WINNER - $90,000 PRIZE

1. **Erin Lavik, University of Maryland Baltimore County**

Recapitulating the architectural and cellular organization of the retina has been challenging, but biology hints at the possibility with the formation of eye cups and organoids. However, these structures are not amenable to high throughput screening approaches which are critical to developing new understanding and therapies, nor do they develop normal structures such as the optic nerve. Approaches for making complex tissue models include photolithography, 3D printing, and bioprinting. These allow one to develop patterns and architectures that are seen in vivo, but they require materials and processes are not always compatible with retinal cells and progenitors due shearing forces associated with many of the 3D printing technologies and UV light for the photopolymerizable approaches. We have developed an alternative approach based on screen printing tissue models that avoids UV light and the shearing issue. It is simple, reproducible, and highly scalable, making it suitable for high throughput assays. We have shown that we can print both a range of gels and cells in complex patterns with high resolution and reproducibility. This allows us to recapitulate the layers of the retina and to provide the matrix cues to promote the critical polarization of the cells types and promote the formation of appropriate synapses in the system, and enhanced survival of target neurons. By coupling this approach with human RPE cells and human adult neural stem cells derived from the eye and optic nerve which have been shown to express markers for the major retinal cell types, we can make a system that models the 3D retina and optic nerve structures in a scalable and reproducible manner that is exceptionally well suited to high throughput screening approaches for understanding and treating diseases of the retina.

HONORABLE MENTION (NO MONETARY PRIZE)

1. **David Gamm, University of Wisconsin**

Protocols for generating 3D neural retina (NR) organoids from human pluripotent stem cells (hPSCs) are capable of producing laminated tissues during early differentiation, with later development of highly organized outer NR structures. However, over time inner NR laminae are often lost and/or become disorganized, beginning with the retinal ganglion cell layer. A likely explanation for the selective survival of the outer NR in organoids lies in the dual blood supply of the retina, where the outer NR is fed via diffusion while the inner NR requires a separate microvasculature that is absent in organoids. We propose to address this disparity in outer vs. inner NR survival and function in vitro by incorporating a perfusable inner retinal microvasculature using hPSC-derived vascular progenitor cells and microfluidic technology. Similar strategies were employed by members of our group to vascularize forebrain organoids and perform screens for toxic agents, which is amenable to scale up for high throughput assay development. Besides the potential to maintain inner NR, a major advantage of our approach is the capacity to model retinal microvascular diseases such as diabetic retinopathy (DR), one of the leading causes of vision loss in the U.S. Physiologically relevant functional readouts for the resulting full thickness, vascularized NR organoids will include electrophysiological recordings, metabolic and functional imaging, and perfusate analysis. If successful, our approach may also address other shortcomings of current organoid culture methods, including low production efficiencies and high inter-organoid variability, which further hinder their practical use for therapeutic and toxicological screening. Our UW-based team of stem cell biologists, bioengineers, tissue imagers, electrophysiologists, and vascular retinopathy model experts is well-suited for this challenge, and our association with leading hiPSC tools manufacturers (Cellular Dynamics International and StemPharm) and a retinal cell therapeutic developer (Opsis Therapeutics) offer future avenues for industrial scale-up and quality systems assurance.

2. **Rebecca Carrier, Northeastern**

Recently, methods to produce 3D retinal organoids, potentially tremendously useful tools for developmental and regenerative research, from iPSCs and ESCs have been developed. However, current approaches are limited in their ability to create retinal organoids that recapitulate the complexity and functionality of the retina. In particular, current retinal organoids cultures lack proper spatial organization of a retinal pigmented epithelium (RPE) relative to neural retina and organization of retinal layers. In addition, current retinal organoid systems lack vasculature, and thereby are limited in availability of nutrients and oxygen supply to the culture system, constraining the size of the organoids that can be grown and the disease states that can be meaningfully explored. To address the shortage of spatial control within developing retinal organoids, we propose to create a biomimetic gradient of chemical and physical cues recapitulating those present during retinal development, rather than an isotropic environment lacking spatial variation in cues. To overcome the lack of vascularization and aid in establishment of these gradients, a bioengineering approach using microfluidic chambers will be employed to enable in situ development of vasculature driving the flow of fluid through a biomimetic hydrogel-based "choroid" on which retinal organoids will be grown. The hydrogel properties, including chemical composition and stiffness (elastic modulus of the retina is 0.431 MPa), will be systematically varied to promote the selective attachment and proliferation of the retinal pigment epithelium cells within growing organoids, and the spatiotemporal control afforded by the microfluidic platform will be used to impart a gradient of signaling cues across the growing organoids to allow proper orientation of layers recapitulating a physiologically relevant laminated retina. The engineering of a system enabling examination of interaction of blood vessels and retinal tissue during organoid development and culture offers high potential for understanding development of the eye and modeling retinal diseases including age related macular degeneration (AMD).

3. **Katja Schenke-Layland @ Fraunhofer Institute for Interfacial Engineering and Biotechnology**

We have developed a prototype of a human induced pluripotent stem cell (hiPSC) based 3D Retina-on-a-chip challenging several current disadvantages of stem cell based in vitro systems. It allows for 3D co-culture of retinal organoids, retinal pigment epithelium (RPE) and further cell types in a defined and reproducible microenvironment, featuring a physiological vasculature-like perfusion. The system enables the maintenance of viability and functionality of the retinal 3D tissue over multiple weeks. Moreover, our first results indicate a substantial improvement of photoreceptor outer segment formation and a functional interplay of photoreceptor and RPE as shown by segment phagocytosis. In the framework of the 3-D ROC Challenge, we propose a parallelization of our 3D Retina-on-a-chip system and the integration of further hiPSC-derived cell types (e.g. endothelial cells) to create a next generation Retina-on-a-chip 2.0 system. The Retina-on-a-chip 2.0 will feature 48 individual units in an integrated chip with standard well plate-dimensions and will be amenable for high content drug screening as well as disease modeling.

4. **Daniel Pelaez, University of Miami**

Proper organogenesis relies on the orchestrated spatial and temporal presentation of graded stimuli for cell fate commitment and maturation. This represents a major obstacle to deriving complex tissue structures, like the fully developed retina, in the laboratory. Typically, in-vitro culture systems deliver uniform stimuli to induce homogenous tissue differentiation at established time points; but spatial distribution of differentiation cues relies mainly on interactions occurring within the culture environment, which are not solely adequate for proper differentiation of all retinal layers. To overcome this obstacle, we propose a novel tissue bioreactor system that allows for the compartmentalization and gradation of stimuli designed to separately induce the maturation of inner and outer retinal cell

phenotypes. Our bioreactor includes an air-tight upper chamber which allows the establishment of physiologically-relevant oxygen tension gradients across the developing retina. Thus, we can recapitulate the normal physiology in which the retina is exposed to a steep gradient of oxygen tensions across the highly oxygenated outer retina, and the hypoxic inner retina. We will use this solution to model Retinoblastoma (Rb), which is one of the leading causes of childhood cancer death worldwide. Rb is not an age-related degenerative disorder, but rather a developmental disease, making it ideal for studies in a platform of retinal development. Using CRISPR/Cas9 technology, we have established RB1-knockout iPSC lines in our laboratory (RB1KO). The availability of wildtype and RB1KO cells from the same parental cell lines, lets us model somatic and germline manifestations of the disease, and study how retinoblast cells interact with normally-developing retinal tissues. This approach allows us to characterize the phenotype(s) from which Rb tumors originate, which is still a matter of debate. Similarly, using our custom bioreactor system, we can study how retinoblastoma tumors transition from oxygen-dependent growth to their most aggressive phenotype, hypoxic-adapted vitreal seeds.

5. **Wei Liu, Albert Einstein College of Medicine**

Here we present our solution to the 3-D ROC. Retinal structures from hESCs or hiPSCs have been generated using a number of protocols, but the requirement of manual manipulation, low efficiency, immaturity and variability limit their applications. We have established a retinal differentiation protocol for generating and isolating large quantities of retinal organoids that produce stratified mini retinas from hESCs. The novelties in our solution are the ease of use, scalability, robustness, reproducibility, and the maturity of photoreceptors. The advantages of our protocol are the efficient generation of early retinal epithelium through Matrigel-induced cyst formation and scalable isolation of self-organized retinal organoids through Dispase-mediated cell detachment and subsequent floating culture. Our retinal organoids produce stratified neuroretinal tissues with all five neuronal retina cell types. Notably, outer segments of photoreceptors and outer limiting membrane are evidenced by immunostaining and electron microscopy. We are using the retinal organoids to model optic cup invagination and Leber Congenital Amaurosis. We propose solutions to faithfully recapitulate the complexity of the retina through tissue engineering.

OTHERS

1. **Kinsang Cho, Schepens Eye Research Institute**

This research plan proposes to use a biomaterial approach to address the challenge of reproducibility of retinal organoid culture This proposed study is sparked by the recent finding during the collaboration among Drs. Dong Feng Chen, Kinsang Cho (Schepens Eye Research Institute), Dr. Taching Chen (ophthalmologist of Taiwan National University) and Weifang Su, a seminal biomaterial scientist and Professor of Taiwan National University. Our goal is to develop a reproducible RO model to investigate the mechanism of retinal degenerative diseases such as glaucoma, interactions between retinal neurons and drug screening.

2. **Tom Reh, University of Washington**

Individuals with USH experience loss of both hearing and vision. USH is a rare disease; however, the loss of both vision and hearing is particularly debilitating. Considerable effort has gone into the identification of the genetic defects underlying USH, and more than 10 genes have been implicated in the disease. Despite significant advances in our understanding of the function of these genes in the inner ear, we know far less about the normal and pathological functions of USH genes in the retina, primarily because the human USH retinal phenotypes are not present in mouse models of these mutations. The potential to produce a model retina in vitro, from USH patient-derived iPSCs is an attractive next step to both (1)

advance our understanding of this disease in the retina, and (2) to use as a screening tool to evaluate the effectiveness of potential therapies, like viral gene therapy. The generation of USH model retinas will allow us to study the pathophysiology of the disease from the very earliest stages. The model will contain (1) a neural retinal organoid (2) a layer of RPE, and (3) perfused vasculature for both the neural retina and RPE. The neural retina and RPE will be derived from patient-derived iPSCs, while the vasculature will be generated from primary fetal human retinal and choroidal endothelial cells. The vessels will be generated in a microfabricated device that allows us to separately establish the RPE and the neural retina, and then the two layers will be placed adjacent to one another. The ability to perfuse the vasculature allows us to maintain the neural retina and RPE in their natural configuration. We predict that perfusion of the RPE and retina through their own microvessel networks will facilitate their differentiation, and reduce cell death that occurs as the retinal organoids mature.

3. **Magdalene Seiler, University of California, Irvine**

Retinal degenerative (RD) diseases that affect photoreceptors and/or retinal pigment epithelium (RPE) affect millions of people worldwide. Stem cell-based therapy is being pursued as a potential approach for those incurable diseases. Our team has assembled expertise in 3 areas: (1) Development of 3D retina organoids (RO's) from human embryonic stem cells (hESCs) that contain most of the retinal cell types (Dr. Seiler, UCI). When transplanted into immunodeficient RD rats, the RO's developed lamination, matured into photoreceptors and inner retinal neurons, integrated and restored some visual function. (2a) Our team at USC has developed a unique technique to grow hESC-RPE as a polarized monolayer on ultrathin parylene (hESC-RPE implant) that has functional similarities to a healthy Bruch's membrane (BM) and is now employed in FDA–approved phase1/2a clinical trials (Dr. Thomas, USC). (2b) The RO sheets can be maintained over the hESC-RPE implants in culture together and can be used as a co-graft for transplantation experiments. Transplantation of RO's together with polarized RPE supported by an artificial Bruch's membrane is highly advantageous since the parylene membrane can act as a barrier between the co-graft and the pathological BM surface to prevent BM abnormalities from unfavorably altering the behavior of the transplanted cells. By using this co-graft in an in vitro model system, it is possible to study the influence of RPE on the survival and maturation of RO sheets as required for different disease conditions. (3) By microfluidic bioengineering, capillary networks will be developed by endothelial cells forming an artificial choroid underneath the RPE-retina construct (Dr. Lee, UCI). – This protocol is advantageous because it will combine three different tissue layers of the eye, and can be usable both for drug testing and disease modeling, dependent on the cell source.

4. **Nandor Garamszegi, Natural To Artificial Genomes And Biosystems LLC.**

In response to the NEI-3D-ROC Idea Competition Challenge, we provide description of three major idea Strategies: I] taking Organoid and Multicellular Spheroid Models to the next level by incorporation of a new media circulatory system, II] provide new alternative method to improve advanced 3D cell culture system addressing the gaps where organoids potentially fail, III] describe new retinal architecture layering for problems unsolvable with I and II. These advances are independent, complementary, interconnected, and feasible combinations of already published methods with customized new protocols. The introduced strategies are capable of generating physiological retinal architecture, modeling multiple diseases, and can be utilized as convenient high throughput drug screening platforms. Our innovation and intellectual property (IP) is relevant to all "3D culture/organ systems" not just "Eye". It is protected by provisional patent applications (pending). No confidential information provided at idea description level, detailed protocols will be shared/available for research when our solutions are selected for further implementation as a winner.

5. **Deepak Lamba, Buck Institute**

The outer retina, consisting of the light-sensing photoreceptors and the overlying retinal pigment epithelium (RPE), are affected in a number from retinal degenerative conditions. Developing strategies to maintain the function and cellular homeostasis of the outer retina requires a better understanding of the degenerative process. The creation of valid in vitro disease model for a number of retinal degenerations would require an organized 3D state such as that existing in the eye in order to mimic its highly-differentiated characteristics. Our solution to this challenge is to combine our expertise in retinal differentiation of iPSCs and combine it with our recent work on using hydrogels as bioscaffolds to promote 3D organization and lamination. This in turn should lead to maturation and generation of organized bilayered retina upon printing over a monolayer of mature RPE. We have previously published work on generating various retinal cells from human pluripotent stem cells (embryonic stem cells and induced pluripotent stem cells) in 2D monolayer culture setting. In this proposed solution, we will develop technologies to generate human retina in a dish by combining human induced pluripotent stem cell technologies with hydrogel based biomaterials. We propose to generate a Gelatin methacrylate (GelMA) based-bioink for three reasons: (i) it is biocompatible with human retinal cells, (ii) it provides support to the retinal cells through ECM adjuvants to promote lamination and (iii) it is extrudable through 3D printers. By optimizing this hydrogel-based bioink, we aim to print human retinal tissue over iPSC-derived retinal pigment epithelial layer to promote tissue interaction which is critical for maturation of photoreceptors. Long-term implications of this solution include the ability to use the proposed technology to study both retinal development as well as human disease modeling in a dish. It also has high-throughput screening applications as it is adapted to be used with multi-well 3D bioprinters.

TRAINEE CATEGORY – NO AWARDS GIVEN

1. **Kun-Che Chang, Stanford University (TRAINEE)**

Glaucoma is the leading of irreversible blindness with an estimated 64 million affected worldwide in 2013 and projected 111 million by 2040. Glaucoma is a neuronal degenerative disease associated with retinal ganglion cell (RGC) apoptosis and optic nerve degeneration. Once RGCs are lost, they cannot regenerate or be replaced in humans or other mammals, and vision loss is irreversible. RGC regeneration and axon reconnection would be approaches to restore vision; however, little is known about which gene or signaling pathway participating in human RGC degeneration/regeneration, neurons' interactions and axon reconnection. Although studies have been done by using other animals such as mouse or rat, are the same mechanisms working in humans? Many protocols have been reported for RGC generation from induced pluripotent stem cells (iPSCs) or embryonic stem cells (ESCs) (Gill et al., 2016; Tanaka et al., 2015), but two-dimension (2D) culture limits our understanding of neurons' interactions in the retina. Unfortunately, 3D retinal organoids (RO) studies have largely ignored questions of RGC differentiation and survival. In this proposal, I aim to address both of these unmet needs by analyzing 3D ROs derived from hiPSCs with a specific application of our RGC cell biology expertise. The goal of this project will be to differentiate more organized and retinal specific RO from hiPSCs, and then use this model to study human RGC degeneration after axon injury using laser axotomy technique, applying a variety of innovative imaging and molecular methods. Finally, I will study approaches to promote human RGC survival and regeneration in this organoid model, as a step towards translating molecular manipulations previously studied in rodents now into the human retinal model.

2. **Julia Oswald, Schepens Eye Research Institute (TRAINEE)**

To date full recapitulation of in-vivo retinal development and structure within hiPSC derived 3-dimensional organoids is limited with respect to both, synchrony of retinal maturation across cell types as well as the characteristic retinal layering. Furthermore, retinal organoids derived from current culture

protocols are extremely sensitive to high-throughput handling and require labor-intensive selection procedures to achieve reproducible yields of differentiation, as batch variations in differentiation and maturation remain high. In order to improve the handling of hiPSC derived retinal organoids on a high-throughput scale this proposal envisions the encapsulation of retinal organoids in stiffness tunable hydrogel to enhance resistance to sheer stress during manual/automatic handling. Furthermore, to bridge the discrepancy between cell type specific maturation and lamination observed within current culture systems, it proposes the generation of 3-dimensional organoids from 3D-printed, multilayered spheroids, using differentially aged retinal cell populations. While all neuronal cell types of the retina will be differentiated from hiPSC according to already existing protocols, the here proposed protocol envisions the isolation of each cell type from those “native” organoids, followed by the reassembly into 3D-printed, multilayered spheroids to achieve the characteristic laminar structure of the retina. To enhance cell viability and to mimic in-vivo extracellular matrix conditions each layer of the proposed spheroid will be derived from a stiffness tunable hydrogel matrix, adjusted for each individual cell type. In conclusion, by disentangling the process of cellular differentiation and neuronal network formation, this proposal aims to provide an organoid system that can be generated and maintained within high-throughput setups and provides the ability to simultaneously maintain all neuronal cell types of the retina within an in-vivo representative laminar scaffold.