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Engineering the Inner Blood-Retina Barrier for Disease Modeling and Therapeutic Discovery

Globally, 1.1 billion people suffer from vision impairment, with 43 million of those afflicted experiencing complete blindness. Retinal vascular diseases, the primary contributor to vision reduction and loss, are characterized by compromised inner blood-retina barrier (iBRB) functionality, inducing vascular leakage. The iBRB is a vascular network containing retinal endothelial cells and pericytes that are tightly associated and regulated via cell-cell surface proteins, maintaining ocular homeostasis. Despite this central role, iBRB dysfunction remains poorly understood. This is due to the inability of in vitro and in vivo systems to mimic the biological complexity and molecular interactions present within the iBRB and the limited studies utilizing human iBRB cells. In vitro models that contain human retinal endothelial cells and pericytes are thus critical to recapitulating the iBRB in health and disease to advance cerebrovascular understanding and treatment of retinal vascular diseases. To generate a model of the physiological iBRB that is capable of accelerating research and uncovering therapeutics, we first established human induced pluripotent stem cell-derived retinal endothelial cells (iRECs) and retinal pericytes (iRPCs). We confirmed their phenotypic integrity and physiological functionality at the gene- and protein-level and with functional assays. Specifically, the iRECs contained canonical endothelial, junctional, and retinal protein markers and physiological barrier properties, while the iRPCs expressed canonical pericyte proteins, validating their biological accuracy. Using the iRECs and soft lithography techniques, we engineered 3D microphysiological systems (MPS) that robustly recapitulated the iBRB. The iRECs self-assembled into morphologically and phenotypically accurate networks with perfusable lumina that are tightly associated via canonical membrane-bound tight and adherens junction proteins. Integrating this system with iRPCS, we observed the formation of luminal iREC networks supported and encapsulated by iRPCs,

mimicking physiological organization and cellular interactions. Taken together, we conclude that the iBRB MPS faithfully recapitulate healthy, functional iBRB barrier properties and physiological endothelial-pericyte interactions, demonstrating their biological fidelity. Our functional and perfusable iBRB MPS can thus be harnessed to investigate iBRB development, elucidate pathophysiological mechanisms underlying retinal vascular diseases, advance precision medicine, and accelerate therapeutic discovery.