Barriers

ENDOTHELIAL BARRIER

Microfluidic Solution to Study in Depth the In Vitro Mechanisms of Endothelial Barrier Integrity

Background

The key mechanism of endothelial dysfunction is the imbalance of endothelium-derived nitric oxide (NO) production and reactive oxygen species (ROS) generation, resulting in a decline in the bioavailability of NO and excessive accumulation of ROS. This finally leads to oxidative stress and cellular injuries.

Pathology Model

Endothelial cells (i.e. Human Umbilical Vein Endothelial Cells HUVEC-2 or aortic endothelial cells HAEC) will be exposed to metabolic stress (i.e. oxygen glucose deprivation) in the presence/absence of the client's compound.

Both static and dynamic in vitro microfluidic models will be taken into consideration in order to evaluate endothelial barrier integrity.

Readouts

The following quantitative parameters will be taken into consideration:

Molecular Biology

Gene expression profile: endothelial cells or aortic endothelial cells will be cultured in vitro and tested for their angiogenetic properties in the presence/absence of CLIENT's compound. Ranibizumab will be used as control.

The expression of main angiogenic molecules will be quantitatively evaluated.

Biochemical Characterization

- Total ROS production (i.e. DCF-DA fluorescent assay)
- NADPH-dependent superoxide formation (i.e. Dihydroethidium (DHE) staining)
- Cell viability and toxicity: (i.e. MTT assay)
- Mitochondrial damage (i.e HCS Mitochondrial Health assay)
- Inflammatory profile. A detailed analysis of pro inflammatory and angiogenic factor production will be characterized (i.e. IL-1a, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, TNFa, IFN-γ, EGF, MCP-1 etc).