**Cover page**

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**Team members:**

Biju B. Thomas, Ph.D.

Assistant Professor

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Submission will be applicable both to disease modeling and drug testing

**Comprehensive Description of the Proposed Solution**

***Abstract:***

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.

***Background***

**A. Retinal degenerative diseases, causes and treatment strategies:**

Age-related macular degeneration (AMD) and retinitis pigmentosa (RP) lead to a profound loss of vision in millions worldwide. Many of these patients require replacement of both lost retinal pigment epithelium (RPE) and photoreceptors (PR’s). Currently, there are no FDA-approved, effective pharmacologic therapies for the above diseases; multivitamins only slow the progression during early phases of AMD. In AMD, although the primary source of pathologic insult is not clear, histologic, biochemical, and genetic analysis suggests a role for oxidative damage, inflammation, and accumulation of toxic by-products within the RPE (Swaroop, Chew, Rickman, & Abecasis, 2009; Zarbin & Rosenfeld, 2010). This suggests that RPE transplantation has great potential to treat AMD (Tezel, Del Priore, Berger, & Kaplan, 2007). Of the other major retinal degenerative (RD) diseases, RP is perhaps the best characterized (Bird, 1995), with an incidence of 1 in 3500-4000 (Bunker, Berson, Bromley, Hayes, & Roderick, 1984). This group encompasses significant genetic and phenotypic heterogeneity (Sorrentino, Gallenga, Bonifazzi, & Perri, 2016). A wide variety of causes have been documented; these include disruption of a number of genes that are involved in phototransduction, including the biosynthesis and folding of the photopigment molecule, rhodopsin, as well as primary failure of the support cells within the retina, particularly the RPE (Sorrentino et al., 2016). This heterogeneity means that RP has highly variable clinical presentation and progression. However, the majority of patients initially experience problems in night vision, since the rod photoreceptors are typically damaged first, followed by a progressive loss of peripheral vision, leading to tunnel vision. This condition may further progress to affect the central visual field and blindness. During the early stages of RD diseases, when a majority of the photoreceptors are still preserved in the retina, the cell transplantation approach has demonstrated positive effects, which are mostly the result of introduction of neuroprotective growth factors into the eye (Jayakody, Gonzalez-Cordero, Ali, & Pearson, 2015). Since the impact of PR degeneration is more severe at later stages of the disease when various retinal cell types, including photoreceptors, RPE and inner retinal neurons have degenerated, neuroprotective approaches will then not be effective.

**B. Differentiation of retina organoids (RO) and retinal pigment epithelium (RPE) from stem cells:** In 2006, Lamba et al. generated photoreceptors (PR) from hESCs-derived embryoid bodies grown in serum free media supplemented with IGF1, noggin and DKK1 (Lamba, Karl, Ware, & Reh, 2006). Similar protocols have also been reported from other groups and have differing success rates (Mellough, Sernagor, Moreno-Gimeno, Steel, & Lako, 2012; Osakada, Ikeda, Sasai, & Takahashi, 2009)*.* Furthermore, photoreceptors have also been generated from mouse and human iPSCs (Hirami et al., 2009).

The next step was to generate 3D retinal structures. Several laboratories have successfully differentiated both ES and iPS stem cells into **optic vesicle**-like (Meyer et al., 2011; Nistor, Seiler, Yan, Ferguson, & Keirstead, 2010; Phillips et al., 2012) and even **optic-cup-like structures** (Eiraku et al., 2011; Gonzalez-Cordero et al., 2013; Nakano et al., 2012; Singh et al., 2015; Zhong et al., 2014) that develop lamination and even some light responses after long-term culture (Singh et al., 2015; Zhong et al., 2014).

Differentiation of RPE monolayers from hESCs is also an established procedure (e.g., (Idelson et al., 2009; Klimanskaya et al., 2004; Lane et al., 2014; Thomas et al., 2016)). We have shown that hESC can be differentiated into highly polarized RPE cells with molecular and functional attributes similar to that of the normal RPE (Lu, Tai, & Humayun, 2014; Lu, Zhu, Hinton, Humayun, & Tai, 2012). The product, termed CPCBRPE1 implant, is being evaluated in a FDA–approved phase1/2a clinical trial (NCT 02590692) for dry AMD, and has been tested successfully long-term in athymic nude and RCS rats (Diniz et al., 2013; Thomas et al., 2016). In addition, RPE can be isolated from retina organoids (Zhong et al., 2014). Because of different media requirements, RPE need to be fully matured separately before attempting retina-RPE co-culture.

Therefore, we propose to differentiate the tissues separately before assembling a choroid-RPE-retina organoid “sandwich”.

**C. Bioengineering of microvascular networks and co-culture with RPE:**

Microfluidic technologies have been developed extensively for generating organs-on-a-chip (reviews: (An, Qu, Liu, Zhong, & Luo, 2015; Haring, Sontheimer, & Johnson, 2017; Jackson & Lu, 2016). Several groups have developed retina-on-a-chip models (Mishra, Thakur, Redenti, & Vazquez, 2015; Su et al., 2015; Zhang et al., 2016), and microfluidic co-cultures of RPE with vascular endothelial cells (L. J. Chen et al., 2017; Kaji et al., 2014). Dr. Lee’s laboratory at UC Irvine has developed the technology of microphysiological organ-on-a-chip platform of microvessels from endothelial cells for large scale drug screening applications (Phan et al., 2017; Wang et al., 2016). The advantage of this system is that it is highly reproducible and based on 96-well dishes so that experiments can be performed with a minimal amount of medium.

**D. The Challenge: How to combine the different components together**

As mentioned above, the different elements of the “eye-on-a-chip” have been produced separately from stem cells; however, nobody has achieved yet to combine them together.

***Technologies and protocols needed***

**Material:** Devices and capillary networks inside will be developed by Dr. Lee’s lab.

3D retina organoids will be provided by Dr. Seiler’ lab; polarized RPE will be grown in Dr. Thomas’ lab.

***hESC culture*:** A H9 (NIH 0043) derived CRX-GFP expressing hESC cell line (Collin et al., 2016) through an MTA with University of Newcastle will be used for differentiation of retina organoids, modified after the protocol of (Zhong et al., 2014). The standard H9 hESC line will be used for differentiation of RPE and endothelial cells.

Cells are regularly verified for normal karyotype and SSEA4 expression. hESCs are maintained on Vitronectin XF (Stemcell Technologies) at a concentration of 10 μg/mL. The cells are typically split 1:24 and passaged every 5-7 days at ~80% confluency using ReLeSR reagent (Stemcell Technologies) to select for passage of pluripotent colonies only, with the addition of 5uM ROCK inhibitor to enhance cell survival during passage. The cells are maintained in TeSR-E8 medium (Stemcell Technologies), and the media are changed daily. 

***Differentiation of retina organoids*:** hESCs at 80% confluency are aggregated to embryoid bodies (EBs) which then are gradually transitioned to neural induction medium (NIM: DMEM-F12, 1% N2, 1x non-essential amino acids, 2ug/ml Heparin) over 3d. On d7, EBs are seeded on 1% matrigel at a density of 20 EBs/cm2. On d 16, they are switched to NIM2 composed of DMEM/F12 (3:1), supplemented with 2% B27, 1x NEAA, and 1% antibiotic/antimycotic).

From day 21-38, eye field (“horse shoe”) structures are cut out from the dish under an inverted microscope, using fine tungsten needles abd forceps. The resulting retinal spheres (containing retinal progenitors and RPE) (**Fig. 1**) are maintained in suspension culture until day 35-60 when they will be used for assembly with the RPE and microvessels after meeting quality control criteria such as transparency and size.

**Fig. 2** shows the procedure of obtaining sheets from retinal organoids.

***Differentiation of RPE:*** H9 hESC will be cultured and differentiated to derive RPE cells. The hESC-RPE obtained from the primary cultures will be isolated and further cultured. Passage 3 of hESC-RPE cells will be seeded and cultured around 30 days on ultrathin parylene substrates (**Fig. 3**) to produce polarized monolayer RPE implants for transplantation experiments. Parylene substrates without any cells will be used as control.



***Developing retina-RPE co-culture, and preliminary data for co-grafts:*** Recently, we received a pilot grant award from California Institute of Regenerative Medicine (CIRM) to investigate the feasibility of developing an RO+RPE co-graft (CIRM Discovery grant award DISC1-09912, Thomas, P.I). This novel tissue engineering technique can enable complete replacement of a diseased retina. Preliminary data from this study support our ability to grow RPE+RO co-grafts that can be surgically placed in the rat’s subretinal area (**Fig. 4**). 

***Capillary networks inside microfluidic devices***

The microfluidic device will be fabricated by bonding two layers of PDMS. Medium channels and retina organoid chambers are in upper layer. Tissue chambers are in bottom layer (Figs. 5 and 6). Regarding the construction of capillary networks, the cell–matrix mixture is first prepared by suspending endothelial cells (ECs) and human lung fibroblasts (NHLFs) in fibrinogen solution. The suspension is then mixed with thrombin and quickly injected into tissue chambers through the gel loading port using a micropipettor. The gel is allowed to polymerize in the incubator at 37 °C for 15 minutes. Next, laminin is introduced through the culture medium channels inlets to coat the inner surface of the medium channels as the basement membrane. The EGM-2 with full supplements is loaded through the medium reservoirs at the culture medium inlets.

Precoating the microfluidic channels with laminin before adding culture medium is crucial to stimulate EC adherence to PDMS when lining, as well as EC migration outward from the tissue chamber during vasculogenesis. The initial pressure drop to maintain the physiological level of mechanical stimuli on vasculogenesis is established by filling the reservoirs to different culture medium heights. Angiogenesis will happen within 6-8 days of culture and perfusable capillary networks will be developed in the tissue chambers.

***Model system to be achieved by this protocol***

Use double-layered configuration, develop capillaries in Bottom layer; then place mature, polarized RPE on a substrate (either parylene or PDMS) on top. Differentiate retina organoid, then cut out clearly retinal pieces and place in the upper layer, to create an Eye-on-a-Chip that mimics the interface between choroid (capillaries), RPE and retina.

***Disease chosen for modeling***

Initially we will use normal hESC (H9) as starting material. When the system is set up and functional, we will test iPSCs derived from AMD or retinitis pigmentosa patients.

**Applications:** A retinal co-graft made from RO and hESC-RPE proposed here will be beneficial for translation applications and for answering basic questions regarding its potential success in translation applications.

***Translational Applications***

1. **Transplantation of RO sheets with or without RPE for the treatment of RD diseases.** This approach can be applied in the treatment of retinal degeneration diseases such as AMD and RP. The feasibility of this approach can be tested in different RD models using our already established techniques.
2. **A total retinal patch made of hESC-RPE and RO sheets to reconstruct a damaged retina**. This approach is applicable for situations in which severe retinal damage is occurred as in battle fields or due to accidents. Since we have already demonstrated a technique to transplant hESC-RPE+RO co-grafts, the above application can be tested using chemically induced (Enzmann et al., 2006; Hariri et al., 2013) or photic induced (Ben-Shlomo et al., 2006; Cai, Xu, & Mo, 1989; E. Chen, 1993; Harris, Lincoln, Amoroso, Stuck, & Sliney, 2003; Seiler et al., 2000; Smiljanic & Vicic, 1996; Thomas et al., 2007; Whitmer & Stuck, 2009) retinal disease models.

***Basic Science Applications.*** An *in vitro* model system consisting of hESC-RPE+RO+choroid can be useful for studying the maturation of the RO sheets under different transplant situations (transplanted alone or together with RPE).

1. **To study survival and maturation of RO sheets transplanted into retinas with normal and healthy RPE**: This model system will assess survival and maturation of RO sheets transplanted alone into a host retina that harbors normal and healthy RPE. The above approach will be applicable for disease conditions where RPE functionality is normal but photoreceptor replacement is a requisite. The survival and maturation of RO sheets transplanted over healthy RPE will be studied as follows. RO sheets will be cultured together with healthy RPE cells (hESC-RPE implants) over the capillary layer (bottom layer). This condition will mimic a retina without any normal photoreceptors but having functional RPE. The survival and maturation of RO sheets cultured under the above condition can be assessed at different time points.
2. **To study co-transplantation approach for the replacement of both RPE and photoreceptors:** This study will help to understand maturation of RO sheets transplanted as a co-graft into a diseased retina. This model system represents conditions where replacement of both photoreceptors and RPE is a requisite as in the case of advanced AMD. To ascertain the feasibility of this approach, RO+hESC-RPE graft will be cultured over diseased RPE cells (Sinha, Phillips, Joseph Phillips, & Gamm, 2016) and maintained over the artificial capillary layer (Fig 3). The RO sheet survival and maturation can be monitored at different time points. 

***Innovation statement – Advantages and pitfalls***

***Innovation:***Our team combines expertise in retinal transplantation and microdissection, retina organoid and RPE differentiation from stem cells, and Bioengineering of vascular networks. The combination of microvessels (artificial choroid), RPE and retina organoid has not been accomplished before although several groups have been creating an artificial choroid with RPE (L. J. Chen et al., 2017; Kaji et al., 2014), and retinal explants with RPE (from primary tissue (Kaempf, Walter, Salz, & Thumann, 2008; Yanai, Laver, Gregory-Evans, Liu, & Gregory-Evans, 2015)), Culturing all three tissues layers together will help to create a new model system for better understanding of cell based therapies in the eye.

***Pitfalls, challenges:*** There is nothing that holds the retina sheet (dissected from the organoid) and the RPE together (as the retina organoid has not yet developed photoreceptors with outer segments). Both tissues have to be embedded together which requires micromanipulation in which Dr. Seiler has extensive experience. Embedding could be aided by bioadhesives.

In addition, we also do not know the best timing for combination of RPE, retina organoid and vascular network. This can be clarified by adding retina organoids of different stages of differentiation (35 or 60 days).

For **photoreceptor differentiation**, extended culture times will be necessary (at least 4 months). The retina organoid pieces need to be kept flat for the duration of the culture and not roll up. This could be accomplished by using embedding matrix with sufficient stiffness that does not hinder diffusion of nutrients.

**Polarized RPE** need to be completely differentiated and polarized before adding the retina organoid piece. We have already established that this is possible by 30 day culture. The RPE need to form a barrier between the artificial choroid and the retina organoid. This needs to be verified by testing for transepithelial resistance using an Ussing chamber.

We anticipate that no endothelial cells or blood vessels migrate into the Upper layer of the culture (where RPE and retina organoid piece reside).

In our model, we have not introduced the formation of retinal vessels which could come at a later phase. During retinal development, blood vessels coming from the optic disc spread out on the retinal surface and later invader the inner retinal layers. However, no blood vessels penetrate the outer nuclear layer.

If it is difficult to image the different components of the eye-on-a-chip together *in vivo* we will use our already established techniques based on histology and immunostaining to understand the morphology of the constructs.

**Biographical Sketches**

**Magdalene J. Seiler, Ph.D.**; Assistant Professor, Dept. Physical Medicine & Rehabilitation, Sue & Bill Stem Cell Research Center, UC Irvine

EDUCATION/TRAINING

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| --- | --- | --- | --- |
| INSTITUTION AND LOCATION | DEGREE | Completion Date | FIELD OF STUDY |
| Inst. Zoology, Univ. of Munich, FRG | Diploma | 06/1982 | Biology |
| Max Planck-Institute for Psychiatry, Munich, FRG | PhD | 12/1985 | Neurochemistry |
| Wake Forest University, Winston-Salem, NC | Postdoc | 11/1987 | Retinal Transplantation |
| Eye Research Institute, Boston, MA | Postdoc | 12/1988 | Retinal Transplantation |

**Positions and Employment**

1989 - 1992 Scientific Associate, Eye Research Institute, Boston, MA

1993 - 2002 Assistant Professor, Univ. Louisville Medical School, Dept. Ophthalmology & Visual Sciences, Dept. Anatom. Sciences & Neurobiology, Louisville, KY

2002 - 2006 Assistant Professor of Research Ophthalmology, Doheny Eye Institute and Depts. of Ophthalmology and Cell & Neurobiology, Keck School of Medicine, University of Southern California, Los Angeles, CA

2007 - 2014 Project Scientist, Dept. Anatomy & Neurobiology, UC Irvine

2014 - 2015 Project Scientist, Dept. Physical Medicine & Rehabilitation; UC Irvine, Irvine CA

2015 (Oct.) Assistant Professor; Dept. Physical Medicine & Rehabilitation; UC Irvine

**Selected Publications** (from a toal of 57)**:**

1. **Seiler MJ**, Aramant RB (1998). Intact sheets of fetal retina transplanted to restore damaged rat retinas. *Invest. Ophthalmol. Vis. Sci.*, **39**:2121-2131. PMID: 9761291
2. Radtke ND, Aramant RB, Petry HM, Green PT, Pidwell DJ, **Seiler MJ** (2008). Vision Improvement in Retinal Degeneration Patients by Implantation of Retina Together with Retinal Pigment Epithelium. *Am J Ophthalmol*, **146:**172-182. PMID: 18547537
3. **Seiler MJ**, Aramant RB, Thomas BB, Peng Q, Sadda SR, Keirstead HS. Visual restoration and transplant connectivity in degenerate rats implanted with retinal progenitor sheets. *Eur J. Neurosci*, **31**(3):508-520 (2010). PMID: 20105230; PMCID: PMC2875871
4. **Seiler MJ**, Aramant RB. Cell replacement and visual restoration by retinal sheet transplants. *Prog Retin Eye Res,* **31**:661-687 (2012). PMID: 22771454; PMCID: PMC3472113
5. **Seiler MJ**, et al. (2017). Vision recovery and connectivity by fetal retinal sheet transplantation in an immunodeficient retinal degenerate rat model**.** *Invest. Ophthalmol Vis Sci*, **58**:614-630. DOI 10.1167/iovs.15-19028

**Biju B. Thomas, Ph.D.**, Assistant Professor, Dept. Ophthalmology, USC

EDUCATION/TRAINING

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| INSTITUTION AND LOCATION | DEGREE | Completion Date | FIELD OF STUDY |
| University of Kerala, India | MS | 05/1990 | Zoology |
| University of Kerala, India | Ph.D | 10/1997 | Behavioral Neuroscience |
| University of Louisville, KY, USA | Post-doc | 01/2001 | Retinal Transplantation |

**Positions and Honors**

1998 - 1999 Guest Lecturer, Post-graduate Department of Zoology, St. Stephen’s College, Pathanapuram, Kerala, India

1999 - 2000 Guest Lecturer, Post-graduate Research Department of Zoology, St. Berchman’s College, Changanacherry, Kerala, India

1998 - 2000 Postdoctoral Research Associate, University of Kerala, India

2001 - 2002 Postdoctoral Research Associate, University of Louisville, Louisville, KY

2002 - 2006 Postdoctoral Research Associate, Doheny Eye Institute, Los Angeles, CA

2007 – Present Assistant Professor of Ophthalmology Research, Keck School of Medicine of the University of Southern California, Los Angeles, CA

**Selected Publications** (from a total of 34)**:**

1. **Thomas BB**, Seiler MJ, Sadda SR, Aramant RB. Superior colliculus responses to light--preserved by transplantation in a slow degeneration rat model*. Exp Eye Res* 2004;79:29-39.
2. **Thomas BB**, Arai S, Ikai Y, Qiu G, Chen Z, Aramant RB, Sadda SR, Seiler MJ. Retinal transplants evaluated by optical coherence tomography in photoreceptor degenerate rats. *J Neurosci Methods* 2006;151:186-193.
3. **Thomas BB,** Seiler MJ, Aramant RB, Samant D, Qiu G, Vyas N, Arai S, Chen Z, Sadda SR. Visual functional effects of constant blue light in a retinal degenerate rat model. *Photochem Photobiol* 2007;83:759-765
4. [**Thomas B.B**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Thomas%20BB%5BAuthor%5D&cauthor=true&cauthor_uid=27233037), [Zhu D](http://www.ncbi.nlm.nih.gov/pubmed/?term=Zhu%20D%5BAuthor%5D&cauthor=true&cauthor_uid=27233037), [Zhang L](http://www.ncbi.nlm.nih.gov/pubmed/?term=Zhang%20L%5BAuthor%5D&cauthor=true&cauthor_uid=27233037), [Thomas P.B](http://www.ncbi.nlm.nih.gov/pubmed/?term=Thomas%20PB%5BAuthor%5D&cauthor=true&cauthor_uid=27233037), [Hu Y](http://www.ncbi.nlm.nih.gov/pubmed/?term=Hu%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=27233037), [Nazari H](http://www.ncbi.nlm.nih.gov/pubmed/?term=Nazari%20H%5BAuthor%5D&cauthor=true&cauthor_uid=27233037), [Stefanini F](http://www.ncbi.nlm.nih.gov/pubmed/?term=Stefanini%20F%5BAuthor%5D&cauthor=true&cauthor_uid=27233037), [Falabella P](http://www.ncbi.nlm.nih.gov/pubmed/?term=Falabella%20P%5BAuthor%5D&cauthor=true&cauthor_uid=27233037), [Clegg D.O](http://www.ncbi.nlm.nih.gov/pubmed/?term=Clegg%20DO%5BAuthor%5D&cauthor=true&cauthor_uid=27233037), [Hinton D.R](http://www.ncbi.nlm.nih.gov/pubmed/?term=Hinton%20DR%5BAuthor%5D&cauthor=true&cauthor_uid=27233037), [Humayun M.S](http://www.ncbi.nlm.nih.gov/pubmed/?term=Humayun%20MS%5BAuthor%5D&cauthor=true&cauthor_uid=27233037). Survival and Functionality of hESC-Derived Retinal Pigment Epithelium Cells Cultured as a Monolayer on Polymer Substrates Transplanted in RCS Rats. *Investigative Ophthalmology and Visual Science* 2016, 57:2877-2887.
5. Lin T, Seiler MJ, [Zhu D](http://www.ncbi.nlm.nih.gov/pubmed/?term=Zhu%20D%5BAuthor%5D&cauthor=true&cauthor_uid=27233037), Falabella P, Hinton RD, Clegg D, Humayun MS, **Thomas BB.** Assessment of Safety and Functional Efficacy of Stem Cell-Based Therapeutic Approaches Using Retinal Degenerative Animal Models. *Stem Cells International* 2017 (in press)

**Abraham Lee, Ph.D.**

EDUCATION/TRAINING

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| INSTITUTION AND LOCATION | DEGREE | Completion Date | FIELD OF STUDY |
| University of California at Berkeley | M.Sc. | 5/15/1989 | Mech. Engineering |
| University of California at Berkeley | Ph.D | 10/15/1992 | Mech. Engineering |
| Lawrence Livermore National Laboratory | Postdoc | 11/1/92-03/31/95 | Medical Technology Program |

**Positions and Honors**

|  |  |  |
| --- | --- | --- |
| 1994 - 1999 | PI/Group Leader, Lawrence Livermore National Laboratory | |
| 1999 - 2001 | Program Manager, DARPA |
| 2001 | Senior Technology Advisor, National Cancer Institute |
| 2002 - present | Professor, BME/MAE, University of California, Irvine | |
| 2010 - present | William J. Link Professor and Chair, BME Department |

**Selected Publications** (from a total of 100)**:**

1. Xiaolin Wang\*, et al., **Abraham P. Lee**+,[Engineering anastomosis between living capillary networks and endothelial cell-lined microfluidic channels](http://pubs.rsc.org/en/Content/ArticleLanding/2016/LC/C5LC01050K?utm_medium=email&utm_campaign=pub-LC-vol-16-issue-2&utm_source=toc-alert#!divAbstract)", Lab Chip, 2016, **16**, 282-290
2. Yu-Hsiang Hsu, et al., **Abraham P. Lee**\*. [Full range physiological mass transport control in 3D tissue cultures](http://pubs.rsc.org/en/content/articlelanding/2013/lc/c2lc40787f), *Lab Chip, 2013,13, 81-89*.
3. Duc T. T. Phan, et al.,**Abraham P. Lee**\* and Christopher C. W. Hughes\*, ["A vascularized and perfused organ-on-a-chip platform for large-scale drug screening applications](http://pubs.rsc.org/en/content/articlelanding/2017/lc/c6lc01422d#!divAbstract)", Lab Chip, 2017,17, 511-520

**Feasibility Assessment**

*Describe the ability to execute the proposed solution and address the Evaluation Criteria. Include estimated timeframe, supporting precedents, and any special resources that are needed. If proposal requires expertise in divergent areas of research, the ability of the team to execute solution will be considered. If applicable, address protections for human subjects, compliance with policies related to use of human stem cells, biosafety issues, and use of technologies covered by patents or other intellectual property protection.*

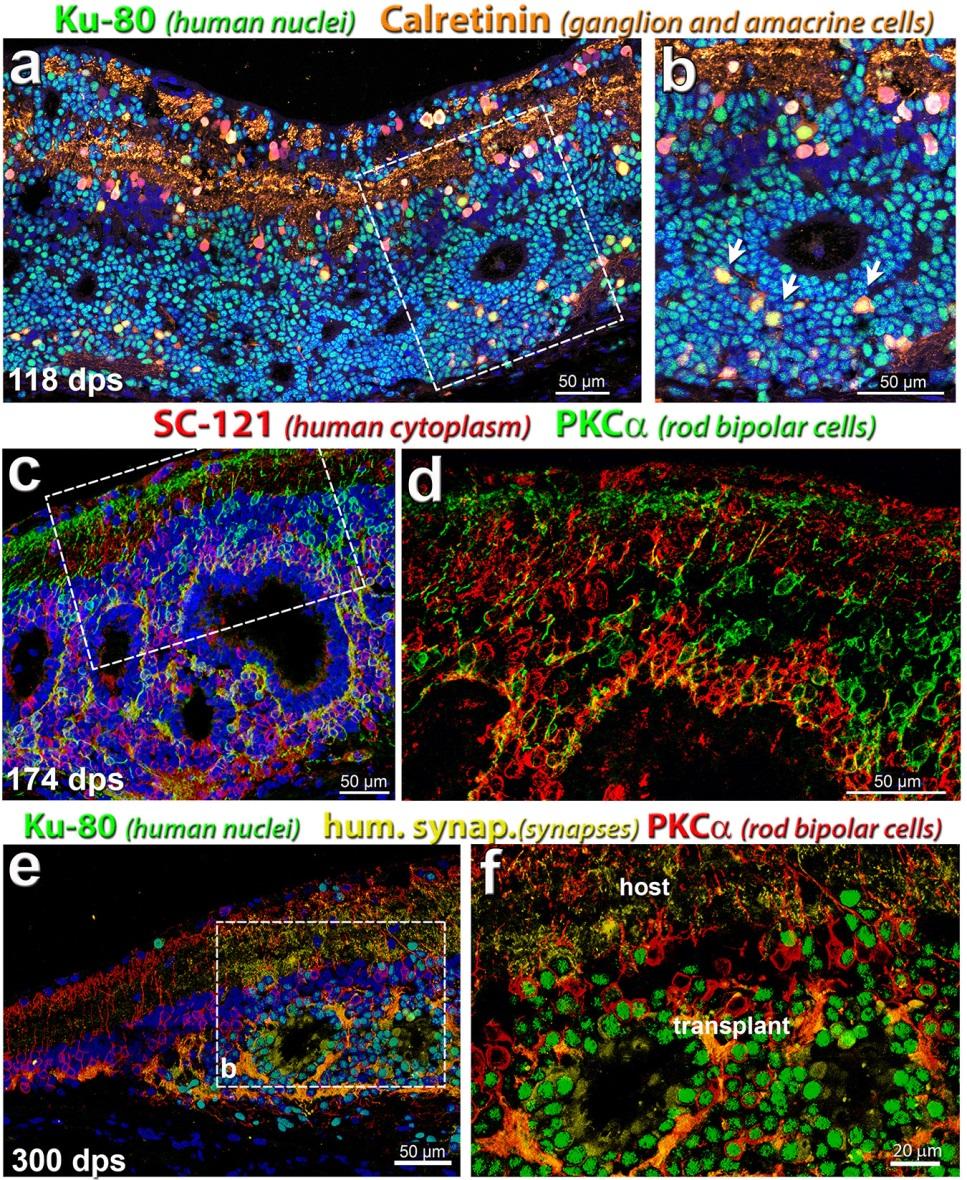
**Estimated Time frame**

The estimated time frame for this project is 1 year. In the first 3 months, components will be differentiated separately from H9 hESCs, then combined step-by-step.

Retina organoids will be differentiated for 30-60 days (at least) before combining them with the other tissues.

**Supporting precedents**

Dr. Seiler’s lab has shown that sheets dissected from retina organoids transplanted to retinal degenerate rat models develop all major retinal cell types *in vivo* (**Fig. 8**).





**Special resources**

There are no special resources required. Each lab (Dr. Seiler, Dr. Thomas, Dr. Lee) has access to a special tissue culture room with biosafety cabinets, incubators, inverted and stereo microscopes, and technicians with expertise in cell culture.

**Team expertise in divergent areas of research**

Dr. Seiler is expert in retinal transplantation, development, and microdissection. Her lab has developed expertise in differentiating retina organoids from hESCs.

Dr. Thomas is expert in studying retinal degenerative diseases and conducting transplantation experiments in rodent models. His laboratory developed the technique to transplant RPE cultured as a polarized monolayer on ultrathin substrates.

Dr. Lee is expert in microfluidic technologies for 3D cell cultures. Collaborating with a vascular biologist, he has developed a microfluidic platform for perfused vascularized tissue that are connected with microchannels intravascularly. He has developed a wide range of technologies for cell sorting, single cell analysis, molecular detection, and therapeutic particles.

**Protections for human subjects**: n/a

**Human stem cells:**

Dr. Seiler’s studies are covered by hSCRO protocol 2006-5316, last approval date 7-21-17 (expiration date 7-20-18).

Dr. Thomas’ SCRO protocol approval is pending

Dr. Lee;s hSCRO protocol is pending.

**Biosafety issues:**

Dr. Seiler’s biosafety protocol is last approved 10/25/2016 through 10/24/2019, with annual report requirements.

Dr. Thomas biosafety protocol is approved July 27, 2017 through July 26 2020

Dr. Lee s biosafety protocol is last approved 05/12/2016 through 05/11/2019, with annual report requirements.

**Use of technologies covered by patents or other intellectual property protection**

N/A

**References**

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Cai, Y. S., Xu, D., & Mo, X. (1989). Clinical, pathological and photochemical studies of laser injury of the retina. *Health Phys, 56*(5), 643-646.

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