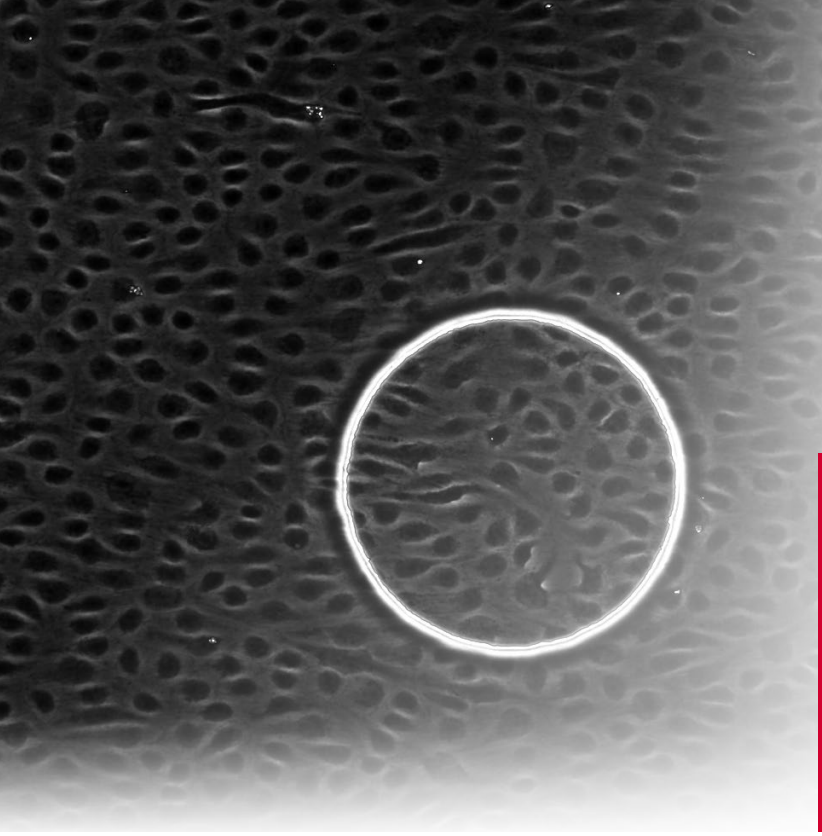


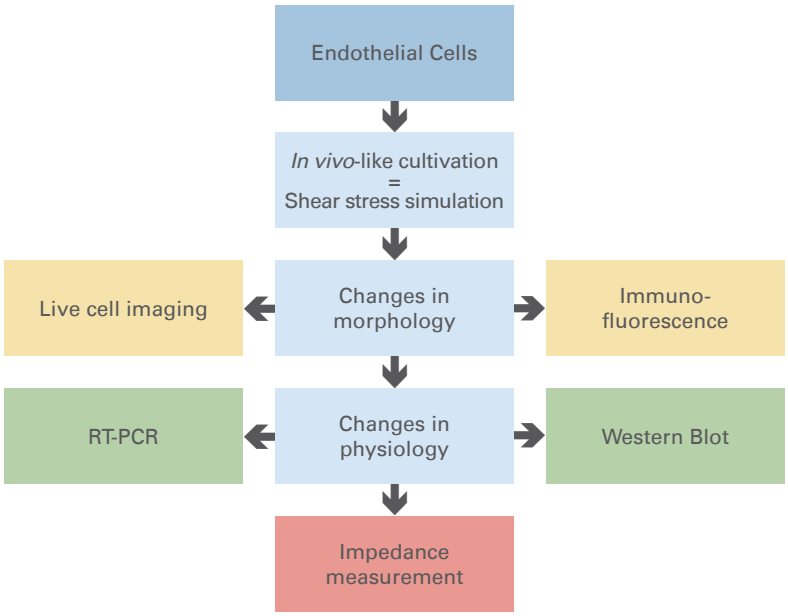
ibidi®
cells in focus

Mimic *in vivo*-like Flow Conditions and Achieve Reliable Experimental Results

Advance Your Endothelial Cell Studies by Combining Shear Stress Cultivation with Impedance Measurements

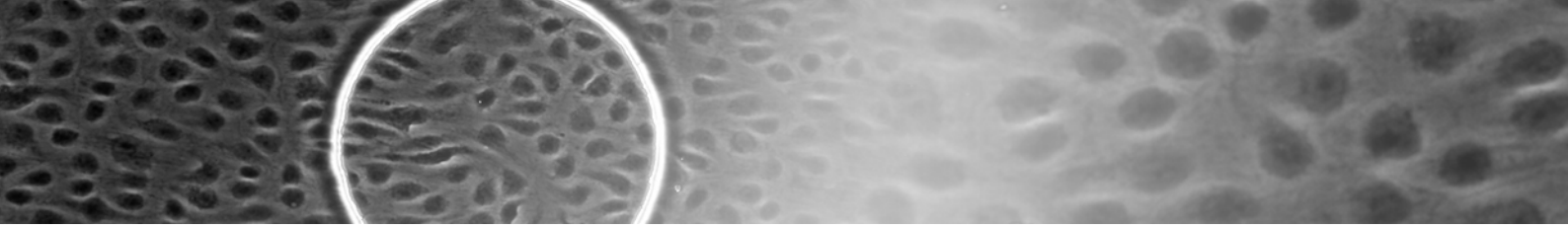
In vivo, endothelial cells (e.g., small and large vessels, lymphatic vessels) are exposed to flow and shear stress conditions.

The *in vitro* analysis of endothelial cells exposed to flow reveals diverse effects on cell division rate, morphological changes, cytoskeletal reorganization, cytokine production, the expression of adhesion molecules, macromolecular permeability, and barrier function.



For *in vitro* investigations, ibidi provides a unique, integrated solution that combines:

- ✓ Cultivation of endothelial cells under physiological, shear stress conditions
- ✓ A sensitive, real-time analysis method for investigating the resulting biological impact on a morphological and physiological level

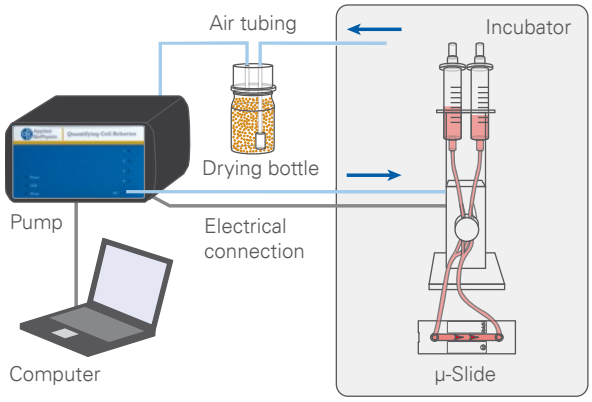


The Challenge: Mimic the Physiological Environment of Cells Under Flow Conditions

In their natural environment, endothelial cells are constantly exposed to physical and biochemical stimuli that can alter cell layer permeability. Laminar shear stress from blood flow is a principal regulator of systemic endothelial cell gene expression, morphology, and the production of soluble mediators. Its importance is highlighted by pathological processes associated with reduced or disturbed laminar shear stress, including atherosclerosis.

The Idea: Combine the ECIS Flow Module with the ECIS System

The **ECIS Flow Module**, together with the **ECIS System** (Electric Cell-Substrate Impedance Sensing), allows researchers to study endothelial permeability *in vitro* under complex shear stress conditions.



System overview: ECIS Flow Module for the cultivation of cells under flow conditions

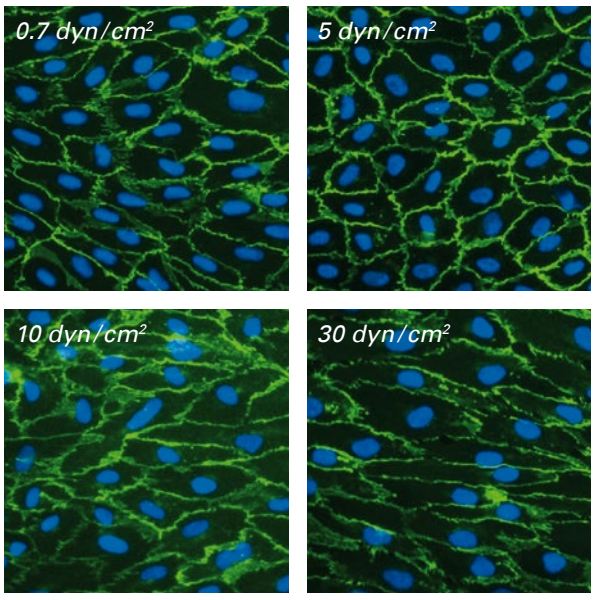
Measure Changes in Cell Morphology

Microscopy:

Microscopy-based techniques, such as live cell imaging and immunofluorescence, can be used to investigate changes in cell shape and cell movement, and to visualize cytoskeletal rearrangements. The ECIS Flow Module and the ibidi µ-Slides are designed for combining shear stress assays with **live cell imaging** or subsequent **immunofluorescence microscopy**.

Impedance:

Impedance measurements offer an additional, label-free, and very sensitive readout of cell morphology changes that might not be detected under the microscope. The ECIS Zθ System works as a real-time sensor to detect changes in cell morphology and barrier function.



Immunofluorescence microscopy of HUVEC under various flow conditions (green: VE-Cadherin, blue: cell nucleus)

Mimic *in vivo*-like Flow Conditions and Achieve Reliable Experimental Results

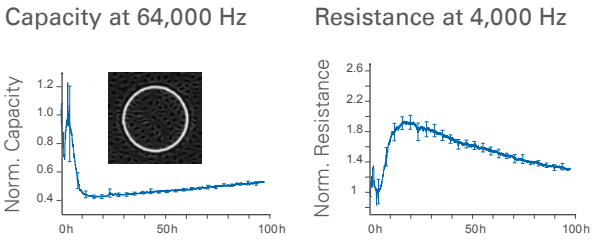
Measure Changes in Cell Physiology

Shear stress changes gene and protein expression levels

Shear stress also has an impact on the molecular level of cells. For example, the expression of ECM (extracellular matrix) genes and proteins changes under long-term shear stress cultivation of endothelial cells.

Application notes on how cells that are cultivated under shear stress can be used for subsequent **RT-PCR** and **Western Blot analysis** are available from the ibidi website.

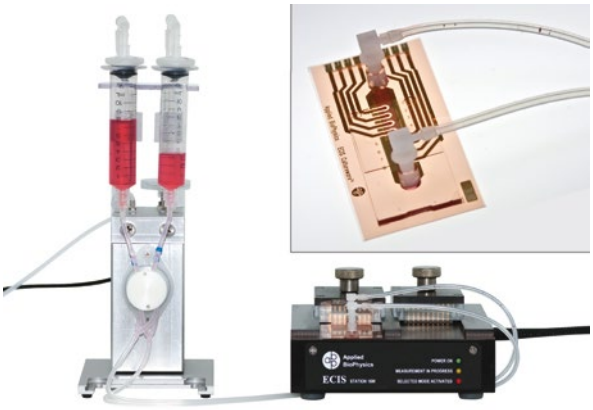
5 dyn/cm²



HUVEC tested under shear stress conditions; all measurements: 8 electrodes per µ-Slide I, Luer

Shear stress changes physiological properties of a cell layer

A single cell or a group of non-connected cells has different properties than a confluent cell monolayer. To study the development from a single cell to a confluent cell layer, impedance measurements enable characterization of the changes over time. The physiological properties of a confluent cell layer can be studied in detail, including time-course changes in the barrier function (permeability) of the cell layer as well as the degree of constricted current flow under the cells.



The ECIS Flow Module and the ECIS System Provide a Versatile, Integrated Solution for Combining Shear Stress Studies with Impedance Measurements.

Ordering Information:

Cat. No.	Description
71616	**ECIS Model Z:** ECIS Model Z station controller, Elevated Field Module (i.e., a pulse system for automated wound healing or electroporation studies), software for data gathering and analysis, laptop
71617	**ECIS Model Z Theta:** ECIS Model Z Theta station controller, Elevated Field Module (i.e., a pulse system for automated wound healing or electroporation studies), software for data gathering and analysis, including model calculations, laptop
71612	**ECIS 16 Well Station:** a holder with two slots for ECIS Cultureware 8 well arrays, and a data processing station
71001	**ECIS Flow Module:** perfusion system for ECIS flow arrays, ideal for defined flow rates and shear stress studies of endothelial cells
70101	ECIS **Flow array 1E** channel µ-Slide with 8 x 1 electrodes: specially designed for endothelial cell studies and cell - cell interaction at defined flow rates (ca. 0.35 mm channel height, other channel heights available on request)
70110	ECIS **Flow array 10E** channel µ-Slide with 8 x 10 electrodes: specially designed for endothelial cell studies and cell - cell interaction at defined flow rates (ca. 0,35 mm channel height, other channel heights available on request)

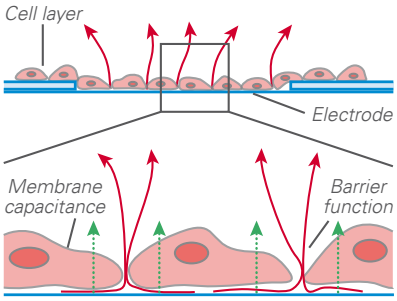
Mimic *in vivo*-like Flow Conditions and Achieve Reliable Experimental Results

What is impedance measurement?

Cell function modulates cell morphology directed by the cell's cytoskeleton. ECIS is capable of detecting and quantifying morphology changes in the sub-nanometer to micrometer range. In ECIS, a small alternating current (I) is applied across an electrode pattern at the bottom of an array. This results in a potential (V) across the electrodes that is measured by the ECIS instrument. The impedance (Z) is determined by Ohm's law Z = V/I.

ECIS is a real-time, label-free, impedance-based method to study the activities of cells grown in tissue culture.

What is measured with impedance sensing?



When cells are added to the wells of a culture slide and attach to the electrodes, they act as insulators, thus increasing the impedance. As cells grow and cover the electrodes, the current is impeded and relates to the number of cells covering the electrode, to the morphology of the cells, and to the nature of the cell attachment. The data generated is impedance versus time.

For measuring impedance, the ECIS instrument uses multiple frequencies. At relatively low frequencies (< 2,000Hz) most of the current flows in the channels between adjacent cells (red lines).

At higher frequencies (> 40,000 Hz) more current capacitively couples through the insulating cell membranes and mirrors the attachment and spreading of cells over the gold electrode (green lines).

The high-frequency impedance is more affected by cell-coverage, whereas the low-frequency impedance responds more strongly to changes in the spaces between the cells.

How and when does a cell layer change its physiological properties over time?

The graph on page 3 shows a typical example of a cell layer physiological change: After applying shear stress (5 dyn/cm^2), the HUVEC show a decay in capacity and an increase in resistance followed by a slow decrease.

Combine Impedance Measurements and Flow for Monitoring Physiological Changes Over Time

Long-term, *in-vitro* experiments with the ECIS System display a dynamic modification in the cell morphology of HUVEC and in cell-cell contacts, leading to a change in the physiological behavior of the cell monolayer. The experimental set-up using the ECIS System and the ECIS Flow Module enables the monitoring of these changes continuously and quantitatively.

This easy-to-use set-up saves time and money and elucidates the physiological changes over time in an easy-to-read graphical surface.

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