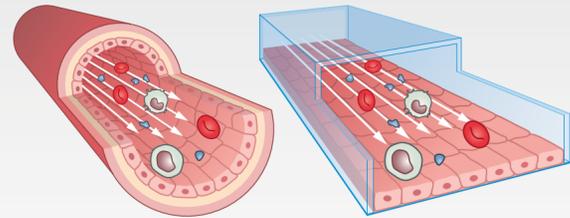


# Cell Culture Under Flow

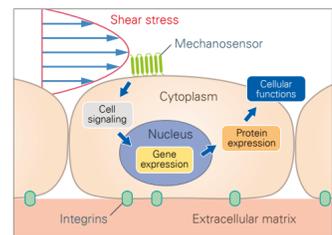
## Enhancing Cellular Physiology and Function Through Dynamic Microenvironments



Traditional static cell culture techniques fail to mimic the dynamic nature of *in-vivo* environments, limiting the physiological relevance of the experimental results. At ibidi, we focus on developing solutions that enable researchers to cultivate and investigate cells under *in vivo*-like conditions.

Here, we provide a comprehensive overview of the features and applications of the ibidi Pump System in combination with specialized channel slides. These slides are designed to stimulate the mechanical force generated by fluid flow, such as wall shear stress in blood and lymphatic vessels. They also support the long-term cultivation of both 2D and 3D cells by enhancing cellular viability and performation through optimal nutrient supply and waste removal functioning as perfusion-based 3D bioreactors.

### Wall Shear Stress



- Simulation of mechanical forces (shear stress) created by fluid flow in biofluidic systems (e.g., blood or lymphatic vessels, nephrons)
- Physiological long-term cultivation of vascular endothelial and epithelial cells
- Analysis of relevant readouts, such as cell morphology, behavior, and physiology

**Cell monolayer on coverslip**

μ-Slide I Luer  
μ-Slide VI  
μ-Slide y-shaped

**Cell monolayer on gel matrix**

Collagen Type I

**Cell monolayer on gel matrix: cells in flow / inside gel matrix**

Collagen Type I

**Co-culture cell monolayers on optical porous membrane**

Upper channel: #1.5 glass coverslip  
Lower channel: 0.4 mm  
#1.5 ibidi Polymer Coverslip (0.18 mm)

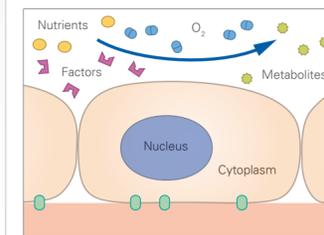
*HUVECs were cultured under flow at 10 dyn/cm<sup>2</sup> for 4 days in a μ-Slide I<sup>®</sup> Luer ibiTreat. Human-Golgin-97 (red), F-actin (green), and nuclei (blue).*

*Phase contrast microscopy of HUVEC cultivated under flow at 10 dyn/cm<sup>2</sup> for 5 days on Collagen I*

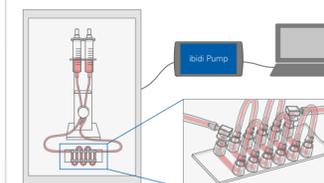
*IF staining of HUVEC after culturing them under flow at 10 dyn/cm<sup>2</sup> for 5 days on Collagen I; alpha-tubulin (red), F-actin (green), nuclei (blue)*

*IF staining of human endothelial cells in the μ-Slide ibiPore coated with Collagen. Overlay image of phase contrast, DAPI (blue), VE-cadherin (green), and F-actin (red)*

### Perfusion-Based 3D Bioreactor



- Physiological long-term 2D and 3D cell culture (e.g., of organoids, spheroids, organs-on-a-chip)
- Continuous medium exchange for constant nutrient supply
- Ensured optimal cell viability and health



Set-up example: Graphical depiction of the ibidi Pump System connected to the ibidi μ-Slide VI 14

**3D spheroids / organoids in confined well geometry**

μ-Slide Spheroid Perfusion

Main well (perfusion-dominated)  
Niche (diffusion-dominated)

Confocal image of a cleared and stained L929 spheroid. Actin (red), nuclei (cyan) in the μ-Slide Spheroid Perfusion.

Data by J. Hofmann & S.J. Keppeler, TUM, Germany.

**3D spheroids / organoids on micropatterned surface**

μ-Slide VI 14

IF staining of RCC-26 (human renal carcinoma) cells cultured in the μ-Slide VI 14 With Multi-Cell μ-Pattern

**3D cell culture inside gel matrix**

μ-Slide III 3D Perfusion  
μ-Slide I Luer 3D

Collagen Type I

IF staining of HepG3 spheroids in a HA-PEG hydrogel in the μ-Slide III 3D Perfusion; viable cells (green), dead cells (red) nuclei (blue)

Data from Christofferson et al 2019 Biofabrication 11 015013; BY CC 3.0 license

**Co-culture of cells/ spheroids on and in gel matrix**

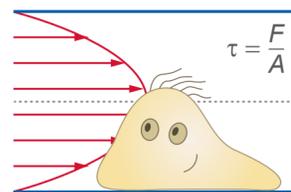
μ-Slide III 3D Perfusion  
μ-Slide I Luer 3D

Collagen Type I

IF staining of a co-culture of MCF-7 spheroids and HUVECs in the μ-Slide I Luer; actin (red), nuclei (blue), CD31 (HUVEC marker, green)

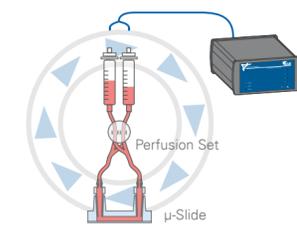
### The ibidi Pump System

#### Defined Shear Stress



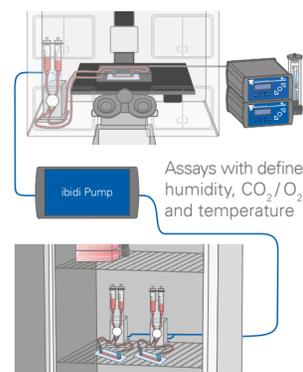
- Software-based flow programming including shear stress and shear rate calculation
- Simulation of all physiological flow patterns with a wide shear stress range (0.1–200 dyn/cm<sup>2</sup>)

#### Closed System



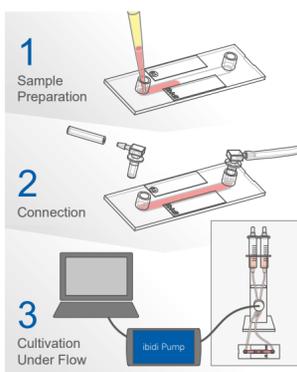
- Long-term sterile setup
- Recirculating medium at low volumes
- No medium contact with mechanical parts

#### Physiological Conditions



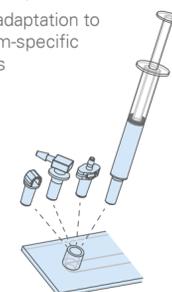
Assays with defined humidity, CO<sub>2</sub>/O<sub>2</sub> and temperature

#### Easy Workflow

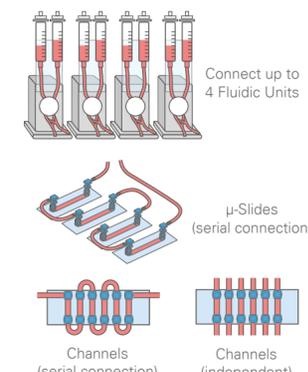


#### Standard Luer Ports

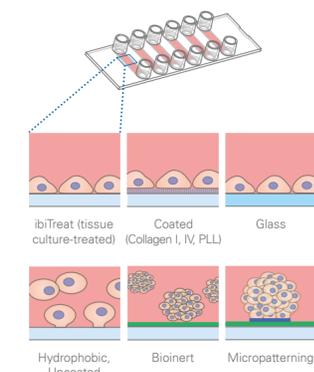
- Plug-and-play connection
- No microfluidic knowledge necessary
- Easy adaptation to custom-specific setups



#### Parallelization



#### Flow Chamber Surfaces



#### Microscopy Readout

