Culture of Human Endothelial Cells Under Shear Stress on a Collagen Matrix in the µ-Slide I Luer 3D

General information: This Application Note is a protocol for how to establish a monolayer of human umbilical vein endothelial cells (HUVEC) on a Collagen Type I gel inside the ibidi µ-Slide I Luer 3D. After coating, gel matrix filling, and cell seeding, the endothelial monolayer is exposed to unidirectional, laminar shear stress using the ibidi Pump System.

This protocol focuses on creating an endothelial barrier on a soft collagen gel matrix without the need of any supportive, artificial filter or membrane.

Related Documents:
Instructions µ-Slide I Luer 3D
Instructions Collagen Type I, Rat Tail
Instructions ibidi Pump System
Application Note 26 Collagen I Gel Protocols for 3D Cell Culture

Keywords:
Endothelial cells, HUVEC, monolayer, barrier, 3-D matrix, Collagen Type I Rat Tail, extracellular matrix, ECM, flow, perfusion, pump, shear stress, microscopy

Material:

- µ-Slide I Luer 3D ibiTreat (87176, ibidi, Germany)
- Collagen Type I, Rat Tail, 5 mg/ml (50201, ibidi)
- 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)
- ibidi Pump System (10902, ibidi, Germany)
- Perfusion Set RED, 15 cm, ID 1.6 mm (10962, ibidi, Germany)
- 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

Note: The coating step increases the adhesion between the well surface and the gel matrix. As it depends on the used gel matrix, this 2D coating step might not be necessary.

- Prepare the Collagen Type I coating solution by diluting it to a final concentration of 40 µg/ml according to the instructions.
- Fill each well of the µ-Slide I Luer 3D with 16 µl of coating solution.
- Aspirate the coating solution completely from the wells.
- Incubate for one hour at room temperature.
- Wash with PBS.
- Aspirate the PBS completely.

2. Gel Preparation

- Prepare the Collagen Type I gel with a final collagen concentration of 2.0 mg/ml according to the ibidi Application Note 26.
- Fill each well of the µ-Slide I Luer 3D with the gel matrix. Mind the flatness of the gel surface. Perform the gel volume optimization procedure in advance. See the instructions for details.
- Close the µ-Slide with the top foil.
- Let the gel polymerize inside the incubator for one hour.

3. Cell Preparation & Seeding

- Cultivate HUVECs in endothelial cell growth medium supplemented with the supplement mix.
- Treat the cells with Accutase for two minutes for detachment.
- Harvest the cell suspension.
- Centrifuge the cell suspension and dilute in full growth medium to obtain the desired concentration.
- Count the cells and adjust to a concentration of 1.5 x 10^6 cells/ml for 100% optical confluency after cell attachment.
- Seed cells into the channel of the µ-Slide.
- Incubate for one hour at 37°C and 5% CO_2 to let the cells adhere.

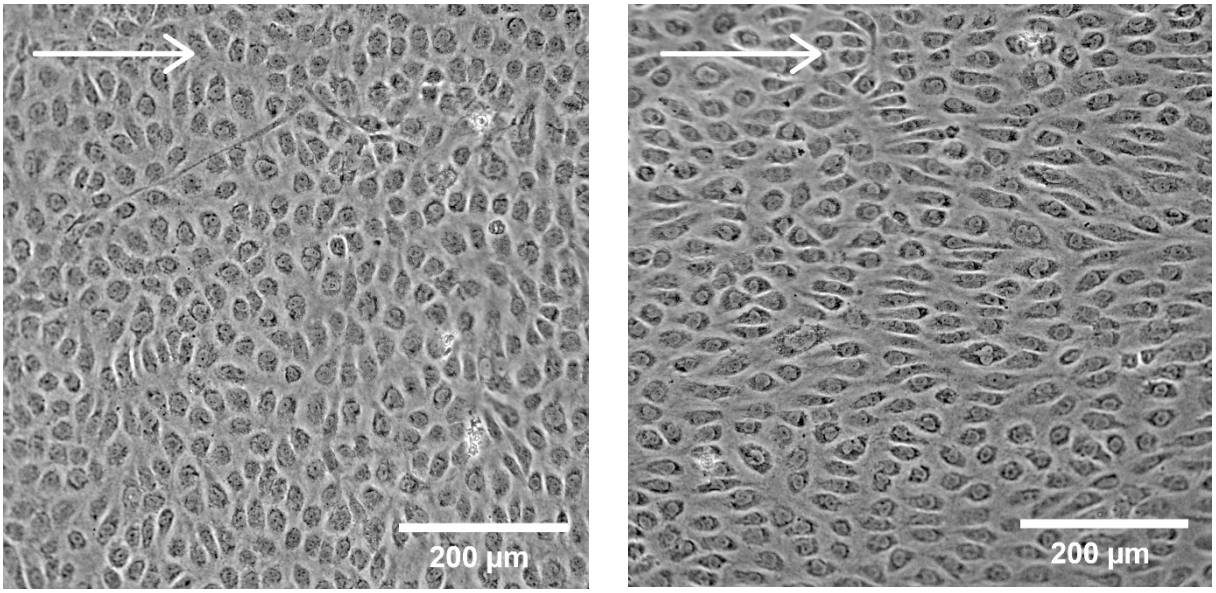
Important Note: Endothelial cells in permanent culture for propagation should never grow to an optical confluency of close to 100%. A confluent monolayer enters into a growth inhibition state, which stops cell proliferation and changes the physiology of the cell. Only in assays, where a monolayer is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

4. Perfusion Experiment

- Control the cell attachment under the phase contrast microscope.
- Prepare the µ-Slide, the ibidi Pump System, and the Perfusion Set for the flow connection according to the instructions.
- To remove air bubbles from the system, let it run 1–2 hours before connecting the µ-Slide.
- Connect the µ-Slide to the tubing and the pump system.
- Start the perfusion experiment using the parameters in the table below. Starting with a low flow before ramping up is mandatory for adapting the cells to the shear stress.

	Pressure	Shear stress	Flow rate	Time span
1	5.2 mbar	2 dyne/cm^2	4.8 ml/min	60 min
2	15.4 mbar	5 dyne/cm^2	11.9 ml/min	60 min
3	33.2 mbar	10 dyne/cm^2	23.8 ml/min	Infinite

5. Results



Phase contrast microscopy of HUVEC after culturing them under flow at 10 dyn/cm² for 2 days (left) and 5 days (right) on a Collagen Type I rat tail (2 mg/ml). Note the cobblestone-like cell morphology after 5 days of culture under flow. 20x objective.