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cells in focus ibidi®

Defining the Critical Shear Stress Range in Long Term HUVEC Cell Culture

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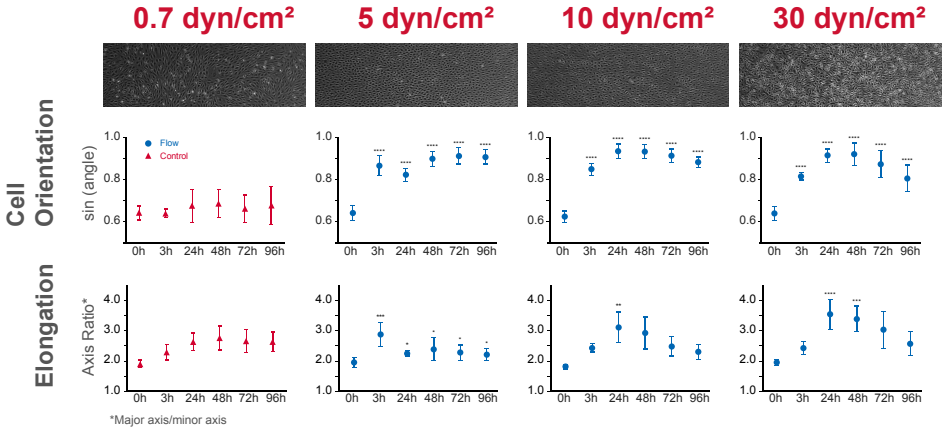
Introduction

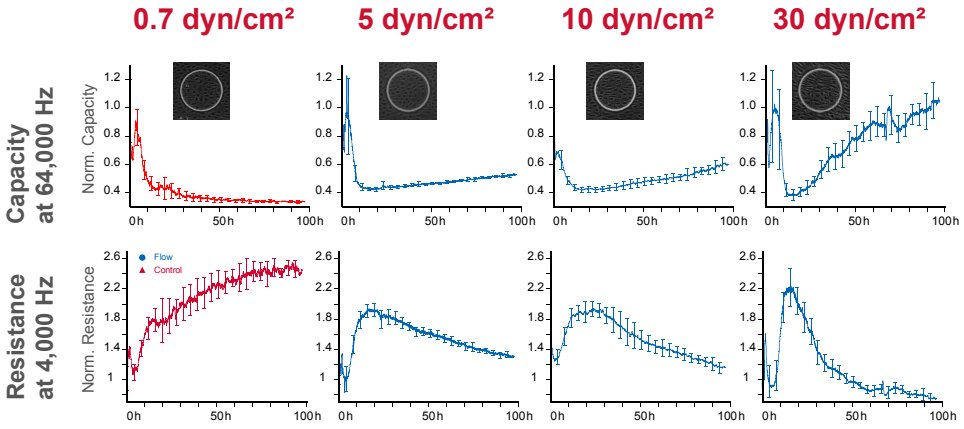
Long term in vitro experiments of HUVEC under shear stress displayed a dynamic modification in cell morphology and cell-cell contacts leading to a dramatic change in the physiological behavior of the cell monolayer.

Up to now it is unknown which shear range is responsible for the observed changes in cell behavior. Therefore we investigated shear stress values between 0.7 and 30 dyn/cm² over 96 hours, regarding the physiological properties and morphological characteristics of the cell layer. In parallel to the long term impedance measurements, a cell-cell contact protein of tight junctions was counterstained with immunofluorescence.

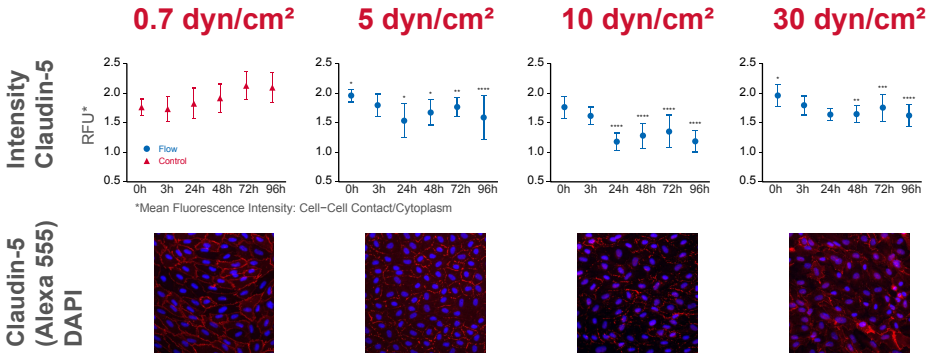
Morphology



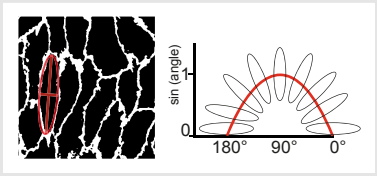
Cell Physiology



Cell-cell Contacts



Long term flow conditioning using the ibidi Pump System



- Flow conditioning of HUVEC (P3/P4) with ibidi Pump System in CollagenIV-coated µ-Slides I Luer over 96 hours
- Flow measurements at 0.7, 5, 10 and 30 dyn/cm²
- Immunofluorescence staining: Claudin-5 (tight junctions)
- Impedance measurements using ECIS ZΘ (Applied Biophysics)

Results

Low shear stress of 0.7 dyn/cm² had no effect on tested parameters and correlates to steady state observations.

Morphology

- Cell orientation was significantly changed at higher shear stress compared to 0.7 dyn/cm².
- Elongation of cells decreases after 24 hours shear stress dependend.

Cell Physiology

- According to the tested parameters, the shear stress of 30 dyn/cm² proved to be far from physiological conditions. We observed a leakage of the cell layer indicated by the sharp and inhomogeneous increase of C and the disturbed optical coherence of the cell layer.

Cell-cell Contact Protein

- The tight junctions were down-regulated under shear stress >0.7 dyn/cm².

Conclusion

Our results highlight the importance of the exact definition of shear stress parameters since only a range between >0.7 and <30 dyn/cm² seems to mimic physiological flow conditions for HUVECs.