

# Changes in cell-cell contacts of endothelial cells (HUVEC) under long term shear stress conditions

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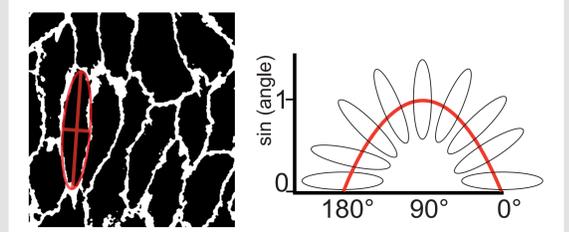
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## Introduction

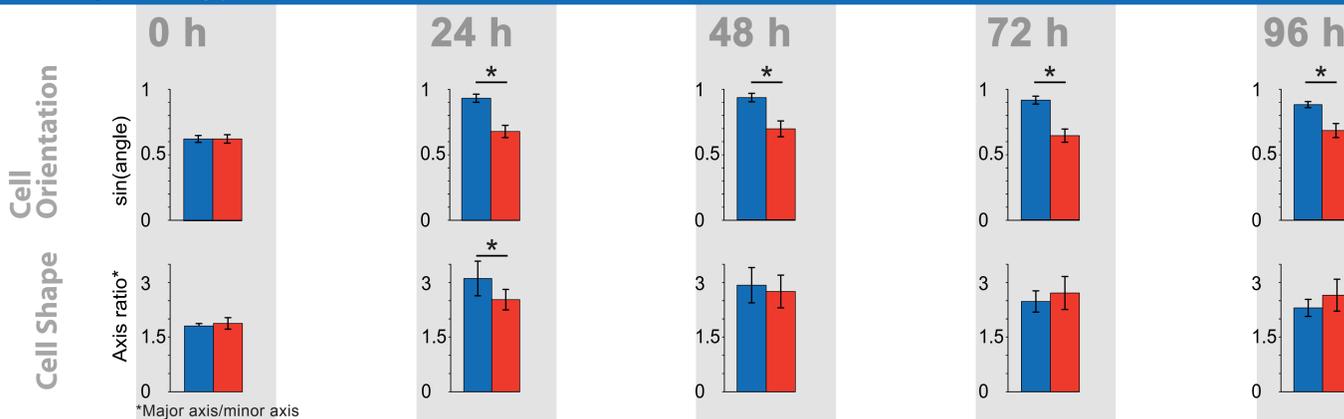
We investigated the long term development of cell-cell contacts in an endothelial cell layer under physiological shear stress conditions. To mimic an *in vivo*-like environment, human umbilical vein endothelial cells (HUVEC) were cultured under unidirectional laminar flow conditions. Cell orientation and cell-cell contacts were measured over the course of 4 days. The highly dynamic physiological behaviour was monitored in impedance measurements.

## Long term flow conditioning using the ibidi Pump System

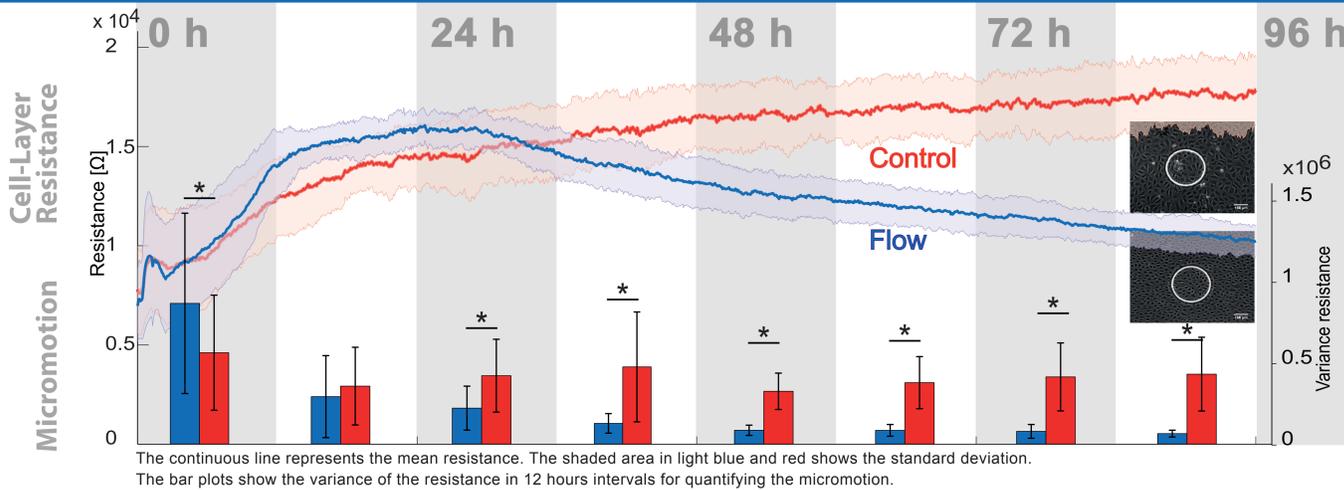
- Flow conditioning of HUVEC (P3/P4) with ibidi Pump System in Collagen IV-coated  $\mu$ -Slides I Luer
- Flow measurements at 10 dyn/cm<sup>2</sup>; Control at 0.75 dyn/cm<sup>2</sup> (perfusion for 8 min every 6 hours)
- Immunofluorescence staining: VE-cadherin (adherence junctions), Claudin-5 (tight junctions), and Connexin43 (gap junctions)
- Impedance measurements continuously over 96 hours using ECIS Z $\Theta$  (Applied Biophysics)



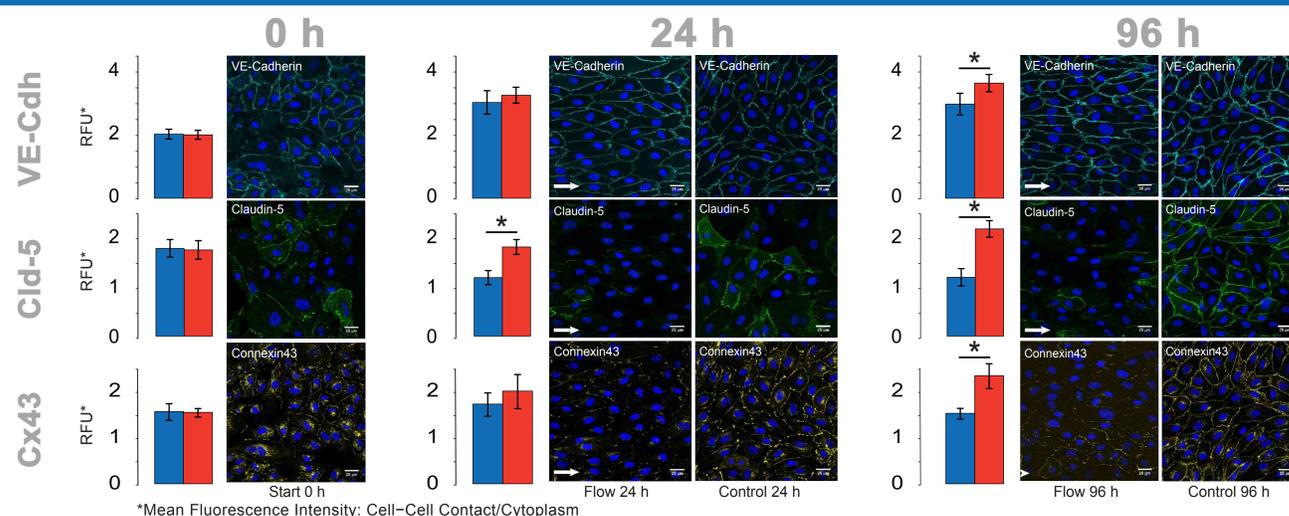
## Morphology



## Physiology



## Cell-cell Contact Proteins



## Results

Cells respond to fluid shear stress as regards:

**Morphology** - Cell orientation in flow direction develops within a few hours and is then conserved over the entire experiment.

**Resistance and Micromotion** - Under static conditions, the resistance rises to a maximum level within 24 hours and then remains constant. Under flow conditions, cells show a continuously decreasing resistance over the next 72 hours. Micromotion also decreases under flow conditions, as seen by the variance of the resistance signal.

**Cell-cell Contact Proteins** - Claudin-5, Connexin43, and to a less degree also VE-Cadherin levels decreased under flow conditions.

## Open Questions

When does the resistance value come up with the minimum?

Does the decrease of the cell-cell contact proteins depend on the magnitude of the shear stress?