



# BLOOD BRAIN BARRIER

## Background

The Blood-Brain Barrier (BBB) is a highly selective permeability barrier that separates the circulating blood from the brain extracellular fluid in the central nervous system (CNS). The Blood-Brain Barrier allows the passage of water, some gases, and lipid soluble molecules by passive diffusion, as well as the selective transport of molecules such as glucose and amino acids that are crucial to neural function

Chronic inflammatory states, as those typically occurring in aging diseases, lead to tissue degeneration and membrane permeability thus favouring immune cell

## Readouts

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

### Biochemical Characterization

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

crosstalk within the central nervous system. This crosstalk is crucial in the onset of neuroinflammatory events which characterize the early steps of neuronal degeneration

## Pathology Model

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.



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## Morphological Characterization

- 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.

## Functional Characterization

- BBB permeability: i.e. FITC-dextran tracer.
- Trans-endothelial electric resistance.