Vascularised Retinal Organoids

It has been recently shown that the dysfunction in the relationship between the neuroretina and the vascular system (neurovascular unit-NVU) plays a crucial role in the pathophysiology of retinal diseases such as diabetic retinopathy and age-related macular degeneration [1]. In vitro retinal model development has gained momentum due to the inadequacy of animal models in replicating the structure and function of the human retina. Human embryonic and induced pluripotent stem cell (hESC and hiPSC)-derived retinal organoids (ROs) have demonstrated diverse applications, such as investigating human retinogenesis, modelling diseases, drug discovery, and potential cell therapy. Multiple protocols have been established to generate ROs, aligning with fundamental principles of forebrain and eye development, in which the consistent laminar organization and the presence of all neural cell types within the retinal structures is significant. However, ROs that have been generated and differentiated from pluripotent stem cells (PSCs) lack vascularization and thus their maturation is impaired [2].

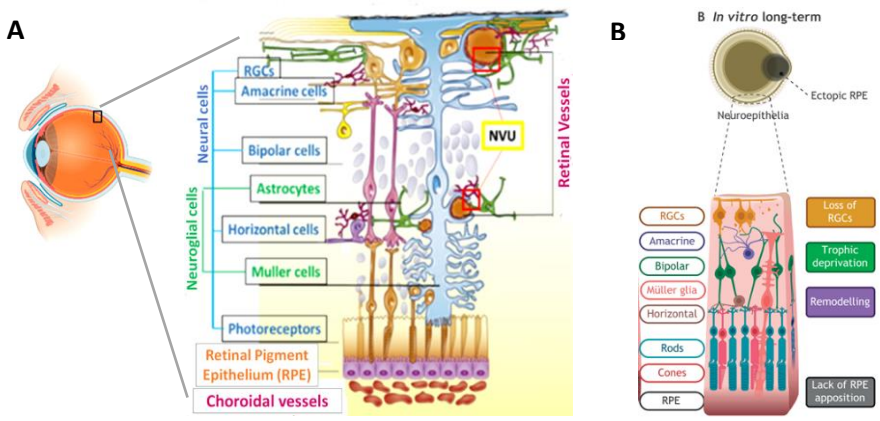

***Figure 1. Anatomy of the retina**. (A) In vivo, the seven main neuroretinal cell types populate the layers of the retina with retinal pigmented epithelium (RPE) next to the outer nuclear layer. Interneurons synapse with photoreceptors in the outer plexiform layer and retinal ganglion cells (RGCs) in the inner plexiform layer to relay signals to the brain. The outer blood-retinal barrier (BRB) consists of the connections between the retinal pigment epithelium (RPE) cells that regulate the flow of substances between the choroid capillaries and the neurosensory retina, while the inner BRB is the result of the strong interaction between endothelial cells (ECs), pericytes/mural cells (MCs), neuroglia, and neurons. (B) In vitro, retinal organoids develop multiple layers and cell types, but RGCs are progressively lost in long-term culture, possibly owing to lack of neurotrophic factors or other ocular structures. Subsequently, interneuron cells are lost and remodelling occurs. In retinal organoids, RPE formation is ectopic [3].*

Therefore, the advancement of reliable experimental model systems in order to study the NVU is an urgent need and the generation of human ROs is the ideal approach to do this, given the limitations of the use of experimental animal models. We have successfully generated and extensively characterized endothelial (ECs) and mural cells (MCs) derived from hPSCs in our lab [4,5] so our work now is focused on the generation of vascularized ROs derived from human PSCs that consist of both neuronal and vascular cells (ECs, MCs)) in order to develop the retinal NVU (rNVU) in the best anatomical layout. Furthermore, in order to recapitulate the microphysiological rNVU environment with a precise control over structural and temporal parameters we are going to merge organoid and Organ-on-chip (OoC) technology and generate an innovative humanized in vitro retinal model (retina on a chip-ROC). In that way, our in vitro rNVU could serve as a model to elucidate the pathophysiology of various retinal diseases. Within this context, we are going to explore the role of rNVU in Retinitis Pigmentosa (RP) (an inherited disease causing blindness) using patient-derived hiPSCs with a PRPF31 mutation, known to be responsible for RP development [6].

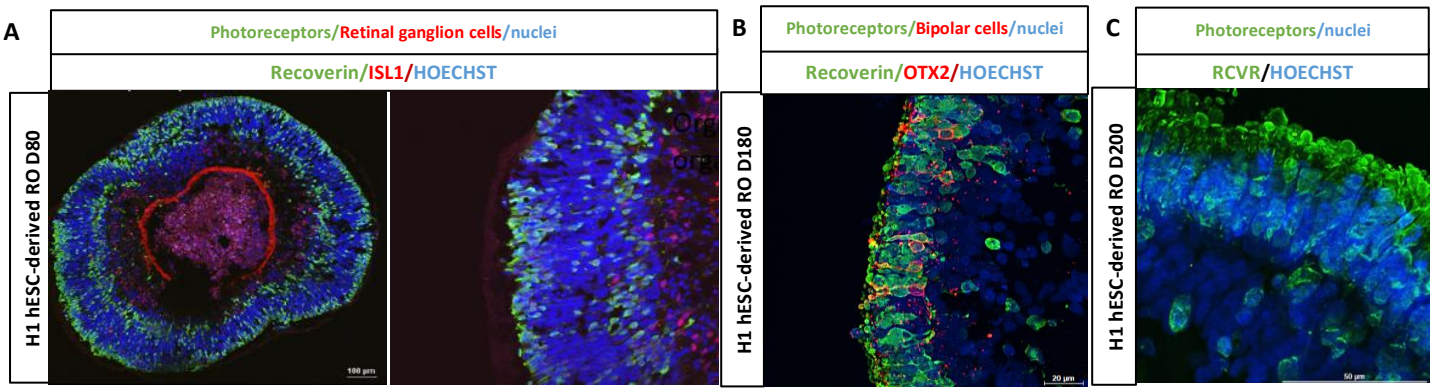

***Figure 2. Generation of Retinal organoids** A) H1 hESCs derived ROs on Day80 of differentiation. ROs consist of a plethora of retinal ganglion cells(red) that localize as expected in the basal layer of the organoid. At the same time a high number of Recoverin positive cells (photoreceptors, green) is present, which seem to migrate to the outer nuclear layer of the organoid. B) H1 hESCs derived ROs on Day180 of differentiation. Photoreceptors(green) have resided the outer apical layer of the organoid. Bipolar cells(red) are also present. Photoreceptors synapse onto bipolar cells in the retina (C) By Day 200 of differentiation mature RO photoreceptors outer segments protrude from the RO surface.*

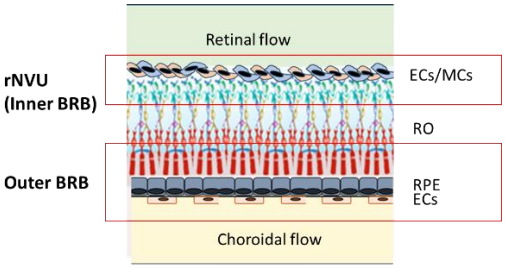

Figure 3 Development of double flow ROC (choroidal and retinal). *Flow applied at the upper level will simulate the blood circulation of the Retina.*

References

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