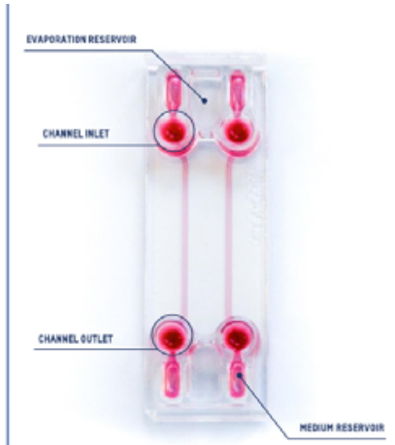
Lung-on-Chip: HUVECs can simulate the vascular compartment of lung-on-chip models, which are used to study respiratory diseases, pulmonary drug delivery, and the effects of airborne pathogens.

Various other examples of OoC models can benefit from a robust endothelial cell culture under perfusion protocol.

2. MATERIALS AND METHODS

Microfluidic Chip

For this application note, we used the BeOnChip Be-flow microfluidic chip (Fig. 1). However, the Omi is a versatile tool that could be used with any kind of commercially available chip, as well as homemade chips.



The BE-FLOW design features two independent channels with inlets and outlets, allowing connectors and tubing to be attached to fluidic controllers. Each channel can be perfused with an independent flow rate using the perfusion system. To prevent culture media evaporation at the start of the experiment, reservoirs next to the medium reservoir are prefilled with water (see scheme).

The channels of the microfluidic chip are first coated with fibronectin protein to enhance cell adhesion.

Figure 1: BeOnChip microfluidic chip, Be-Flow.

	Height	Width	Lenght	Total volume
Each channel	375 µm	1,5 mm	43 mm	28,5 µL
Inlet/Outlet	7 mm	UNF 1/4''-28		130 µL
Medium Reservoir	5 mm	3,6 mm	8,8 mm	185 µL

* The volumes presented on the table are theorical values calculated for the standard products. Changes in the device features of custom chips may modify the exact channel volume.

Protocol of Fibronectin Coating:

1. Prepare Fibronectin at a concentration of 50 µg/mL.
2. Prepare a pipette 50 µL of working solution and inject it directly into the channel's inlet.
3. Incubate at 37°C for 30 min.
4. Remove the solution from the channel + inlet/outlet using a pipette.
5. Wash the channel with DPBS twice by tilting the chip to help the flow.
6. Wash the channel using 100 µL of culture medium.
7. Aspirate the medium before cell seeding.

Human Umbilical Vein Endothelial Cell Culture and Seeding

HUVECs were sourced from (Lonza, Basel, Switzerland,). They were cultured in Endothelial Cell Growth Medium-2 (EGM-2, PromoCell, C-22211, Heidelberg, Germany) at 37 °C and 5% CO2 up to seven days.

Cell Seeding Protocol:

- Prepare the cells at a concentration of 4 million cells/mL.
- Introduce 50 µL of the cell solution in the fibronectin-coated channel, for at least 1 hour at 37°C to allow the cells to adhere.
- After 1 hour, fill in reservoirs with culture media.
- Leave cells overnight (16h-24h) before beginning the experiment the next day.

Omi Setup

The Omi is an advanced automated fluid handling platform designed for precise liquid flow control in organ-on-chip applications. It offers high accuracy and reliability. It's compatible with a wide range of microfluidic chips, tubing and connectors, ensuring versatility for various experimental setups. The system features an intuitive interface for easy operation and protocol creation. The Omi is particularly useful in research areas where controlled fluid dynamics are essential.

The Omi's recirculation system has two fluidic paths: the first one goes from the inlet reservoir through the microchip to the outlet reservoir, and the second links the two reservoirs directly (Fig. 2).

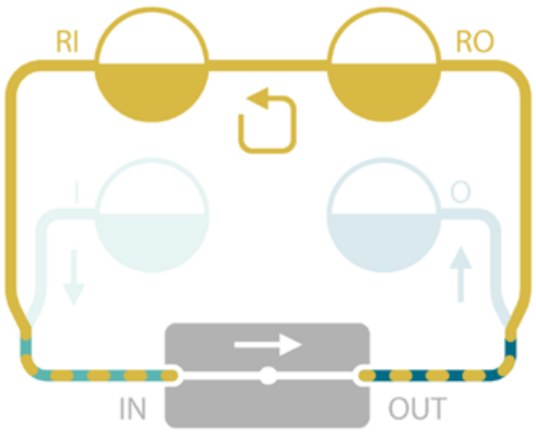


Figure 2: Recirculation system and flow paths in the Omi.

Endothelial Cell Culture Under Perfusion Protocol

Biological protocols should be carried out under sterile conditions. All the steps of the protocol including the setup of the Omi are to be performed under a biological safety cabinet.

Step 1: Omi Preparation

- Clean the Omi with EtOH 70%.
- Plug a sterile, new cassette and a Low Resistance to the Omi.
- Plug in the fluidic circuit (including tubing & connectors) and initiate a reservoir sensor «calibration» with sterile water.
- Run the protocol «Sterilization» to ensure your Omi is sterile and ready to use.
- Replace the low resistance adaptor with a high resistance adaptor, since the flow rate used for this application is very low.
- Run the protocol «Loading» to ensure the whole system is primed with the corresponding culture medium for your protocol.
- Fill RI with fresh medium (3 mL).

Step 2: Connect the Chip and Launch Protocol

- Select the channel to perfuse and make sure the cell monolayer is healthy and confluent.
- Wash cells gently by introducing fresh culture media in the channels to remove old culture media. Ensure that the channel is never dry.
- Add (not directly in the channel) 100 µL of fresh media to the inlet. Tilt the chip to create a slow flow from the inlet to the outlet. Flush the inlet/outlet with media until observing the convex meniscus, before connecting to the Omi.
- Go to the tablet application and load the «recirculation» protocol, allowing users to have a flow rate of 10 µL/min for 7 days.
- Once the flow is settled, incubate the Omi at 37°C for seven days.
- After 4-5 hours of perfusion, pause the protocol and change the medium. This step is to discard the non-adherent cells that remain in the recirculating medium.

Step 3: Maintenance and Monitoring

- The culture medium should be replenished every day to ensure a consistent supply of nutrients.
- At each medium change, the flow must be paused.
- Regularly check the cell monolayer under the microscope, to monitor cell morphology, alignment, and confluence.
- Immunostaining for specific markers can be used to access endothelial cell integrity and junction formation.

3. RESULTS

Endothelial cells naturally experience shear stress in their physiological environment. Therefore, culturing these cells with a continuously circulating medium will accurately mimic their typical physiological conditions compared to static culture.

Endothelial cell culture was sustained for at least seven days under perfusion. During this period, culture media was renewed every day, and the cells were checked under a microscope to monitor cell morphology, behavior and flow alignment.

As observed in the images (Fig.3), the cells exhibit healthy morphology, continue to proliferate and are oriented in the direction of the flow.

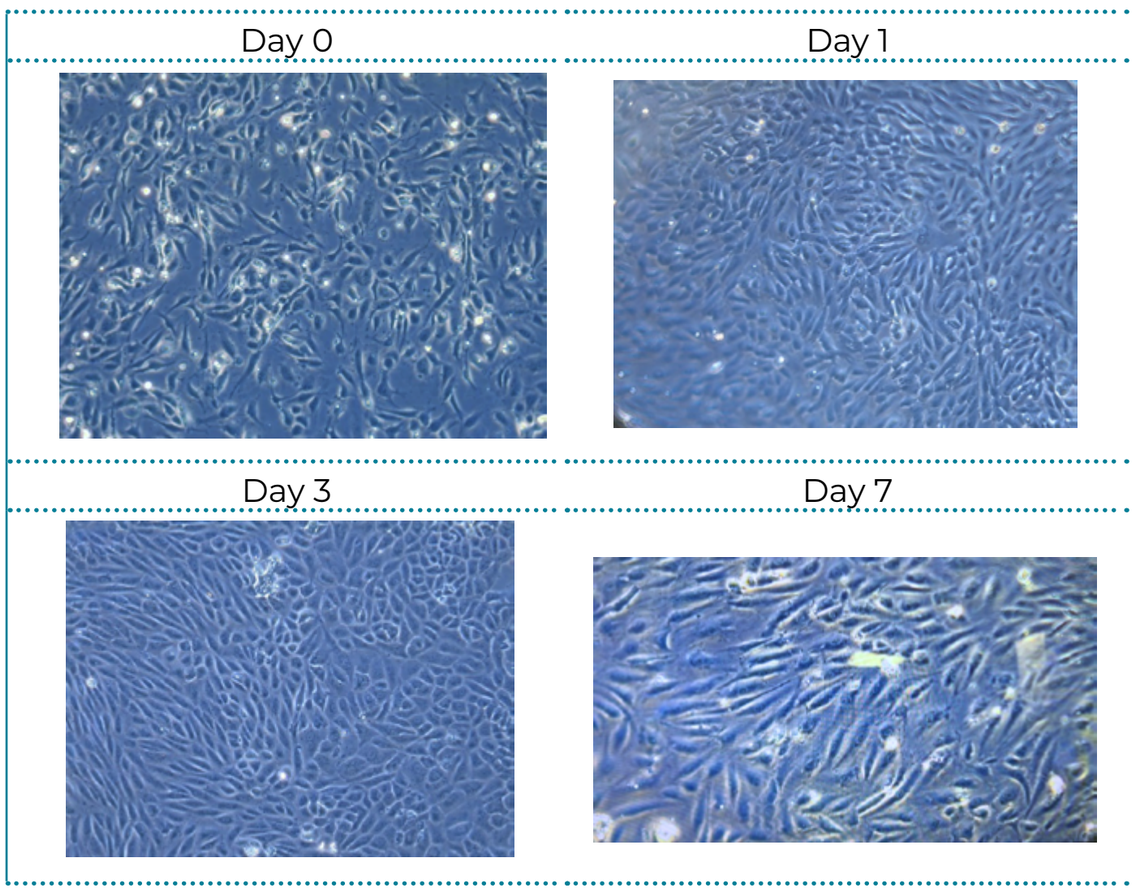


Figure 3: Endothelial cell culture under perfusion, at day 0, day 1, day 3, and day 7 after perfusion.

CONCLUSION

In conclusion, endothelial cell culture under perfusion in microfluidic devices provides a physiologically relevant platform for studying endothelial biology, disease mechanisms, and therapeutic interventions.

The dynamic flow conditions enhance cell functionality and viability, making these systems invaluable tools in vascular research.

The Omi automated OoC platform, offers an ideal solution for the long-term recirculation of cell culture media in various OoC models. It delivers high-precision flow, enabling the development of a robust monolayer suitable for organ-on-a-chip applications. Easy-to-use, autonomous, and portable under a microscope, the Omi is the perfect companion for on-chip biology experiments.

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