Final Report

Project acronym: *BIOMEMBRANE*

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2. Publishable project summary

Age-related macular degeneration (AMD) is a multifactorial chronic eye disease and the leading cause of blindness in millions of elderly people world-wide. In AMD patients, several morphological and structural changes occur in the retinal pigment epithelium (RPE), the Bruch's membrane (BrM), and the underlying choroidal vascular network (CVN) resulting in the progressive vision loss. At present, the exact disease pathogenesis remains poorly understood. As a result, no effective treatments exist to halt or reverse AMD progression. To investigate the pathophysiological process underlying AMD and to validate novel drug candidates, several *in vitro* models have been proposed, but without physiological realism and great predictive value. The concept behind the **BIOMEMBRANE Project** is the design and fabrication of a **novel in vitro model of the outer blood-retinal barrier (oBRB)**, to boost the discovery of new therapeutic strategies for AMD.
The BIOMEMBRANE Project was organized into seven (7) work packages (WP), five related to the scientific activities, one to analysis toward industrial scaling-up, and one to dissemination and management. All WPs were advanced synergistically to achieve a novel 3D microfluidic platform consisting of: i) a bioengineered BrM (WP1-WP2), ii) a biomimetic microfluidic network of the CVN (WP3), iii) a bioreactor reproducing the *in vivo*-like intraocular environment (WP4-WP5).

Gelatin-Methacryloyl (GelMa)-coated electrospun PLGA membranes were chosen as healthy model of the BrM, while GelMA-coated Gelatin membranes crosslinked with GPTMS were used as BrM pathological model. Both BrM models were biologically validated by culturing on top of them human embryonic stem cells derived–retinal pigment epithelium cells (hESc-RPE). Results showed that HESCs-RPE cells seeded adhered and matured in most of the tested membranes, moreover they were polarized and formed a typical cobblestone monolayer.
The microfluidic network mimicking the CVN was designed starting from indocyanine angiography scans and optimized via computational analysis. The resulting CVN model was fabricated on polydimethylsiloxane (PDMS) through a novel manufacturing method established to provide a time-saving and cost-effective alternative to the common lithographic-based techniques.
BrM and CVN were assembled and subsequently housed in a single PDMS-based 3-part bioreactor to enable the co-culture of human RPE and endothelial cells above the BrM and inside the CVN, respectively. Multiphysics simulations, based on digital analysis using the finite element method (FEM), were performed for studying the physical phenomena occurring inside the designed bioreactor, to guide and validate the design choices of the fabricated device. Tests of dynamic seeding and static/dynamic culture of HUVECS endothelial cells were set out to validate the in vitro model.
To better understand AMD *in vitro,* metalloproteinase (MMP)-2/-9 inhibitors (FC-311), which are thought to contribute to the pathology, were tested in the developed in the *in vitro* model.
In parallel, with the attempt to develop new therapeutic approaches innovative drug candidate as anti-inflammatory drugs for AMD were also tested in the devices and compered to a commercially available control. Despite numerous experiments, not successful but encouraging results to pursue this research were achieved, demonstrating the validity of the BIOMEMBRANE approach.
In conclusion, the novel designed, and fabricated *in vitro* model proved to accurately mimic the anatomical and functional structure of the oBRB, thanks to the successful integration of a bioengineered BrM as interface of a microfluidic circuit. This element of innovation makes the developed model appliable for the implementation of other in vitro models where the integration of a membrane and a microfluidic vascular network is needed, such as blood-brain barrier.