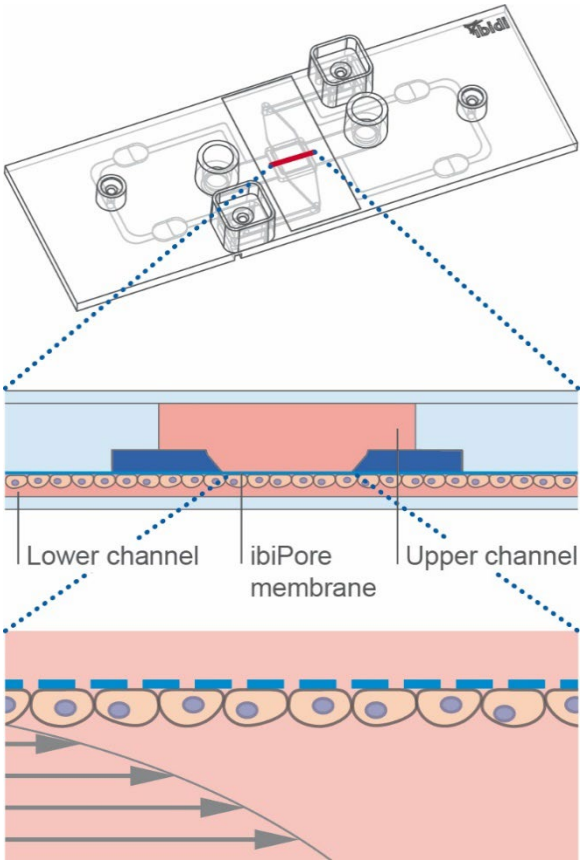
cells in focus ibidi®

Human Endothelial Cells Cultivated Under Shear Stress on the Glass Membrane of the µ-Slide ibiPore SiN

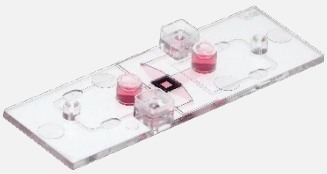
This Application Note is a protocol for creating a monolayer of human umbilical vein endothelial cells (HUVEC) inside the ibidi µ-Slide ibiPore SiN. After coating and cell seeding, the endothelial monolayer is exposed to unidirectional, laminar shear stress using the ibidi Pump System.

This protocol focuses on combining the µ-Slide ibiPore SiN 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 ibiPore SiN and the manual of the ibidi Pump System, shown in the related documents below.



ibidi solutions for transmigration and transport studies under flow or static conditions:

- µ-Slide ibiPore SiN
- ibidi Pump System



Related documents:

- Application Note 08: Coating Protocols for ibidi Labware (PDF)
- Application Note 11: Shear Stress and Shear Rates (PDF)
- Application Note 13: Endothelial Cells Under Perfusion (PDF)
- Application Note 26: Preparation of Collagen I Gels (PDF)
- Instructions µ-Slide ibiPore SiN (PDF)
- Instructions ibidi Pump System (PDF)

1 Material

- µ-Slide ibiPore SiN 0.5 µm/20% ibiTreat (85216-S, 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, PromoCell, Germany)
- 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: One day before the experiment, 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.

2 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 (a detailed coating protocol can be found in the Instructions µ-Slide ibiPore SiN).
- Aspirate the coating solution completely.
- Wash twice with PBS.
- Let the µ-Slide dry at room temperature before seeding the cells.

3 Cell Preparation and 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 2×10^6 cells/ml for 100% optical confluency after cell attachment.
- Seed cells on in the lower channel of the µ-Slide ibiPore SiN (a detailed description of cell seeding can be found in the Instructions µ-Slide ibiPore SiN).
- Incubate for two hours at 37°C and 5% CO_2 to let the cells adhere.

Important Note: Endothelial cells in permanent culture for propagation should never grow to an optical confluency of close to 100%. A confluent monolayer enters 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.

4 Perfusion

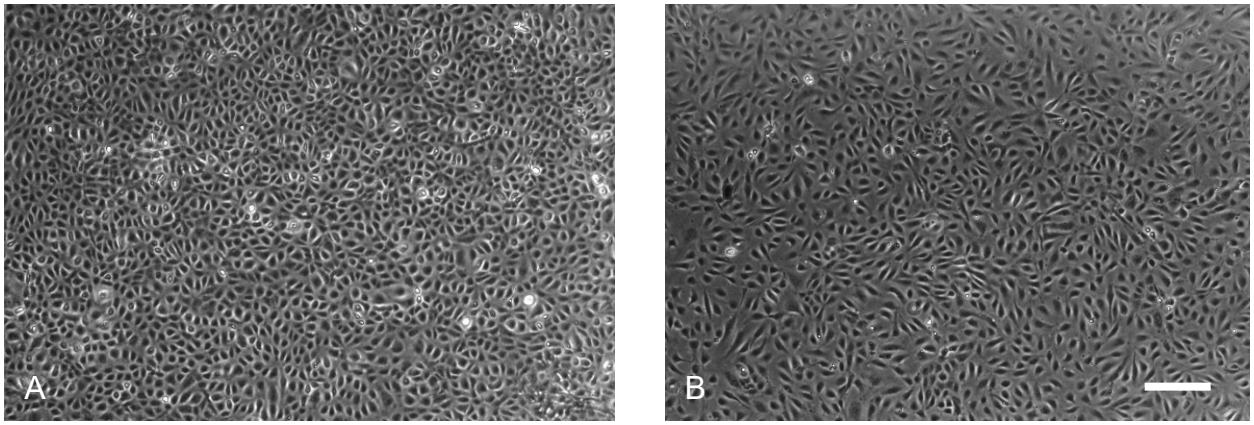
- Control the cell attachment under the phase contrast microscope.
- To remove air bubbles from the ibidi Pump System, let it run for 1–2 hours before connecting the µ-Slide ibiPore SiN.

Important Note: How to remove air bubbles in the system on the day of the experiment is described in the Instructions ibidi Pump System (PDF).

- Pinch off the tubing of the Perfusion Set with the ibidi Screw Clamp or a Hoffmann tubing clamp.
- Connect the µ-Slide ibiPore SiN to the tubing.
- Open the pinched tubing slowly 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
1	5.0 mbar	3.5 dyne/cm^2	3.8 ml/min
2	7.2 mbar	5.0 dyne/cm^2	5.4 ml/min
3	14.9 mbar	10.0 dyne/cm^2	10.9 ml/min

5 Results



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