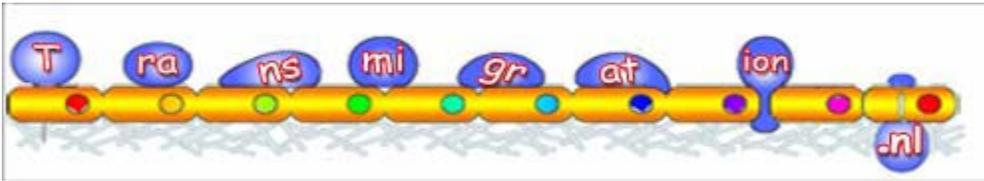


MCB Internship project 1:



Physiological flow as a tool to study shear stress-mediated morphological changes in endothelial cells

Introduction: Physiological flow is more and more recognized as a must have technique when studying endothelial cell function and behavior *in vitro*, as well as studying the process of leukocyte transendothelial migration. Our lab uses IBIDI-microslides to study leukocyte-endothelial cell adhesion and transmigration in real time. Moreover, we focus on the behavior and adaptation of endothelial cells to different flow conditions. We used the (fluorescent) microscope, flow cytometry and Western blotting to analyze changes in endothelial activity and protein expression.

For flow cytometry, the endothelial cells were removed from the microslides immediately after the flow experiment using a special protease: acutase. This protease did not induce apoptosis or shedding of crucial surface markers such as ICAM-1. In fact, the results showed that ICAM-1 surface levels were increased after flow. For Western blotting, the microslides were flushed with 200 μ L of lysis buffer, after which the lysate was diluted in boiling sample buffer. The results showed that, also by Western blotting, ICAM-1 levels were increased after flow.

Endothelial cells respond to flow by aligning to the direction of the flow. Moreover, we observed the formation of stress fibers as well as elongation, both induced by changes in the actin cytoskeleton. This project will look into detail how endothelial cells respond to different flow conditions, i.e. disturbed/turbulent versus laminar versus static conditions. Many techniques will be used to study this central theme.

Techniques:

- Western blotting, flow cytometry: to study protein expression and activation.
- Confocal laser scanning microscopy and scanning electron microscope techniques are used to study the morphological changes on the endothelial cells.
- RNAi techniques, including shRNA or siRNA approaches are used.
- Overexpression of dominant active and negative mutants with adenoviral or lenti-viral delivery system is routinely used.
- Molecular biology is used to create required point mutants or deletion mutants in order to study the involvement of specific proteins.

Duration: at least 6 months. Students from the University or HLO who are looking for a dynamic and interesting internship and are interested in the above project are encouraged to contact the group leader, Jaap van Buul, either by e-mail: j.vanbuul@sanquin.nl or phone: (+31) 20-5121219