Review

Perceiving Retinal Progress: From Anatomy to Bioengineering Organoids

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Review

Perceiving Retinal Progress: From Anatomy to Bioengineering Organoids

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Abstract: The retina, a crucial part of the eye, is made up of various cells and structures that are essential for the conversion of a light stimulus into an electric signal for the brain to develop an image. The different cells of the retina including the retinal pigment epithelium, rods and cones, and the retinal ganglion cells together are involved in the transduction of light and the formation of connections with the optic nerve. This is a complex and efficient system with distinct layers of cells being involved in the absorbing of unnecessary light stimulus and providing physiological support. Retinal disorders are most prevalent in people above the age of 50 and cases of blindness are in the millions, making the need for better treatment options a pressing matter. Shortage of donors and patient incompatibility are reasons for which the focus has moved to the use of stem cells and tissue engineering as an alternative treatment option. Induced pluripotent stem cells can be engineered to differentiate into retinal cells using supplements like SMAD antagonists and retinoic acid. Inactivating motor proteins and using Wnt/BMP4 antagonists facilitate the development of retinal neural characteristics to give three-dimensional retinal organoids. Microfluidics provides vascularization for better nutrient supply to make improved functional tissue models. Retinal organoids are hence promising for drug discovery and development, disease modeling, transplant alternatives, and developmental biology.

Keywords: retina, stem cell, organoid

1. Anatomy of the Retina and Light Transduction

The retina is an essential part of the eye that holds various components responsible for the interpretation of light stimulus into electrical signals for the brain to develop visual images[1]. The three essential cells that comprise the retina are the photoreceptors that connect to bipolar neural cells, and ganglion cells connecting after. This large-scale connection of the retina can further be divided into ten specific cellular sublayers for maintaining cells and interpreting light. The innermost layer, named the inner limiting membrane, is an exception to the composition of retinal layers as it is made up of basement membranes that separate the retina from the vitreous cavity of the eye[2]. Hereafter, all ganglia axons begin to converge to create the next layer called the nerve fiber layer (NFL). These fibers eventually thicken as they enter the optic disc, the gateway from the retina to the optic nerve[3]. The following ganglion cell layer is where ganglion cell bodies reside and where they project their axons to form the optic nerve[1]. The inner plexiform layer follows and is named for the connection between the synapse of ganglion cells and bipolar cells, moreover, bipolar cells are seen to form various types of connections between amacrine cells[4]. Next is the inner nuclear layer which includes bipolar, horizontal, and amacrine cells[1]. The next three layers are counterparts to the inner layers: the outer plexiform layer (OPL), the outer nuclear layer (ONL), and the external limiting membrane (ELM). The OPL describes the synaptic connection between the bipolar cells and photoreceptors, the ONL describes the layer of photoreceptor cell bodies, and the ELM is filled with gap junctions that separate photoreceptor cell bodies from their inner and outer segments[1]. After the ELM is the photoreceptor layer consisting of photoreceptors' inner and outer segments responsible for housing mitochondria for metabolism demands and light-sensitive rhodopsin for light transduction, respectively[1]. The final layer is the retinal pigment epithelial (RPE) which is below

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choroid vascularization and has essential roles in absorbing unnecessary light stimulus, providing physiological support, and secreting growth factors[5].

When light enters the eye, it is first processed by the two types of photoreceptors, rods and cones. The process of light transduction begins with the chemical breakdown of rhodopsin. A quick overlook of the process shows that rhodopsin, composed of protein Opsin and 11-cis Retinal, isomerizes into all-trans retinal and detaches the protein Opsin through photobleaching[6]. A more detailed process shows that when rhodopsin is photobleached, the inactivated protein scotopsin or photopsin, respective to rods and cones, will isomerize through varying opsin intermediates before settling into metarhodopsin, the product in both photoreceptors that activates transducin[7]. Specifically, metarhodopsin exists in equilibrium between metarhodopsin I and metarhodopsin II, where metarhodopsin II is favored in high temperatures and pH is the active form essential for transducin activation[8]. Transducin, the G-protein bounded with GDP, then becomes activated through the photolysis of rhodopsin to exchange GDP for GTP[9]. The protein phosphodiesterase is then activated by GTP and regulated by ATP to turn cyclic GMP (cGMP) to GMP through hydrolysis[10]. This breakdown of cGMP is done through phosphodiesterase, a light-activating enzyme. Mediated by cGMP, the breakdown to GMP closes positive-ion channels and produces a hyperpolarized electrical signal of the photoreceptors[6]. In dark settings, photoreceptors possess sodium and calcium ion channels located in synapse terminals that bring in a constant current of positive ions within the outer segment that trigger the release of the neurotransmitter glutamate[11]. In the presence of light, cation levels consequently drop alongside glutamate release. This change in the rate of neurotransmitter release is then decoded by bipolar cells. Bipolar cells have been seen to differ between rods and cones as each photoreceptor has its respective bipolar cell it interacts with[12]. Regardless of the photoreceptor, all variations of bipolar cells are still mediated by glutamate expression within synapse terminals. Communicating through synaptic potential instead of action potentials, bipolar cells send inputs toward retinal ganglion cells that eventually form a bundle of axons termed the optic nerve that sends information toward the brain.

1.1. Immunological and Homeostatic Role of Retinal Cells

As seen in the immune system, microglia play pivotal roles in the retinal microenvironment. They are tasked with constantly observing the body for immunoregulation, tissue repairment, and defending the body against foreign stimuli[13]. Alongside microglia, the blood-retinal barrier (BRB) and the complement system play roles in retinal immunity. The BRB supports the retina layers through an inner layer comprised of tight vascular endothelial cell junctions and an outer tight junction layer between RPE cells[14]. This defense protects the retina from potential foreign pathogens and any harmful responses from the systemic immune system. In the event of a breach within the BRB, the retina suppresses immune response to reduce inflammation. Lowering immunopathology is achieved through suppressing immune activation mediating by neural-immune regulatory proteins that influence microglia, macrophages, and the activation of the complement system[14]. As part of the innate immune system, the complement system is involved in retinal immunity through retinal microglia. Initially synthesized from hepatocytes, complement proteins are circulated throughout the body awaiting stimuli from BRB injury. Depending on the protein, three pathways may be activated that trigger membrane ruptures through osmotic pressure[15] of impacted cells. Furthermore, past studies show that protein synthesis involved in activating complement pathways is also locally synthesized in retinal cells, demonstrating a higher involvement of retinal cells within immunity[16].

In the context of homeostasis, retinal Müller cells are glial cells that are heavily involved in maintaining the retinal environment. Located in all nuclear and plexiform layers, Müller cells are seen to regulate the excitatory and inhibitory neurotransmitters glutamate and GABA[17].

Additionally, neurotrophic and growth therapeutic agents are synthesized for neuronal and other glial cells that facilitate retinal development and reduce reactive oxygen species[17]. Like microglia, Müller cells can phagocytose external cell factors and debris for microenvironment maintenance and may trigger pro-inflammatory responses when pathogens appear [17]. Moreover, Müller cells behave as a secondary defense when misplaced serum or proteins flow into the cell[18].

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Horizontal and amacrine cells are seen to play roles in homeostasis. Horizontal cells mediate glutamate transmission with photoreceptors while amacrine cells interpret and form signals within the inner retina toward ganglion cells[19].

1.2. Pathology Regarding the Retina

Findings within the year 2020 report that within patients 50 years or older, diseases such as Glaucoma, Age-related Macular Degeneration, and Diabetic Retinopathy were among the highest causes of eye blindness[20]. With over 40 million blind cases, the alternative for addressing irreversible blindness is the transplantation of retinal progenitor, pigment epithelium, and ganglion cells[21]. In this section, we highlight the workings of retinal degeneration diseases that result in blindness.

Age-related Macular Degeneration, or AMD, is the progressive loss of photoreceptors and RPE through the abnormal buildup of drusen, or ECM components seen on RPE and Bruch's membrane BrM[23]. At first, manifesting in moderate drusen sizes termed "early AMD," the progression into larger aggregates is termed Geographic Atrophy (GA) or neovascular AMD[20].

Eventually leading to blindness, AMD causation can originate from aging, genetics, and even biochemical pathways such as the complement system. It has been studied that the complement system heavily influences the retina, and dysregulation of this pathway becomes a main driver for AMD pathology[23]. Specifically, it is through the combination of local and system complement proteins that affects tissues and has been validated through higher complement protein concentration in patients with AMD compared to controls[21]. Moreover, RPE and immune cells influenced by the complement system to activate and secrete pro-inflammatory substances to further cause pathology [21].

Diabetic retinopathy (DR) is one of the implications of both variations of diabetes mellitus, where hyperglycemic blood flow leads to retinal endothelial cell damage. This change in retinal blood flow also leads to basement membrane thickening and blood vessel ischemia which trigger further complications[24]. Through ischemia, the frequent occurrence of endothelial lesions and microaneurysms can result in retinal detachment, ultimately causing vision loss[22].

Regarding inflammation, microglia have been confirmed to play roles in retina pathology-induced animal models, produce factors that induce retinal neuronal death in cell culture, and have been identified in post-mortem DR patients at different pathology progression states[25].

Glaucoma is a retinal disease that damages the foundation of the optic nerve head through the loss of retinal ganglion cell axons[26]. The risk factors that facilitate the development of glaucoma include aging, genetic heritage, and intraocular pressure that ultimately leads to optic nerve damage and vision loss. This degeneration of retinal ganglion cells is caused by elevated intraocular pressure attributed to the unmediated outflow of clear fluid produced in the eye, known as aqueous humor[27]. Though the focus for therapies addresses intraocular pressure, studies have reported that glaucoma pathophysiology changes with disease progression, incorporating aspects of the innate immune response[28]. Thus, demonstrating that mechanical and biochemical origins contribute to disease progression.

Of the retinal diseases mentioned, Retinitis Pigmentosa (RP) arises from genetic predispositions that lead to vision and color impairment through photoreceptor degeneration. RP displays a range of genetic origins such as being inherited through dominant and recessive autosomal chromosomes and X-linked genes and additional genes have been implicated in critical retinal roles such as phototransduction[29]. The presence of these genes leads to the execution of detrimental necrotic and apoptotic signaling which affects rods first, followed by the degeneration of cones[30]. Specifically, apoptosis pathways are characterized as three-caspase- dependent mechanisms that, when altered through gene mutations, become activated. This activation causes many downstream proteins to be cleaved and released which results in irreversible damage to DNA[31]. Additionally, unregulated necrotic pathways are activated through the presence of reactive oxygen species and cell death attributed to ischemia[29].

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2. Commercial Alternatives to Eye Therapeutics

Biobanks provide an efficient way for patients to find alternatives for their best match in tissue replacements. It has been through biobanking that most patients impacted by eye diseases turn to for their therapeutic needs. Moreover, biobanks provide useful information for researchers to study pathologies such as Diabetic Retinopathy or Glaucoma within retinas through gaining thousands of measurements in visual acuity, fundus photography, and refraction[32]. Over the past couple of years, biobanks have been heavily involved in the contribution of organ transplantations for patients through compatible biomarker identification for reducing risk of organ rejection[33]. Termed population-based, these biobanks focus on common to complex diseases, and biobanks defined as disease-oriented possess tissue samples and clinical data[34], both of which have made accessibility to retinal or other eye tissues easier. Although biobanks have made it easier to access compatible tissue sources, these therapies rely on patients or institutions that ultimately do not address the shortage of donors. Additionally, the many databases available for doctors to decide on what may or may not improve patient compatibility are set to have a margin of error as it is not truly personalized to the patient. To address such concerns, stem cells are a viable option for their ability to revert to a pluripotent state from any patient cell, making it truly personalized.

3. Induced Pluripotent Stem Cells to Retinal Cells

Stem cells are the foundational cells of the body that differentiate into different cell types and can also self-renew. These exist in both embryos and adults. Embryonic stem cells are present in the inner cell mass of blastocysts i.e., from the 4th to the 7th day after fertilization.

These stem cells carry the potential to differentiate into the three germinal layers[35]. Adult stem cells (ASCs) are also known as somatic stem cells and are undifferentiated cells that can be isolated from the bone marrow, central nervous system, skin, intestine, etc. These can only be induced to form epithelial cells as they are composed of mature cells that brings higher genetic stability for extended-duration cultures[35].

These cells can also be classified into totipotent, pluripotent, multipotent, oligopotent and unipotent stem cells based off their differentiation potential.

Totipotent stem cells can divide into cells of the entire organism, while pluripotent stem cells are usually specific to a certain germ layer. Multipotent and oligopotent stem cells have narrower differentiation spectrums while unipotent stem cells have the narrowest differentiation capability but with a rapid dividing ability[36].

Yamanaka, Takahashi and their group reprogrammed multipotent adult stem cells to pluripotent stem cells. They successfully induced fibroblasts to become pluripotent, naming them pluripotent stem cells[37]. IPSCs show similar characteristics to embryonic stem cells within their genetic markers and differentiation potential. They can form the three germ layers, hence why they are preferred for developmental studies[38].

Retinal cells are highly specialized cells and hence do not divide. Damage to these cells gives rise to irreversible blindness and other retinal diseases. Stem cell therapy is a treatment option that has recently been explored for retinal disorders. Stem cells originating from blastocyst-stage embryos or adult fibroblasts can be reprogrammed into pluripotent stem cells and utilized for retinal regeneration[39].

The retina is made up of neuroretina and retinal pigmented epithelium. The undifferentiated induced pluripotent stem cells must be programmed toward neuronal lineage. The pivotal stages from iPSCs to retinal cells include the formation of the anterior neural plate, the optic vesicle emerging from the diencephalon and an invagination to form a bilayered optic cup. These iPSCs are introduced to proneural N2 media which involves SMAD inhibition and the addition of IGF1 to promote retinogenesis[40]. SMAD inhibition allows for neuroectodermal development while IGF-1 plays a role in neural/eye field development and Notch inhibition is required for photoreceptor development[41]. Retinoic acid and FGF-2 in B27 and N2 media promote the neural retinal cell types while Activin-A moves the cells from progenitor to maturing neurons[42]. Barnea-Cramer and colleagues introduced retinal induction media and neural differentiation media to pluripotent stem

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cells which became eye field progenitors. Neural differentiation media was then removed to give retinal neuronal progenitor cells and photoreceptor progenitors. Photoreceptor-like cells were formed when BDNF (brain-derived neurotrophic factor), retinoic acid, DAPT, and ciliary neurotrophic factor (CNTF)[43].

3.1. Clinical Trials for Retinal iPSCs

Clinical trials using iPSC replacing RPE cells have been conducted as early as 2013. In the trial and study by Mandai et al, the group utilized a patient's cell line to develop RPE cells that exhibited a consistent genetic profile of the RPE tissue. Moreover, this transplantation aimed to restore conditions from AMD pathology without implementing immunosuppressants[44].

Though this trial only had one patient, it is important not to disregard the impact of the immune system in the aspect of cell rejection. As recent as 2023, various trials have been aimed at minimizing stem cell rejection by immunosuppressing the body through medication and steroids[45]. Through rare trials mentioned in Gowrishankar et al. that showed severe side effects such as pneumonia or urinary tract infections, most trials and patients were successful in their immunosuppression. Though the longevity and efficacy of transplanted retinal stem cells are yet to be elucidated, mediating the immune response is appealing when considering past animal model studies reporting no identification or rejection of hESC RPE cells when no immunosuppression was administered[45]. Table 1 provides up-to-date information on the ongoing clinical trials utilizing iPSCs for addressing retina-associated diseases.

Table 1. Current retinal clinical trials using induced pluripotent stem cells (iPSCs).

Trial ID	Year Started	Trial Phase	Goals	Sample Size	Cell Used	Disease Target
NCT06394232[46]	2024	I/II	Varying doses of suspended human derived iPSC RPEs will be administered to patients to view therapeutic efficacy.	54	iPSC-RPE	AMD-Dry
NCT05991986[47]	2023	Observational	The aim of the trial is to collect patient somatic cells to prepare personalized retinal iPSCs for AMD research	10	Somatic Cells	AMD
NCT05445063[48]	2022	I	Using derived retinal iPSCs, cells will be transplanted onto retinal space for RPE replacement	10	iPSC-RPE	AMD-Dry
NCT04339764[49]	2020	I/IIa	The aim is to transplant a monolayer of retinal iPSCs on a PLGA scaffold into one patient eye and follow up in 5	20	iPSC-RPE	AMD-Dry

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			years.			
NCT03403699[50]	2018	Observational	iPSCs derived mesoderm cells will be generated from peripheral blood cells of diabetic patients to transplant into the vitreous cavity of rodent and primate eyes for observation.	20	IPSCs-mesoderm cells	Diabetic Retinopathy

4. Miniature Organs: Organoids

Cells can grow into three-dimensional structures where they accumulate to form small masses of cells that can self-organize and differentiate into various cell types. These originate from stem cells to form three-dimensional structures called organoids which resemble an organ's functional and structural characteristics. These mimic the organ biochemically better than 2D cultures.

Scientists Smith and Cochrea were the first to use the term organoids for cystic teratomas, in 1946[51]. In 1987, Li et al. used mouse mammary epithelial cells to form ducts, ductules, lumina, and secretory alveoli-like structures that secreted milk proteins[52].

Ronnovjessen et al, in 1990, co-cultured tumor cells along with fibroblasts to recreate breast cancer organoids[53]. 2009 brought a milestone in organoids. Sato et al, used adult intestinal stem cells, that expressed a single leucine-rich repeat containing G protein-coupled receptor 5, to form a 3D intestinal organoid with villus-like structures[54,55]. Following this, we have made organoids of multiple kinds such as the heart, liver, kidney, brain, pancreas, and retina[56].

Stem cells can be induced to take up a certain cell type lineage by intrinsic cellular components, introducing growth factors or environmental cues such as through media or extracellular matrix[57]. Cell-cell interactions and external forces from the extracellular matrix can also provide active and passive biochemical cues[56]. The most used extracellular matrix is matrigel which is composed of collagen, entactin, laminin, and heparin sulfate proteoglycans[58]. However, matrigel is derived from Engelbreth-Holm-Swarm mouse sarcomas and hence contains tumor growth factors that could affect the maturation process of organoids. Other alternatives to matrigel include hydrogels like collagen, alginate, or functionalizing the hydrogel with RGD (Arg-Gly-Asp) peptides, and synthetic polymer hydrogels of poly-ethylene glycol (PEG) or poly lactic-co-glycolic acid (PLGA)[59].

Soluble factors such as biologics, growth factors, or small molecule drugs help activate or inhibit pathways to direct cells to a particular lineage resulting in the differentiation of stem cells to form organoids[60]. Furthermore, the use of microfluidics aids in creating concentration gradients of growth factors or morphogens that allow tissue patterning.

Organoids are formed from human-derived cells with high efficiency and stability, making them advantageous to research human physiological systems. The added benefit of individualization contributes to precision and personalized medicine. The three major organoid applications are drug discovery, transplantation alternatives, and disease modeling.

5. Retinal Organoids

Stem cell-derived 2D cultures are unable to replicate all aspects of the retina and hence an efficient model that offers stability and allows the study of cell-cell interactions and signaling pathways to interpret the development of retinogenesis as well as retinal disorders.

The first successfully developed retinal organoid was done by the Sasai lab in 2011. They used mouse ESCs which spontaneously formed a hollow vesicle of the neuroepithelium and further differentiated into the rostral forebrain tissue to give a retina-forming field. These ESCs self-organized to take up an optic cup structure with six different cell types that included the photoreceptors, Muller cells, bipolar cells, ganglion cells, horizontal cells, and amacrine cells[61]. The

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same research group subsequently demonstrated using human ESCs to form self-organizing retinal organoids with an optic cup structure and layers of distinct retinal cells using a serum-free floating culture-like aggregates method[62].

Earlier work indicated using Noggin and DKK1 for early differentiation in neural induction[63]. The use of antagonists of Wnt, IGF1, and BMP4[64,65,66] is an alternative guided approach for the differentiation of development of the neural retina. The inactivation of motor proteins, myosin, is said to facilitate the invagination of the optic cup[62]. Neural induction media is usually B27 media with antagonists of Wnt/Notch/BMP4 signaling. This influences the cells to take up the neural retinal fate. For the further differentiation and maturation of these organoids, lipid supplements, taurine[67], and retinoic acid[68] are used. All of which help in developing retinal ganglion cells and photoreceptors[69,70].

5.1. Retinal Organoids for Medicine

5.1.1. Age-Related Macular Degeneration (AMD)

IPSC-derived retinal pigmental epithelium from patients has been used to create successful disease models. These are known to have the appropriate biomarkers, the required immune complement systems, and pro-inflammatory factors[71,72]. Studies done using retinal organoids indicated extracellular vesicles in the RPE of AMD as disease messengers and hence could be considered therapeutic targets[73]. The disease model for AMD is in the works along with research being done on potential therapeutics.

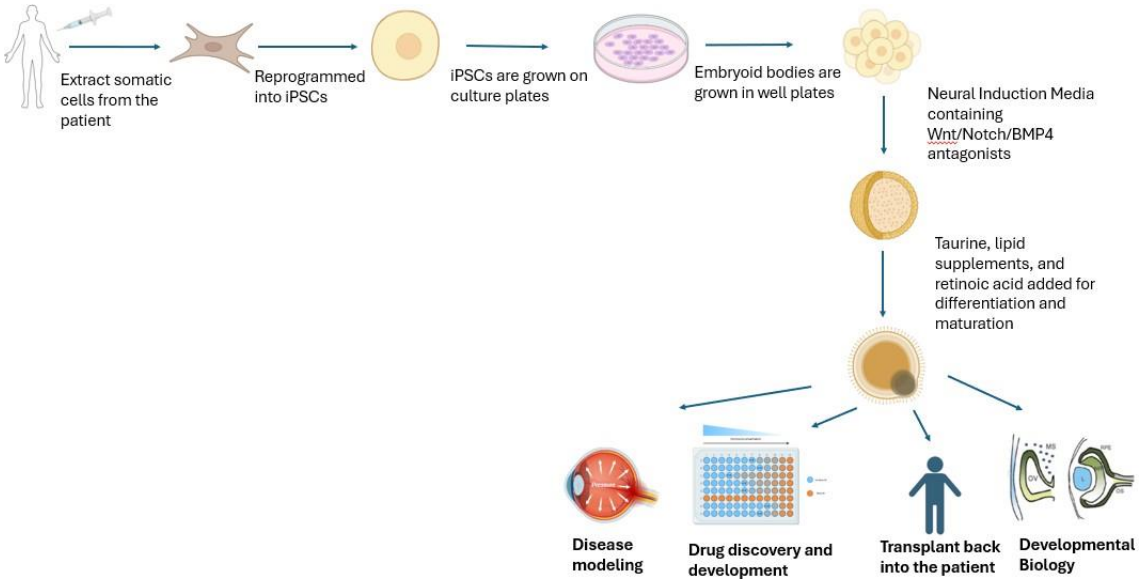

Figure 1. Somatic cells can be extracted from a patient to generate induced pluripotent stem cells. These are grown on culture dishes and well plates to get embryoid bodies (clusters). Neural Induction Media along with supplements allow these to grow into retinal organoids. Drug discovery and development, transplant alternatives, disease modeling, and developmental biology[97] are examples of their applications. (Created in Biorender).

5.1.2. Retinitis Pigmentosa

RP is a hereditary retinal degenerative disorder in which an irreversible loss of photoreceptor cells is observed. Cyclic GMP concentrations are elevated along with an influx of Ca ions through the CNG channels. The use of retinal organoids as a disease model for early- onset and late-onset retinal degeneration has been established using patient-derived iPSCs[74,75,76]. A mutation in *RP2* accounts for 15% of X-linked retinitis pigmentosa, Cheetham and his scientists investigated this mutation and a rescue mechanism using iPSC-derived retinal organoids[77]. These organoids are also now emerging as transplantation alternatives. One such study was done in patients of retinitis pigmentosa, and the grafts led to no adverse effects and helped in improving full-field light stimulus[78].

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5.1.3. Glaucoma

Photoreceptor cells, retinal pigment epithelium cells, and retinal ganglion cells (RGC) have been widely studied using retinal organoids. The genetically inherited form of glaucoma leads to the loss of RGCs. These were rescued using neuroprotective factors introduced to patient-derived retinal organoids[79]. A recent study showed the use of retinal ganglion-like cells from retinal organoids by adding surface markers like CD184 and CD171. These cells survived, integrated with the receptor retina, and even helped with optic nerve damage[80].

5.1.4. Neurodegenerative Disorders

A 3D disease model of the retina was created to study the early-onset phenotypes of Alzheimer's disease[81]. Neurodegeneration and inflammation studies of early diabetic retinopathy have also been modeled in retinal organoids[82].

5.1.5. Bacterial Conjunctivitis

Pharmacological and toxicological studies have also been conducted on these retinal organoids. The drug Moxifloxacin, which is a wide-spectrum antibiotic, when introduced to these organoids had toxic effects, especially on the amacrine cells and photoreceptors[83].

5.1.6. X-Linked Juvenile Retinoschisis

A retinal organoid model for X-linked juvenile retinoschisis was developed and a CRISPR/Cas9 base modification was made to show that the condition could be repaired in that manner[84].

6. Organ – On – A – Chip

Organ-on-a-chips are microfluidic devices in which living single or multiple cell cultures are grown to research organ-level pathophysiology. The first ever device created was a lung alveolus, that had hollow channels like the lung airway. This chip had endothelium-lined channels to mimic vasculature, and a cyclin suction recreated the breathing motions[85,86]. Multi- organ systems can also be developed by creating separate chambers for each organ[87].

Retinal organoids have emerged as a useful tool in developing new therapeutic strategies.

However, these lack vascularization and hence the physiological interactions between matured photoreceptors and retinal pigment epithelium have not been achieved. This led to the advent of the retina-on-a-chip. This enables vasculature and interactions, providing a closer model of the human retina due to the development of outer segment-like structures as well[88].

The lack of the blood-retinal barrier (BRB) leads to a lack of neuronal signaling, resulting in vision loss. Organs on chips facilitate the organization of multi-layer complexes with the different transporters to investigate the entirety of the BRB. Another advantage to this is the assessment of immune infiltration in diseased conditions. Gradients can be created that allow the study of drug thresholds to predict drug dosages[89,90].

The specificity and security of the eye lead to challenges in ocular drug delivery. Topical eye drops or intraocular injection administration seem the best and most widely adopted treatment option in ophthalmology. Organ-on-a-chip provides a platform to study the long-term effects of these treatment options due to their high stability[91].

The platform provides a nutrient supply for the organoids using microfluidics. This allows for a stable and uniform nutrient supply facilitating the longer survival of these organoids[88,92]. This was done to help expand the retinal ganglion cells for drug screening, RGC- related disorders, and cell transplantation[93].

7. Future Directions of Retinal Studies

Notable progress within retinal studies ranges from understanding disease pathology to learning more about retinal development. In a study published in 2024, retinal organoids were

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utilized to uncover the mechanisms behind long or medium cone fate, which dictates red or green cone reception, respectively[94]. Previously two models were proposed that attempted to explain how cone fate was dictated, and by referencing past animal studies[95] and mechanisms observed in this study, researchers report retinoic acid playing a vital role in human cone fate.

Furthermore, a 2023 study uses human-derived retinal organoids to study how the subretinal space of animal models impacts the transplanted cells. The successful transplantation of human retinal organoids demonstrated that the in vivo environment supports efficient rod and cone development compared to in vitro counterparts and follow-up sequencing data exhibited a variety of cells seen in the retina[96].

Scientists Busskamp and Sharma addressed the challenge of vasculature in the retinal organoids by mixing endothelial cells and iPSCs before cell differentiation. At the 30-week mark, the endothelial cells formed branching and even helped to improve the retinal organoid size[97,98]. The integration of vasculature and the immune system in these organoids is still a standing challenge. Another limitation is non-uniform differentiation patterns from iPSCs[99].

8. Conclusion

Retinal disorders are highly prevalent in adults above the age of 60. These disorders do not have complete treatment options and hence the emergence of retinal organoids as a model system to help in the development of drugs and as a transplantation alternative aid in accomplishing that. Their use as disease models to assist in fully understanding the pathogenesis of these disorders is a step towards better treatment options. The role of these organoids in personalized medicine is promising for developing cures and therapy for retinal disorders in the coming years.

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