

Vascularization and microfluidic guidance elevate human retinal organoids to a new level of functional maturity

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ABSTRACT

Retinal organoids from human pluripotent stem cells have emerged as vital tools for research in human retinal development, disease, and therapy. The absence of a vascular system in these cultures along with decreased survival of retinal ganglion cells (RGCs) over extended culture periods have, in many ways, hindered their recreation of physiological processes as they are in an *in vivo* environment. A recent paper published in *Cell Stem Cell*, from the lab of Sharma et al., involving the generation of transiently vascularized retinal organoids (vROs) through the inclusion of stem cell-derived endothelial cells and microfluidic technology, presents a remarkable enhancement in the survival, functional maturation, as well as the light response mediated through photoreceptors of RGCs in comparison to non-vascularized cultures. This development removes many of the caveats prevailing in the history of retinal organoid research, aligning more accurately with the *in vivo* environment. In the following sections, the implications of this directive technology have been highlighted.

KEYWORDS

retina, organoid, stem cell, vascularization, microfluidic technology

During the past decade, human retinal organoids have become established as high-fidelity three-dimensional (3D) cell culture systems that recapitulate several aspects of retinal development, cellular heterogeneity, and initial electrophysiological development *in vivo*. These systems have provided the scope to explore the mechanisms underlying photoreceptor cell differentiation, the formation of layered tissue architecture in the retina, and diseases like inherited retinal dystrophies and retinoblastomas^[1,2]. Despite the success of existing retinal organoid culture systems, their limitation lies in their inability to establish a vascular network. This causes metabolic limitations, hypoxia, and ultimately the loss of specific cell types that are particularly sensitive to hypoxic or metabolic insults, like retinal ganglion cells (RGCs).

RGCs are the only type of projection cells found in the retina, and they carry information from phototransduction to higher centers connected to the optic nerve. Metabolically demanding cells with long axons require vascularization; however, *in vitro* models lack this since they present dense aggregates devoid of vessels, resulting in a diminished population of RGCs after a short time, affecting the ability to model conditions where RGCs are a key aspect, such as glaucoma and optic neuropathies^[3].

Sharma et al. overcome these challenges with a transient system

of vascular support through the generation of vascularized retinal organoids (vROs), where they introduce endothelial cells (ECs) that coalesce and bridge the organoid tissue, facilitating the transport of media components to address issues of hypoxia and apoptosis, and increase the overall organoid size and health^[4]. Importantly, at key developmental time points, vROs had significantly higher populations of RGCs than control organoids lacking vascular integration, as defined by canonical markers POU4F1 and RBPMs.

Outside of survival, the authors utilized microfluidic devices that enhanced directional axonal extension and microelectrode arrays that measured electrophysiology. Compared to controls that showed loss of viable RGC activity over time, vROs showed robust spontaneous activity and synchronized activity, indicative of mature networks. Most impressively, vRO cultures showed an ON, OFF, and ON-OFF light response mediated through the photoreceptors, then through intermediate interneurons to the RGC, which is indicative of vertical retinal networks, particularly by or around week 30.

These results verify three important innovations: (1) vascular support to reduce metabolic stress of dense organoid tissue; (2) microfluidic control of axonal stability to maintain RGC

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process extension; and (3) continued electrophysiological activity of a type that reflects sophisticated retinal circuitry.

Each of these technologies addresses one of the greatest challenges to growing retinal organoids: hypoxic degeneration and inability to maintain RGC-dependent functional outputs beyond early developmental stages.

The implications of vROs range from basic biology to disease modeling and translation. For instance, by supporting the survival and maturation of RGCs, this system will provide a more accurate model for understanding the network dynamics of the human retinal network than before. This will provide unique opportunities for studying aspects such as circuit assembly, neuronal subtype specification, and activity-conditioned developmental processes that were previously inaccessible *in vitro* studies. Additionally, the hypoxia responsiveness and vascular remodeling properties exhibited by vROs will provide opportunities for understanding retinal vascular diseases such as retinopathy of prematurity or diabetic retinopathy.

From a translatability perspective, the prospect of long-term RGC functionality provides a means for preclinical assessment of drugs along with gene therapies and neuroprotective strategies for optic neuropathy and glaucomas. Previous attempts to mature organoid cultures by means of bioreactors or co-culture with supporting cells assisted differentially with photoreceptor differentiation or lamination but failed to promote integrated RGC function output^[3]. The vRO paradigm greatly enhances the translatability of organoid systems in a multidimensional manner for a variety of complex functional assay purposes.

Nevertheless, challenges exist in achieving the same in the future. The vascular structures generated in the current study showed their transient nature and regressed in the longer term in static conditions. Also, the lack of stable endothelial cell clusters in transcriptomic data indicates phenotypic changes in engineered endothelial cells and merits further exploration. Improvements in vascular structure generation through flow perfusion support, the use of pericytes in culture, or immune cell-like microglia can help recreate the *in vivo* environment more accurately and support long-term function of vasculatures in the retina^[5].

Sharma et al., however, point to a paradigm-shifting breakthrough in retinal organoid technology utilizing a combination of transient vascular support and microfluidic guidance to finally address the historical challenges for RGC viability and functional maturation in cultures. Not only does this research improve the state-of-the-art for retinal cell culture models, but it also promises a highly versatile model system for studying human neurovascular development, neurodegenerative processes, and therapeutic responses with unprecedented functional detail. As knowledge pertaining to organoid biology continues to incorporate aspects of *in vivo* biology, vROs point to a key landmark on the path to functional human retina analogs in the laboratory.

Research ethics and patient consent

Not applicable.

Availability of data and material

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Author contributions

Zongming Song conceived and guided the study. Gang Wang, Zixu Huang, and Zongming Song contributed to writing the draft. Wei Deng visualized it.

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