Blood Brain Barrier

A model of in vitro blood brain barrier (BBB) using primary rat brain endothelial cells will be used (Maria Deli ref.). BBB will be subjected to oxygen glucose deprivation (OGD) to mimic stroke conditions and beside ROS, cell viability and eNOS functionality, BBB integrity will be evaluated.

The following morphological, biochemical and functional parameters will be quantitatively assayed:

1. **Biochemical Characterization**
   a. Cell viability and toxicity: (i.e. MTT assay)
   b. Mitochondrial damage (i.e HCS Mitochondrial Health assay)
   c. Total ROS production (i.e. DCF-DA fluorescent assay)
   d. NADPH-dependent superoxide formation (i.e. Dihydroethidium (DHE) staining).
   e. Inflammatory profile: a detailed analysis of pro inflammatory and angiogenic factor production will be characterized (i.e. IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL10, VEGF, TNF-α, IFN-γ, EGF, MCP-1 etc...).

2. **Morphological Characterization**
   a. Membrane integrity: the morphological analysis of membrane integrity will be monitored by means of confocal microscopy on immunofluorescent-labeled cells; zona occludens and tight junction will be quantitatively evaluated.

3. **Functional Characterization**
   a. BBB permeability: i.e. FITC-dextran tracer.
   b. Trans-endothelial electric resistance.