Over the past two decades, advancing techniques for genetic analysis and concerted efforts by scientists and clinicians have led to the identification of a remarkable number of genes involved in retinal degenerative diseases [10]. However, a greater understanding of the biological processes impacted by these disease genes is necessary to focus treatment strategies and evaluate efficacy. Traditionally, efforts to gain such knowledge have relied upon surrogate cell culture systems (often employing heterologous overexpression) and animal models that, while highly valuable, may not reproduce critical aspects of human disease. Human induced pluripotent stem cells (hiPSCs) offer a novel and convenient platform to study cell type(s) targeted by disease from affected patients and test therapeutics, and thus provide a bridge between clinical and bench research.

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Furthermore, hiPSC models are indefinitely renewable and express human genes and proteins at endogenous levels, increasing the likelihood that they recapitulate important disease mechanisms.

Like any tool, however, hiPSCs have limitations that must be taken into account when choosing a disease to model. Indeed, the greatest asset of pluripotent stem cells – the ability to produce a diverse array of cell types derived from all three germ lineages – can be a detriment unless desired cell types can be identified and isolated, or at least greatly enriched, in culture. If not, subsequent experiments are at risk of being confounded by the variable influences of unknown or unwanted cell types that occupy the same culture well.

Should adequate enrichment be achieved, it may also be necessary to expand and reseed cells onto a variety of surfaces and differentiate them to a uniformly mature state. The latter task is particularly important, since pluripotent stem cells are, at their core, dynamic mini-developmental model systems. As such, it is easy to envision differences in maturation levels between or within hiPSC cultures, which in turn will affect physiological behavior and experimental outcomes. To control for this potential source of variability, it is helpful to use cell cultures derived from primary tissue as developmental measuring sticks for their hiPSC-derived counterparts. Finally, there is the question of disease complexity. More specifically, what is the a priori likelihood that a disease phenotype can be recapitulated in a dish with a single cell type? It goes without saying that diseases that require involvement of multiple cell types or organ systems (and perhaps environmental influences) pose greater, although not insurmountable, challenges.

Taking into consideration the concerns outlined above, the retina is an appealing target for hiPSC-based disease modeling, and it is not surprising that the creation of retinal disease-specific hiPSC lines is
in full force. Protocols now exist for the robust generation and isolation of retinal pigment epithelium (RPE) and neuroretinal progeny from human pluripotent stem cell sources, including hiPSCs [1–5]. RPE-based disorders, in particular, appear to be ideal for hiPSC modeling, given the ease and extent to which this cell type can be generated, isolated, cultured, manipulated and tested. hiPSC-RPE grow in distinctive pigmented patches that can be manually dissected, dissociated and expanded on a variety of substrates, where they form monolayers and display numerous critical physiological functions in vitro akin to primary human prenatal RPE [6–8]. As such, hiPSC-RPE offers a host of opportunities to test the cellular and molecular impact of gene mutations and/or environmental perturbations. Furthermore, the maturation state of hiPSC-RPE can be monitored in live cultures using readily discernible morphological features and measurements of transepithelial electrical resistance and proper cell polarization.

Taking advantage of the culture characteristics of human RPE, Singh et al. recently developed a hiPSC-RPE ‘model in a dish’ of Best vitelliform macular dystrophy (BVMD) [9]. BVMD is an inherited macular degeneration caused by autosomal dominant mutations in the RPE-specific protein bestrophin-1 (BEST1) [10, 11]. Interestingly, macular RPE in BVMD remains intact even late in the course of the disease, whereas the photoreceptors they subserve degenerate with time, leading to central vision loss [12, 13]. This finding suggests that BEST1 mutations are not immediately harmful to the RPE cells themselves, but instead lead to disruptions in RPE physiology that affect long-term photoreceptor health. Consistent with this hypothesis, differences were generally not observed between BVMD and control hiPSC-RPE unless a physiological stimulus was introduced, such as exposure to photoreceptor outer segments or the purinergic-receptor agonist, ATP. Following these challenges, BVMD hiPSC-RPE cultures displayed defective photoreceptor outer segment degradation and disposal and reduced fluid transport, as well as increased oxidative cell stress [9]. The authors then used this system to investigate the role of BEST1 and partially define the cellular mechanism underlying BVMD, which appeared to involve disrupted endoplasmic reticulum-mediated calcium homeostasis.

In contrast to hiPSC-RPE, the utility of hiPSC-derived neuroretinal cell types for examining disease pathophysiology remains to be determined. For example, given the lack of production of outer segments by cultured photoreceptors, as well as the general scarcity of rods in differentiating human pluripotent stem cell cultures, it is unclear to what extent the effects of rhodopsin mutations in retinitis pigmentosa (RP) can be investigated using currently available culture methods. However, this problem may be circumvented by differentiating the hiPSC-derived neuroretinal cultures for longer periods of time, coculturing them with RPE or transplanting the photoreceptor-like progeny into retinal tissue [14, 15].

Interestingly, though, some photoreceptor-based RP mutations that target components of the phototransduction cascade may be conducive to hiPSC modeling. Meyer et al. showed that, in addition to expressing key phototransduction component genes, human pluripotent stem cell-derived photoreceptor-like cells exhibited a change in membrane potential in response to exogenous 8-Br-cGMP that mimicked the switch from a light-adapted to a dark-adapted state [1]. Thus, opportunities probably exist to examine the functional effects of photoreceptor-specific gene mutations using hiPSCs, as long as the limitations of the culture system are kept in mind.

Another unique application of hiPSC technology to retinal disease modeling was illustrated by Tucker et al., who used hiPSCs from a patient with sporadic RP to verify the pathogenicity of homozygous Alu insertions uncovered by exome sequencing [16]. In this elegant study, the authors found that the insertion of the Alu sequence into exon 9 of the patient’s MAK gene prevented the expression of a splice variant of MAK that is normally expressed in retinal precursors. In this case, hiPSCs offered an efficient means not only to confirm the gene defect responsible for disease, but they also concurrently provided insight into its mechanism.

In addition to their potential to model disease mechanisms and evaluate the pathogenicity of gene mutations, hiPSC-derived retinal cells can be used for drug testing, using both wildtype and patient-specific hiPSCs to determine the effects of pharmacological agents on human cell function in normal and disease states, respectively. To date, there have been a limited number of publications looking at the effects of drugs on hiPSC-derived retinal cells. Meyer et al. reported the restoration of ornithine aminotransferase activity in gyrate atrophy hiPSC-RPE following vitamin B6 treatment [1]. Vitamin B6 supplementation is a known treatment for gyrate atrophy; however, the particular patient whose fibroblasts were used to generate the hiPSC line had been deemed unresponsive to vitamin B6 supplementation by virtue of traditional surrogate tests. Therefore, this study underscored the potential importance of using custom hiPSCs to test the actual cell types (in this case, RPE) targeted by a disease in order to more accurately assess drug efficacy.

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In a second report by Jin et al., hiPSC lines were created from patients with mutations associated with RP, including RP1, PRPH2, RHO and RP9 [17]. In this study, the authors noted death of rhodopsin-positive cells in all disease lines between days 120 and 150 of culture. However, treatment with α-tocopherol led to a statistically significant preservation of rhodopsin-positive cells only in retinal cultures carrying the RD9 mutation. Therefore, this study provided the first evidence that hiPSC technology may

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be useful in screening for drug responses across numerous related, yet genetically distinct, retinal diseases. This application is particularly important for genetically and phenotypically heterogeneous disorders such as RP, and could ultimately help narrow the list of disease targets for experimental drugs and improve clinical trial design. Beyond customized testing of known therapeutics, hiPSC technology also offers an unlimited source of material for automated, high-throughput drug screening systems to interrogate libraries of pharmacological compounds. Such efforts may rapidly uncover new drugs for previously untreatable disorders.

Gene therapy testing is yet another area in which hiPSCs will likely play a significant role in the near future. Functional correction of a retinal disease-causing gene defect has been demonstrated in patient-specific hiPSCs using bacterial artificial chromosome-mediated homologous recombination [1,18], and this approach could be used to repair genes in hiPSCs prior to autologous transplantation. However, restoration of normal gene function in hiPSC cultures should also be attainable using viral vectors that are currently being employed in human gene therapy trials, provided the target gene can be appropriately packaged and expressed. Having hiPSC-based culture systems for gene therapy testing would be particularly useful for retinal diseases that do not have corresponding animal models for demonstration of treatment efficacy.

While modeling and testing hiPSC-derived retinal progeny as adherent cultures is preferable for RPE-based disorders and perhaps more convenient for those affecting neuroretinal cells, it may be more beneficial in the latter situation to generate a layered, retina-like structure. Such 3D tissue structures could promote interactions between retinal cell types normally found in vivo, and thus provide a more accurate representation of the effects of retinal disease. Indeed, recent reports have demonstrated the formation of laminar, retina-like structures from multiple pluripotent cell sources, including mouse embryonic stem cells, human embryonic stem cells and hiPSCs, which have the added capacity to form synapses [2,5,19]. The ability to generate these pluripotent stem cell-derived retinal tissues, along with the potential to coculture them with RPE, offers the potential to model complex human retinal diseases (e.g., age-related macular degeneration) at a level not previously possible in culture.

Looking to the future, it is likely that hiPSC technology will have a broad and significant impact in the area of in vitro disease modeling, drug discovery and gene therapy testing. However, it is a rare clinical or laboratory tool that can stand completely on its own. Therefore, we anticipate that data from hiPSC models will be combined with information from clinical observation and animal models to provide much-needed insights into the pathophysiology of human retinal diseases and the strategies needed to overcome them.

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