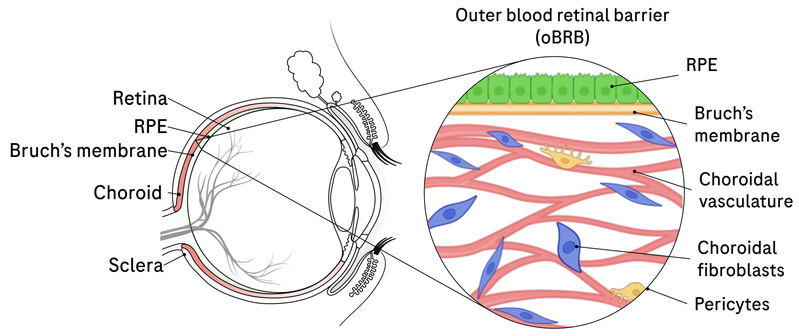
A human outer-blood-retinal barrier (oBRB) microphysiological system as a platform for disease modeling and target assessment for subretinal fibrosis

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Introduction

Age-related Macular Degeneration (AMD) is a leading cause of global vision loss, impacting over 200 million people. The pathogenesis is driven by the breakdown of the outer blood-retinal barrier (oBRB), a complex comprising the vascular choroid, Bruch's membrane, and the Retinal Pigment Epithelium (RPE). Under physiological conditions, the RPE maintains tight junctions that form a selective seal, regulating the transport of nutrients and waste essential for photoreceptor health. When this barrier is compromised, the ensuing choroidal neovascularization and maladaptive wound-healing responses could progress into subretinal fibrosis. In this pathological state, the activation and differentiation of tissue resident cells into extracellular matrix (ECM)-producing cells such as myofibroblasts triggers collagen I accumulation causing irreversible scarring associated with aggravating vision decline. To elucidate these mechanisms and validate prospective drug targets, we have developed an in vitro oBRB model featuring a mature RPE monolayer with functional tight junctions integrated over a perfusable choroidal vasculature network. This vascular component is composed of primary human retinal microvascular endothelial cells (HRMVECs), retinal pericytes (hRPs), and ocular choroidal fibroblasts (HOCFs).



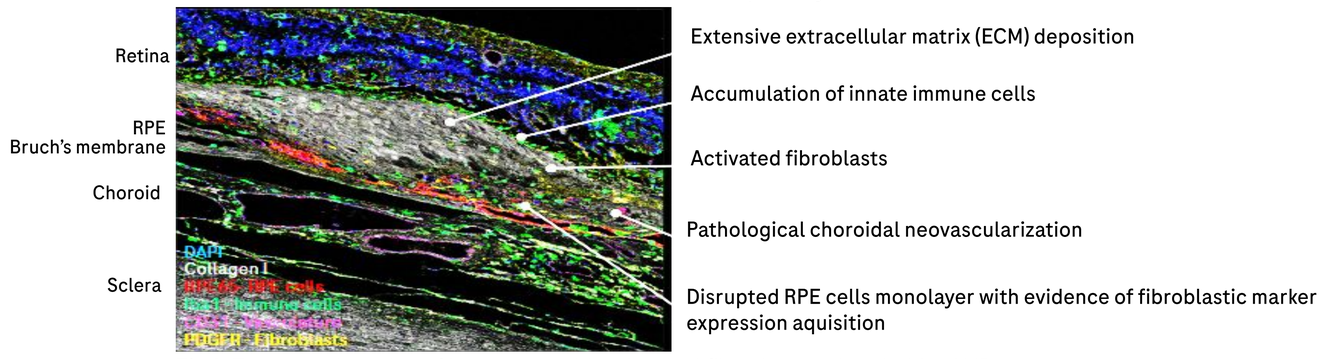
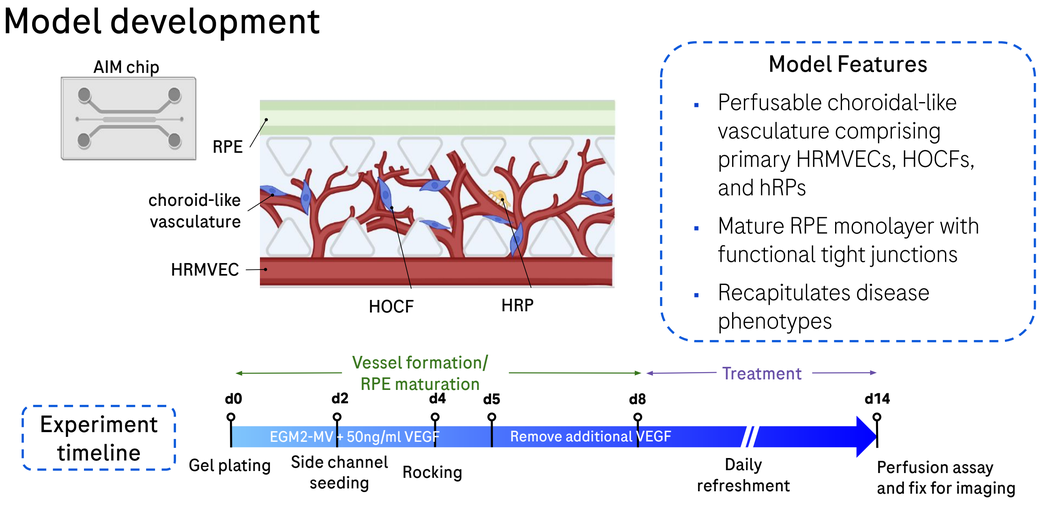


Fig1: Transversal section of human eye with subretinal fibrosis associated with nAMD.

Model development



Outer blood-retinal barrier on-a-chip

We have successfully established a stabilized 3D choroidal-like vascular network that achieves physiological homeostasis. The model integrates HRMVECs, HRPs, and HOCFs to self-assemble into complex, luminized vessels supported by a native extracellular matrix. This platform provides a robust, perfusable baseline that accurately recapitulates the structural and functional complexity of the human outer blood-retinal barrier.

Establishment of choroidal-like vessels

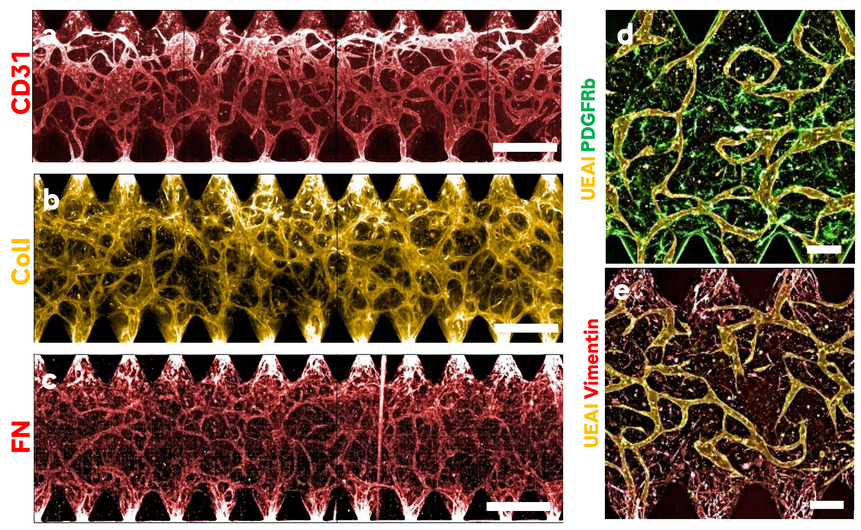


Fig2: Representative immunofluorescence of the stabilized 3D vascular network (HRMVEC, HRP, HOCF). **(a)** Endothelial cells (CD31) form vessels which span the inner channel of the chip. Scale bar 500 µm. **(b,c)** Deposition of collagen I (Coll) and fibronectin (FN) at the basement membrane and the intra-vascular space. Scale bar 500 µm. **(d,e)** Retinal pericytes (PDGFRb) and ocular choroidal fibroblasts (Vimentin) support the vasculature and in close interactions with the vessels. Scale bar 100 µm.

Perfusable vessels with tight RPE barrier

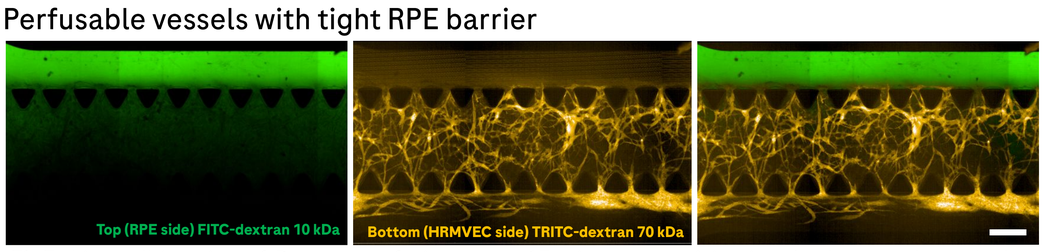


Fig3: Live-cell imaging of Dextran perfusion assay 15 minutes post-injection. In the top channel, the retention of 10 kDa TRITC-Dextran demonstrates a tight RPE barrier that prevented perfusion and diffusion of fluorescence dye. The retention of the 70kDa TRITC-Dextran within the self-assembled lumenized microstructures demonstrates a functional and stable vascular barrier. Scale bar 500 µm.

Disease modeling

Increase in number of aSMA positive myofibroblasts

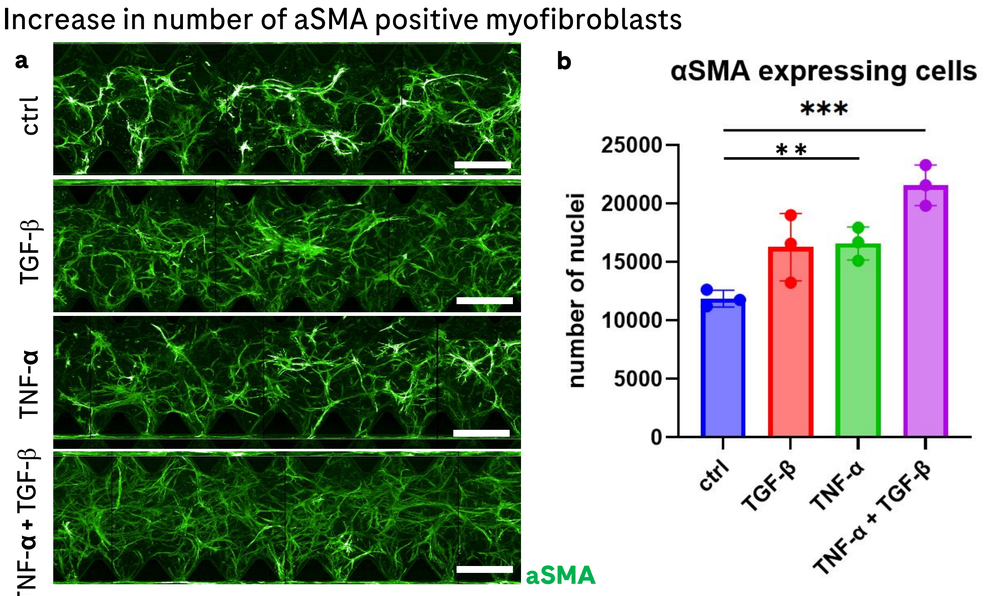


Fig4: Pro-fibrotic activation of choroidal fibroblasts. **(a)** Representative immunofluorescence images showing αSMA expression in myofibroblasts across different treatment groups. Scale bar 500 µm. **(b)** Quantification of αSMA-positive nuclei. Both TNF-α and TGF-β stimuli trigger a significant shift from physiological homeostasis (ctrl) to a pathological fibrotic phenotype, with TNF-α + TGF-β inducing the most robust myofibroblast differentiation.

Fibroblasts invasion across RPE barrier

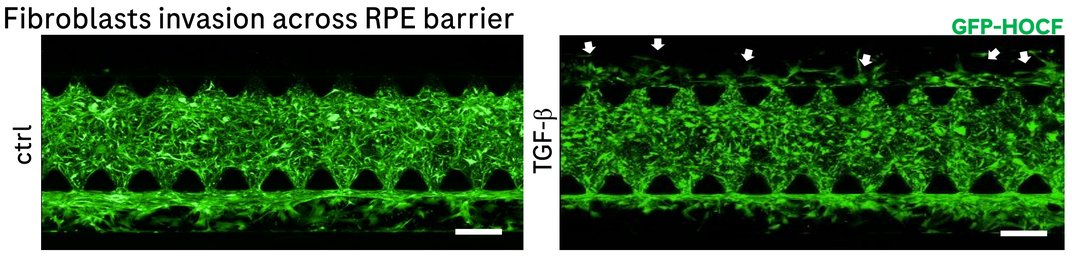


Fig5: TGF-β induced barrier breakdown and cellular invasion. Under TGF-β stimulation, fibroblasts exhibit increased motility and successfully breach the RPE barrier (arrow), an essential step in the development of sub-retinal fibrosis. Scale bar 500 µm.

Increased extracellular matrix deposition

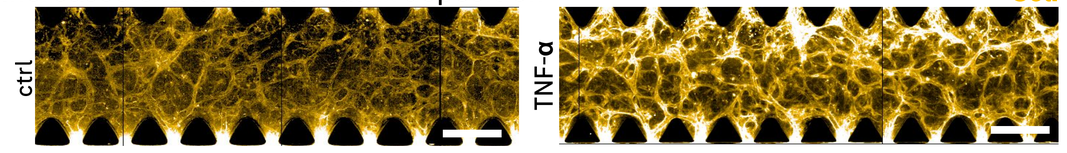


Fig6: Pathological collagen I accumulation as a hallmark of sub-retinal fibrosis. While the control (left) maintains physiological homeostasis, TNF-α triggers an aberrant up-regulation of collagen I (right). This dense accumulation leads to thickening of the choroid and a transition to a pathological state. Scale bar 500 µm.

Conclusions and outlook

This 3D oBRB platform successfully recapitulates the critical characteristics of the outer blood-retinal barrier and replicates key cellular and extracellular matrix hallmarks of subretinal fibrosis. It enables the evaluation of antifibrotic targets and testing of candidate molecules capable of mitigating pathological ECM remodeling and preserving oBRB integrity.

- Disease modeling: The model can recapitulate fibrotic phenotypes:
  - RPE barrier disruption
  - Fibroblast migration and increase in myofibroblasts (α-SMA expressing cells)
  - Increased ECM deposition
- Future Directions: Utilizing the model for target validation and incorporating cell types specific to diseases of interest to further elucidate complex pathological mechanisms

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