Human endothelial cells under shear stress cultivated on the glass membrane of the µ-Slide Membrane ibiPore Flow

General information: This Application Note is a protocol for how to create a monolayer of human umbilical vein endothelial cells (HUVEC) inside the ibidi µ-Slide Membrane ibiPore Flow. After coating and cell seeding, the endothelial monolayer is exposed to unidirectional, laminar shear stress using the ibidi Pump System.

This protocol focuses on combining the µ-Slide Membrane ibiPore Flow with the application of shear stress using the ibidi Pump System. Detailed instructions on coating, seeding cells, and tube connection are provided in the instructions of the µ-Slide Membrane ibiPore Flow and the manual of the ibidi Pump System. Please read those two documents before using this protocol. Whenever this protocol refers to those documents, a special marker is used (->).

Related Topics:
Application Note 08 Cell culture coating
Application Note 13 Endothelial Cell Culture under Perfusion

Keywords:
Endothelial Cells, HUVEC, monolayer, porous glass membrane, flow, perfusion, pump, shear stress, microscopy

Material:

- µ-Slide Membrane ibiPore $^{0.5\ µm/20\%}$ Flow ibiTreat (85116, ibidi, Germany)
- ibidi Pump System (10902, ibidi, Germany)
- Perfusion Set RED, 15 cm, ID 1.6 mm (10962, ibidi, Germany)
- Screw Clamp (10861, ibidi, Germany)
- Human umbilical vein endothelial cells, HUVEC, (C-12203)
- Endothelial Cell Growth Medium (C-22010, PromoCell, Germany), supplemented with Endothelial Cell Growth Medium Supplement Mix (C-39215, PromoCell, Germany)
- Accutase (A1110501, Gibco)
- Collagen Type IV (354233, Corning)
- Standard cell culture equipment (sterile working bench, cell culture incubator, culture flasks, PBS, etc.)

Important Note: Equilibrate all required materials, such as µ-Slides, culture medium, and tubing (Perfusion Sets), **overnight** inside the incubator at 37°C and 5% CO_2. This is essential for keeping air bubbles from emerging over time.

1. Coating

- Prepare the Collagen Type IV coating solution by diluting it to a final concentration of 40 µg/ml according to the instructions of the manufacturer.
- Coat the µ-Slide (->).
- Aspirate the coating solution completely.
- Wash twice with PBS.
- Let the µ-Slide dry at room temperature before seeding the cells.

2. Cell Preparation & Seeding

- Cultivate HUVECs in endothelial cell growth medium supplemented with the supplement mix.
- Treat the cells with Accutase for 2 minutes for detachment.
- Harvest the cell suspension.
- Centrifuge the cell suspension and dilute in growth medium to obtain the desired concentration.
- Count the cells and adjust to a concentration of 2 x 10^6 cells/ml for 100% optical confluency after cell attachment.
- Seed cells on the membrane in the lower channel of the µ-Slide (->).
- Incubate for two hours at 37°C and 5% CO_2 to let the cells adhere.

Important Note: Endothelial cells in in permanent culture for propagation should never grow to an optical confluency of close to 100%. A confluent monolayer enters into a growth inhibition state, which stops cell proliferation and changes the physiology of the cell. Only in assays, where a monolayer is desired, 100% confluency is recommended.

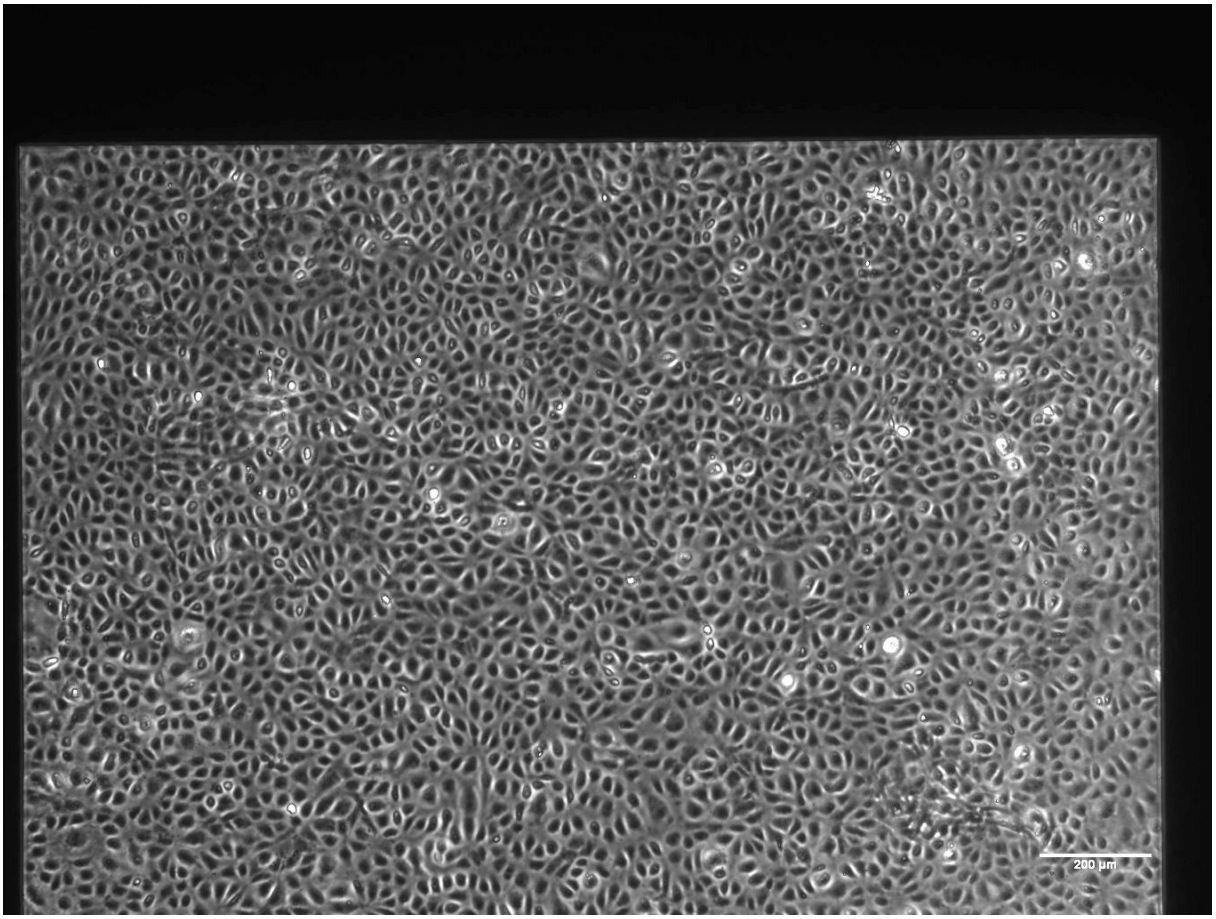
3. Perfusion Experiment

- Control the cell attachment under the phase contrast microscope.
- Prepare the µ-Slide, the ibidi Pump System, and the Perfusion Set (->).
- To remove air bubbles from the system let it run 1-2 hours before connecting the µ-Slide (->).
- Pinch off the tubing of the Perfusion Set with the ibidi Screw Clamp (#10861) or a Hoffmann tubing clamp.
- Connect the µ-Slide to the tubing (->).
- Open the pinched tubing slowly in order to minimize the pressure peak.
- Start the perfusion experiment following the parameters in the table below. Starting with a low flow is mandatory for adapting the cells to the shear stress.

	Pressure	Shear stress	Flow rate	Time span
1	5.0 mbar	3.5 dyne/cm^2	3.8 ml/min	60 min
2	7.2 mbar	5 dyne/cm^2	5.4 ml/min	60 min
3	14.9 mbar	10 dyne/cm^2	10.9 ml/min	Infinite

Important Note: After connecting the µ-Slide to the pump, re-open the pinched tubing slowly in order to minimize the pressure peak.

4. Results



Human umbilical vein endothelial cells (HUVEC) after 24 hours at 10 dyne/cm² in the µ-Slide Membrane ibiPore Flow, 0.5 µm porous membrane, window size 2 mm x 2 mm, 4x objective lens, phase contrast, scale bar 200 µm.