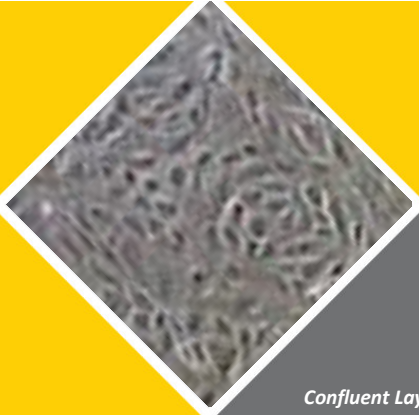
Ocular Diseases

# AGE RELATED MACULAR DEGENERATION



*Confluent Layer of Human Retinal Pigmental Epithelial Cells*

## Background

Recent evidence implicates the immune system in the development of Age-Related Macular Degeneration (AMD), as immune-related proteins are found in drusen from AMD eyes. Excessive activation of inflammatory and immunological cascade with subsequent induction of damage, persistent activation of resident immune cells, accumulation of byproducts that exceeds the normal capacity of clearance giving origin to a chronic local inflammation, alterations in the activation of the complement system, infiltration of macrophages, T-lymphocytes and mast-cells from the bloodstream, participate in the mechanisms which originate the drusen.

## Pathology Model

In order to evaluate the CLIENT's compound ability to modulate retinal degeneration in an in vitro model of AMD, a human line of retinal pigmental epithelial cells, microglia, macrophages and T-lymphocytes (all of human origin) will be cultured in separate chambers in a microfluidic platform. Optimal cell density will be evaluated for each cell population. Cell purity and viability will be tested by specific markers. Immunoreactive cells will be activated by either LPS or IFN gamma and put in microfluidic communication soluble factor, cell-cell mediated crosstalk. Particular attention will be given to the quantification of oxidative stress. Measuring oxidative stress is crucial for the pathogenesis of AMD; in fact, recent evidence showed that the autoimmunity component might be related to oxidative stress. Furthermore, complement components will be quantified in supernatant and cellular lysates. It has been postulated a role for an alteration of the complement pathways in the pathogenesis of AMD. In fact, drusen include elements of the complement system belonging to all pathways: classical, alternative and lectin pathways. In addition, a strong association between advanced AMD and high levels of the complement components C3, CFB, CFI, CFH, and factor D (CFD) and activation fragments Bb, C3a, C5a, iC3b, and SC5b-9 has been found in the peripheral blood of patients..

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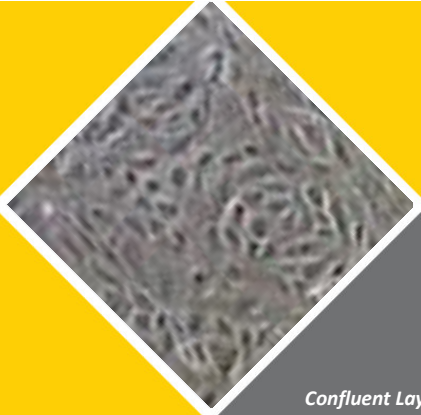

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## Readouts

The following parameters will be analyzed:

- Cell purity (immunocytochemistry)
- Cell viability (MTT assay)
- Deposition of druse (i.e. quantification by electron microscopy
- Quantitative evaluation of proliferation rate
- Quantitative evaluation of cell's activation state (i.e. membrane permeability)
- Mitochondrial damage (i.e. HCS Mitochondrial Health assay)
- NO production (i.e. Griess assay)
- Total ROS production (i.e. DCF-DA fluorescent assay)
- Cytokine production (multiplex ELISA)