Screen Printing Retinal Models

Team Lead: Erin Lavik, ScD
Professor, Chemical, Biochemical, and Environmental Engineering
University of Maryland, Baltimore County

Team Member: Steven Bernstein, MD, PhD
Professor and Vice Chair of Ophthalmology and Visual Sciences
University of Maryland Medical School

Team Member: Adam Day
Graduate Student, Chemical, Biochemical, and Environmental Engineering
University of Maryland, Baltimore County

Team Member: Bryan Ibarra
Undergraduate Student, University of Miami

This submission is a solution that is best suited to high throughput screening and drug testing

Comprehensive Description of the Proposed Solution: Screen Printing Retinal Models

ABSTRACT

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.

BACKGROUND

There have been substantial developments in the retinal organoid field in the last several years with more and more labs generating the major retinal cell types and forming retinal-like structures, albeit without the organization of the retina.

3D printing offers an exciting approach to facilitate organizing the cell types into retinal models, but it is not without limitations particularly in relation to stem cells and retinal cells. Bioprinting allows printing of cells and hydrogels into complex architectures (Jung, Bhuiyan, & Ogle, 2016; Yue, Liu, Coates, & Wallace, 2016). This allows one to develop the kinds of patterns and architectures that are seen in vivo, but it requires extruding the materials and cells through fine openings as well as specialized equipment. While the cost of bioprinters has come down, the extrusion process requires bioinks that are compatible with the shearing associated with the approach as well as materials that protect cells during this process (Dubbin, Hori, Lewis, & Heilshorn, 2016).

There is a real need to develop new approaches to make complex tissues that are simple, cost effective, and avoid the extrusion issues associated with bioprinting approaches and the UV light source in photolithography.

We have developed an approach that is highly scalable and avoids these extrusion issues, especially the need for specific bioinks to protect cells during the bioprinting process. Taking a cue from the electronics industry (Peng et al., 2016; Suikkola et al., 2016), our approach uses screen printing to develop highly scalable hydrogel-cell tissue models.

Screen printing offers several advantages. (1) It can model tissue comprising many types of materials and cells including those that are sensitive to UV light and extrusion. (2) These materials and cells can be patterned within a wide range of three-dimensional, multilamellar structures. (3) By varying the screen structure, layers can be created across a wide range of thicknesses. (4) These models can be built quickly and at low cost. A schematic of the screen printing process is shown in Figure 1.

As a foundation for this process, we have synthesized hydrogels based on poly(ethylene glycol) (PEG) for this system coupled with polylysine. By tuning the ratio of PEG with polylysine we can achieve a wide range of moduli (Hynes, Rauch, Bertram, & Lavik, 2009; Zustiak & Leach, 2010). The presence of polylysine enables us to absorb proteins of interest into the gels, considerably reducing time and cost compared to building each protein material directly for a gel (Hynes et al., 2009).

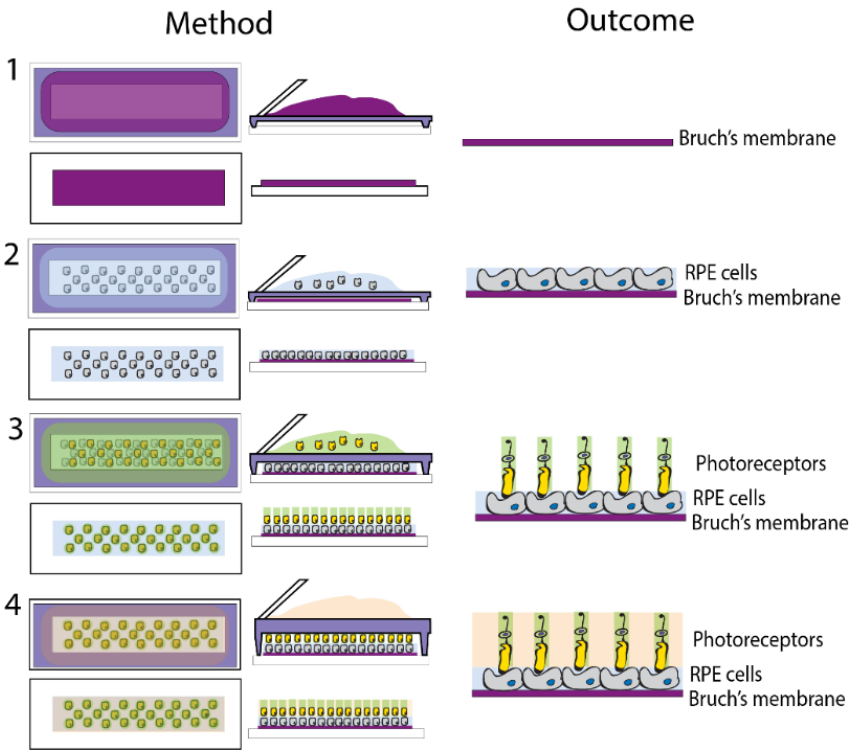

Figure 1: Schematic for making the gel for screen printing and screen printing Bruch's membrane and two of the cellular layers of the retinal model. In this schematic, the green gel is the cell-permissive gel, and the yellow is the PEG-based gel that does not support cells.

TECHNOLOGIES AND PROTOCOLS FOR THIS SYSTEM

Human optic nerve neural progenitor cells

We have isolated adult neural progenitor cells (aNPCs) from young and mature optic nerve. These cells are SOX2(+)/Nestin(+)/GFAP(+) (Fig 2), and can be distinguished from their surroundings by: 1) Their ability to be cultured continuously for >10 replications. 2) Their ability to give rise to astrocytes, oligodendrocytes and rarely, neurons. 3) Their ability to form neurospheres (Fig 3A and B). 4) Their age-associated depletability. These cells are maintained in a high concentration matrix medium. Withdrawal of growth medium and replacement with fetal bovine serum results in differentiation into largely glial cells. Our data suggests that these cells are used during ON growth in adulthood, to maintain gliogenesis in high stress areas. Loss of these cells results in segmental hypomyelination and ON hypoplasia.

While these cells will implant on biosurfaces and grow, these surfaces are non-optimal. The hydrogel screen printing process will allow us to build and identify the most appropriate extracellular matrix, an artificially constructed surface is appropriate. By using an aNPC-artificial matrix construct, in conjunction with a retinal ganglion cell (RGC)-survival assay, we can determine the optimum conditions for aNPC survival, autologous growth factor expression, and RGC-axonal development; all critical conditions required for normal eye development and growth.

Structure

Hydrogel facilitates retinal cell differential and organization

One of the challenges with bioprinting is engineering matrices that can be sheared through fine needles, protect cells, and still be an appropriate matrix to support cellular organization. The screen printing process avoids the shearing aspect which allows a far broader range of materials and gels to be used. The major requirement for screen printing is that the gel be able to set up, crosslink, or phase separate on a timescale on the order of the printing process which means within seconds to a minute. This is easy to achieve with a range of coupling chemistries. We focus on the vinylsulfone-thiol chemistry because it is easily performed and highly biocompatible avoiding light or toxic coupling agents (Jin et al., 2010; Nah, Yu, Han, Ahn, & Kim, 2002; Zhou, Stukel, Cebull, & Willits, 2016). The material is like a classic epoxy. The oligomer containing vinylsulfone (PEG-polylysine) is mixed with a dithiol-PEG oligomer.

Polarization of the cells in the layers

One of the major questions regarding this approach is how will a layer of cells printed using this system achieve the necessary guidance to polarize appropriately. With the screen printing approach, we can print vertical channels of permissive matrix such as laminin absorbed hydrogel surrounded by non-permissive matrix such as a pure PEG gel that resists protein absorption and provides structural guidance cues to orient the cells. By patterning the cell-permissive protein gels with non-permissive gels, we are able to create interfaces that will act as architecture to guide the retinal cell types to polarize in the matrix.

Resolution of Features and Reproducibility

The resolution of features in the 3D printing process is dictated primarily by the resolution of the screens. The 110 mesh has pores on the order of 130 um. A 200 mesh screen has pores on the order of 74 um. In the electronics industry, resolution down to 20 um is common (Hyun, Secor, Hersam, Frisbie, & Francis, 2015), but 100 um is more than adequate for printing of the structures we are interested in. As a comparison, bioprinting resolutions are on the order of 50 um have been achieved under very tightly controlled circumstances, but most instruments and features resolutions are on the order of 75-150 um depending on the material being printed (Goldberg, Brown, Liu, & Meyerhoff, 1994). When cells are combined, the resolution decreases to features on the order of hundreds of microns except when laser systems are used which exposes cells to high energy light (Datta, Ayan, & Ozbolat, 2017; Ozbolat & Hospodiuk, 2016; Ravnic et al., 2017).

Screen printing cells

We have tested meshes with 100 micron pores to determine the viability of cells post printing. We have used iPS cells differentiated down a neural linage from our colleague, Michael Nestor. 84+/-2.6% of the cells are viable post-printing in the absence of any matrix which typically augments survival (Lozano et al., 2015).

PROTOCOL FOR SCREEN PRINTING THE RETINAL MODEL FOR HIGH THROUGHPUT SCREENING

The following are the key steps in engineering the 3D model of the retina using the screen printing technique:

1. **Preparing the cells.**

Cells are obtained from human donor optic nerves and eyes for which we have obtained using an UMB IRB exemption, and are currently available in the lab. These cells are grown and stored as low-subculture passage cultures, and express appropriate markers (Chx10, MBP, GFAP, SOX2, NeuN). Following replating of the subcultures, they are subsequently dissociated with elastase and placed in the appropriate fluid. Retinal cells are prepared from using triturated retinae digested with Papain. Retinal ganglion cells (RGCs) and amacrine cells are isolated following removal of microglia using Thy-1 immuno-linked beads (Miltenyi) (Barres, Silverstein, Corey, & Chun, 1988; Meyer-Franke, Kaplan, Pfrieger, & Barres, 1995).

Photoreceptors are isolated in a similar manner from the Thy-1 (-) eluate, but utilizing the appropriate surface markers for photoreceptors (glycosylphosphatidylinositol (GPI)-anchored cell surface molecule ecto-5'-nucleotidase (CD73) for rods). The purified populations (>65%) are then employed in the preparation of cell printing. The RGCs are also utilized for co-incubation with the aNPC surface assays. These assays are prepared in triplicate, and are compared against both commercially available artificial matrices, as well as laminin-coated surfaces.

2. **Preparing the screens.**

The mask for the screen is printed on an inkjet printer on a transparency. The screens are coated with the emulsion, the transparency is applied, and the screen is exposed to a UV light source followed by rinsing to remove uncrosslinked emulsion. The screens are then autoclaved and ready for use. Using these standard materials traditionally used for screen printing t shirts, we can obtain reproducible features on the order of 100 um.

3. **Choice of support structure for the retina: glass coverslips versus oxygen transport membrane**

One can print on a range of materials including glass coverslips and gas permeable membranes such as a silicone (polydimethylsiloxane) membrane. We focus on coverslips and glass plates because of the ease with which they can facilitate mutliwell culture and high throughput screening.

4. **Printing Bruch's and ON membrane surfaces**

The basement membrane that interfaces with the RPE layer consists of laminin, fibronectin, collagen type VI, and glycosaminoglycans (Booij, Baas, Beisekeeva, Gorgels, & Bergen, 2010). We will print a cocktail of these molecules on the polylysine-PEG printed gel as a model for the healthy Bruch's membrane and ON surfaces. In future work, we can investigate alterations to the chemistry of these different surfaces. We will characterize the subsequent concentrations of these molecules using immunocytochemistry for the proteins and expect it to reflect the ratio in the feed solution based on our previous work (Hertz, Robinson, Valenzuela, Lavik, & Goldberg, 2013; Hynes et al., 2009; Rauch, Michaud, Xu, Madri, & Lavik, 2008).

5. **Printing RPE and ON aNPCs.**

We will print human RPE and aNPCs cells in a laminin-coated matrix. We will characterize these cells by looking at cell survival (live/dead assay), formation of tight junctions (BEST1; ZO-1) (Brandl et al., 2014; Shadforth et al., 2015), expression of RPE- and mature glial specific markers (RPE65), MBP, CNPase, Glut-1 (Ahmado et al., 2011), and the response to variations in Bruch's membrane layer.

6. **Printing photoreceptors in the matrix**

We will print the photoreceptor progenitors in the laminin-coated matrix. We will assess marker expression as well as phagocytosis of the outer segments by the RPE cells.

7. **Printing bipolar, horizontal, and amacrine cells**

We will use three masks to pattern the horizontal, bipolar, and amacrine cells. The bipolar cells will be aligned with the photoreceptors. The horizontal and amacrine cells will be offset. All will be in the laminin-coated matrix.

8. **Printing ganglion cells**

The ganglion cells will be aligned with the bipolar cells in the laminin-coated matrix.

9. **Culturing the constructs**

The materials completely set within 3 minutes. At that point, the constructs are immersed in media and cultured at 37 C with media changes twice per week or as needed.

10. **Characterizing the structures**

The constructs are characterized structurally as described below immunohistochemically using epifluorescent microscopy, confocal, and a high throughput imager, the acumen cellista. The Acumen cellista uses laser scanning technology to quantitatively assess fluorescent signals. While it can create images with a resolution equivalent to 200x, the strength of the system is that it can do rapid quantification of multiple fluorescent signals through several millimeters of tissue. This will allow us to not only rapidly image many structures but to also quantify the fluorescence and, therefore, the number of cells and their marker expression using immunocytochemistry without having to section the constructs.

11. **Assessing functionality**

One of the attractions of the screen printing process is that it can be used on a range of surfaces. To assess function, we will print the retinal and ON structures on multichannel electrode array systems to do the equivalent of ERG in a dish. This is coupled with validation by mimicking aspects of disease including alterations in Bruch's membrane that are associated with AMD. Details are outlined below.

The screen printing process increases survival of cells, particularly those which are sensitive to shear forces and UV-light by eliminating the shearing associated with more traditional 3D printing and using materials that gel without UV exposure. The open matrices lead to robust proliferation and organization of both neural and retinal cells. The approach and the materials provide are exceptional for building the multilamellar structure of the retina and providing the extracellular matrix cues and support to foster healthy cells and tissue organization.

VALIDATING THE RETINAL MODEL

Describe the technologies and protocols needed to develop the model systems, including a validation scheme that outlines reproducibility and transferability or ease of use of proposed protocol.

Structural assessment and reproducibility

We will screen 48 replicates per experiment. We will use immunocytochemistry for the appropriate markers as noted above along with epifluorescence and high throughput screening using a laser scanning system and three lasers (blue, green, Cy5). Synapses in the system will be characterized using an antibody for PSD95 (Schaefer et al., 2016).

Functional assessment

We will use multielectrode recording for functional assessment, essentially an in vitro version of an ERG. We can screen print the system on a multichannel electrode array (multichannel systems) and screen the response from different structures in response to light exposure using an LED-based stimulation system (Stett et al., 2003).

Challenging the model

Alterations in Bruch's membrane

The changes in the extracellular matrix, particularly associated with Bruch's membrane, play a significant role in the pathology of diseases including age-related macular degeneration (AMD) but the methods to investigate this have been limited (Al Gwairi, Thach, Zheng, Osman, & Little, 2016; Booij et al., 2010; Hollyfield et al., 2008). By patterning our Bruch's membrane structure and then building the retinal model, we will be able to model the healthy retina with tight RPE junctions as well as the diseased retina with the degenerating RPE layer. We will use high throughput screening to assess the junctions as well as the health of RPE cells in response to changes in the matrix.

Calcification of the elastin layer, crosslinking of the collagen layers, and overall thickening have been associated with a reduction in elasticity that may play a role in AMD progression (Fernandez-Godino, Pierce, & Garland, 2016; Kaluzny, Purta, Poskin, Rogers, & Fawzi, 2016). Increases in glycosaminoglycans may also play a role in disease (Booij et al., 2010; Fernandez-Godino et al., 2016; Kaluzny et al., 2016). Will the PEG-polylysine system as the support, we can vary these molecules and look at the cellular response of the RPE and retinal cells.

2-Aminophosphonobutyric acid

2-Aminophosphonobutyric acid (AP4), is a blocker of the on-signal pathway in the retina. By using the multielectrode array system, we will be able to measure the B-wave amplitude before, during and after wash out of the drug. The B-wave is primarily due to Muller cell and bipolar cell activity (Stett et al., 2003).

3D MODEL FOR HIGH CONTENT SCREENING OF THE RETINA

The screen printing process allows one to build up patterned, lamellar systems cheaply and easily with high resolution. All of the materials are readily available and autoclavable including the hydrogel components since they are not hydrolytically degradable. Screen printing allows one to make many replicates in a single printing process that preserves the viability of the cellular components and provides the critical extracellular matrix cues to build retinal structures in 3D.

By printing on a plate followed by the addition of a silicone gasket, we can print up to 96 replicates on a single plate in minutes, making this an extremely efficient method for building up retinal models quickly.

INNOVATION STATEMENT

The screen printing process combined with the adult neural progenitors will allow us to rapidly build retinal structures and determine the impact of structural, molecular, and cellular cues on retinal development and lead to a system that extremely amenable to high throughput screening. This approach lays a foundation for facilitating the development of printed tissue models more broadly in that the techniques are easily taught and the materials are readily available and low cost.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Lavik, Erin

eRA COMMONS USER NAME (credential, e.g., agency login): EBLAVIK

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Massachusetts Institute of Technology	BS	06/1995	Mat. Sci. and Eng.
Massachusetts Institute of Technology	MS	06/1997	Mat. Sci. and Eng.
Massachusetts Institute of Technology	SCD	06/2001	Mat. Sci. and Eng. Poylmers
Massachusetts Institute of Technology	Postdoctoral Fellow	06/2003	Health Sci. and Tech. Program

A. Personal Statement

My lab focuses on the development of materials for drug delivery and tissue engineering applications. We have been engineering novel materials and processes to make model tissues for over a decade with the majority of our experience focusing on neural and retinal models in vitro and in vivo.

B. Positions and Honors

Positions and Employment

1997 - 2003 Research Fellow, Harvard School of Dental Medicine
2000 - 2000 Teaching Assistant, MIT
2001 - 2003 Postdoctoral Research Fellow, MIT
2002 - 2002 Teaching Assistant, MIT
2002 - 2003 Associate Research Scientist, Yale University
2003 - 2008 Assistant Professor, Biomedical Engineering, Yale University
2008 - 2009 Associate Professor, Biomedical Engineering, Yale University
2009 - 2016 Associate Professor, Biomedical Engineering, Case Western Reserve University
2014 - present Associate Editor, Bioconjugate Chemistry, an ACS journal
2014 - 2020 Standing Member, NIH Gene and Drug Delivery Study Section
2015 - 2015 Professor, Case Western Reserve University, Cleveland, OH
2016 - present Professor, University of Maryland, Baltimore County (UMBC), MD

Other Experience and Professional Memberships

Honors

1995 Graduate Fellowship, NSF
1995 Best Senior Thesis, Dept. Mat. Sci. and Eng. MIT
1998 NIDR Training Grant in Biomaterials Fellowship, Harvard School of Dental Medicine/MIT
2000 John Wulff Award for Excellence in Teaching, MIT
2000 Graduate Student Gold Award, Materials Research Society
2003 TR100. 100 top innovators under 35., Technology Review
2004 Rave Award Nominee, Medical Scientist, Wired Magazine
2004 Invited Participant, National Academy of Engineering Frontiers of Engineering Symposium
2007 Sheffield Distinguished Teaching Award, Yale University
2007 Sloan Foundation Scientific Advisory Board, Manhattan Theatre Club

2007	Invited Participant, National Academy of Engineering Keck Futures Initiative, Engineering the Future of the Human Healthspan
2008	Academic Leadership and Innovation Award, Connecticut Technology Council
2008	Poorvu Family Award for Interdisciplinary Teaching, Yale University
2009	Seton Elm-Ivy Award, Yale University
2010	Director's New Innovator Award, NIH
2011	Research Award, Case School of Engineering
2011	Flora Stone Mather Award, Case Western
2013	Editorial Advisory Board, Macromolecules and ACS Macro Letters
2014	Fellow, American Institute of Medical and Biological Engineers (AIMBE)

C. Selected Peer-reviewed Publications (of 61)

1. Robinson R, Bertram JP, Reiter JL, Lavik EB. New platform for controlled and sustained delivery of the EGF receptor tyrosine kinase inhibitor AG1478 using poly(lactic-co-glycolic acid) microspheres. J Microencapsul. 2010 May;27(3):263-71. PubMed PMID: 20055747; PubMed Central PMCID: PMC4431618.
2. Hynes SR, Lavik EB. A tissue-engineered approach towards retinal repair: scaffolds for cell transplantation to the subretinal space. Graefes Arch Clin Exp Ophthalmol. 2010 Jun;248(6):763-78. PubMed PMID: 20169358.
3. Grozdanic SD, Lazic T, Kuehn MH, Harper MM, Kardon RH, Kwon YH, Lavik EB, Sakaguchi DS. Exogenous modulation of intrinsic optic nerve neuroprotective activity. Graefes Arch Clin Exp Ophthalmol. 2010 Aug;248(8):1105-16. PubMed PMID: 20229104; PubMed Central PMCID: PMC3678383.
4. Lavik E, Kuehn MH, Kwon YH. Novel drug delivery systems for glaucoma. Eye (Lond). 2011 May;25(5):578-86. PubMed PMID: 21475311; PubMed Central PMCID: PMC3171267.
5. Robinson R, Viviano SR, Criscione JM, Williams CA, Jun L, Tsai JC, Lavik EB. Nanospheres delivering the EGFR TKI AG1478 promote optic nerve regeneration: the role of size for intraocular drug delivery. ACS Nano. 2011 Jun 28;5(6):4392-400. PubMed PMID: 21619059; PubMed Central PMCID: PMC3136352.
6. Williams C, Rauch MF, Michaud M, Robinson R, Xu H, Madri J, Lavik E. Short term interactions with long term consequences: modulation of chimeric vessels by neural progenitors. PLoS One. 2012;7(12):e53208. PubMed PMID: 23300890; PubMed Central PMCID: PMC3531360.
7. Shoffstall AJ, Atkins KT, Groynom RE, Varley ME, Everhart LM, Lashof-Sullivan MM, Martyn-Dow B, Butler RS, Ustin JS, Lavik EB. Intravenous hemostatic nanoparticles increase survival following blunt trauma injury. Biomacromolecules. 2012 Nov 12;13(11):3850-7. PubMed PMID: 22998772; PubMed Central PMCID: PMC3496064.
8. Kador KE, Montero RB, Venugopalan P, Hertz J, Zindell AN, Valenzuela DA, Uddin MS, Lavik EB, Muller KJ, Andreopoulos FM, Goldberg JL. Tissue engineering the retinal ganglion cell nerve fiber layer. Biomaterials. 2013 Jun;34(17):4242-50. PubMed PMID: 23489919; PubMed Central PMCID: PMC3608715.
9. Hertz J, Robinson R, Valenzuela DA, Lavik EB, Goldberg JL. A tunable synthetic hydrogel system for culture of retinal ganglion cells and amacrine cells. Acta Biomater. 2013 Aug;9(8):7622-9. PubMed PMID: 23648573; PubMed Central PMCID: PMC3722500.
10. Shoffstall AJ, Everhart LM, Varley ME, Soehnlen ES, Shick AM, Ustin JS, Lavik EB. Tuning ligand density on intravenous hemostatic nanoparticles dramatically increases survival following blunt trauma. Biomacromolecules. 2013 Aug 12;14(8):2790-7. PubMed PMID: 23841817; PubMed Central PMCID: PMC3758899.
11. Sethi R, Sethi R, Redmond A, Lavik E. Olfactory ensheathing cells promote differentiation of neural stem cells and robust neurite extension. Stem Cell Rev. 2014 Dec;10(6):772-85. PubMed PMID: 24996386; PubMed Central PMCID: PMC4238386.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	
Bernstein, Steven Lance	Professor of Ophthalmology, Anatomy And Neurobiology, and Genetics
eRA COMMONS USER NAME slbernst	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Phila. Coll. Pharm. Sci.; Philadelphia, Pa.	B.Sc.	1971-75	Microbiology
Cornell University, NYC, NY	Ph.D.	1975-82	Neurobiology
SUNY-Downstate Med. Ctr. Brooklyn, NY	M.D.	1981-85	Medicine
Maimonides Medical Center., Brooklyn, NY	Internship	1985-86	Medical Internship
Medical Center of Virginia-VCU	Residency	1986-89	Ophthalmology
National Institutes of Health-National Eye Institute, Bethesda, MD	Postdoctoral Training and Fellowship	1990-95	Retinal Cell Biology and Ophthalmic Genetics

A. Personal Statement

I am interested in identifying the critical mechanisms of repair and regeneration for common optic nerve (ON)- and retinal diseases. My lab generated the first clinically relevant models of nonarteritic anterior ischemic optic neuropathy (NAION) in rodents and nonhuman primates. My work is translational in nature, utilizing cell, molecular biological, and functional analyses, as well as transgenic and knockout rodent models, nonhuman primate and human tissues. We have discovered the first compound (PGJ_2) with the potential to treat early clinical NAION. The most surprising new work in the lab is identification of an adult neural stem cell niche in the lamina (the junction of the eye and optic nerve) of both rodents and humans. I identified these cells while beginning my Sabbatical (in 2011) in Dr. Sally Temple's lab (NY Neural Stem Cell Institute) in Albany, NY. This new finding has many implications for treatment of anterior optic nerve diseases such as primary open angle glaucoma (POAG) and NAION, and will likely result in potent new treatments. My interests focus on the early- and later changes associated with optic nerve damage following POAG, stroke/ischemic injury, and generating selective treatments to improve early treatment and recovery. I utilize statistically robust methods to analyze ganglion cell injury and death. We have now perfected the isolation and cultivation of rodent laminar stem cells. My lab's work on repair of ON-ischemic injury is enhanced by my clinical experience as a comprehensive Ophthalmologist. I incorporate and routinely utilize and interpret OCT images, and other clinical testing methods such as fundoscopy, electroretinography and visual evoked potential. The human stem cell project is complementary to other work being done in the lab, and will provide new insights into human optic nerve disease and effective treatment.

B. Positions and Selected Honors

Professor, Dept. Ophthalmology; UMAB School of Medicine 2009-Current
Associate Professor, Dept. Ophthalmology; UMAB School of Medicine 2002-2009
Assistant Professor, Dept. Ophthalmology; UMAB School of Medicine 1996-2002
Adjunct Prof., Dept. Anat. and Neurobiology, UMAB School of Medicine 2002-current Adjunct Professor, Dept. Genetics 2002-current
Comprehensive Ophthalmologist, UMAB Department of Ophthalmology.
Director, Molecular Research Laboratory, UMAB Department of Ophthalmology.
Director, UMAB Ophthalmic Genetics Clinic
Senior Staff Fellow, National Eye Institute 1990-1996

Editorial board, *Molecular Vision;* External Reviewer, *Invest. Ophth.. Vis. Sci.*; *Arch. Ophthalmol; J. Neurosci; Foundation Fighting Blindness*, *American Health Assistance Foundation*, *American Federation of Aging Research (AFAR)*

C. Selected Peer-reviewed publications (In chronological order from more than 78 publications)

1. **Bernstein, S.L**., Meister, M., Zhuo, J., Gullipalli, R.P. (2015) Postnatal growth of the human optic nerve. Eye **30**,1378-80 doi: 10.1038/eye.2016.141
2. Miller N.R.., Johnson, M.A., Nolan, T., Guo, Y., **Bernstein, S.L**. (2015) A Single Intravitreal Injection of Ranibizumab Provides No Neuroprotection in a Non-Human Primate Model of Moderate-to-Severe Nonarteritic Anterior Ischemic Optic Neuropathy. Invest. Ophth. Vis. Sci. **56**(13) 7679-7686.
3. **Bernstein, S.L**. and Miller, N.R. (2014): Ischemic optic neuropathies and their models: Disease Comparisons, Model Strengths and Weaknesses. Jap.J. Ophth. **59**(3), 135-147
4. Miller, N.R., Johnson,M.A., Nolan, T., Guo, T., Bernstein, A.M., and **Bernstein, S.L.** (2014) Sustained Neuroprotection From a Single Intravitreal Injection of PGJ_2 in a Nonhuman Primate Model of Nonarteritic Anterior Ischemic Optic Neuropathy. Invest. Ophth. Vis.Sci. **55**, 7047-7056
5. Touitou V, Johnson MA, Guo Y, Miller NR, and Bernstein SL (2013): Sustained Neuroprotection From a Single Intravitreal Injection of PGJ2 in a Rodent Model of Anterior Ischemic Optic Neuropathy. Invest Ophthalmol Vis Sci. 2013 54, 7402-7409. PMID: 24106118 PMC-in process
6. Slater, B.J., Vilson, D., Guo, Y., Weinreich, D., Hwang, S. and Bernstein, S.L. (2013) Optic nerve Inflammation and demyelination in a rodent model of nonarteritic anterior ischemic optic neuropathy. Invest Ophthalmol Vis Sci. 54, 7952-7961. PMC Journal ID: 24065807 PMC in process
7. Nicholson J.D., Weinreich D., Guo Y., Slater B.J. and **Bernstein, S.L**. (2012) PGJ2 provides prolonged CNS stroke protection by reducing white matter edema. PLoS-One, 7(12): e50021. Published online 2012 December 20. doi: 10.1371/journal.pone.0050021 PMCID: PMC3527449
8. Salgado C., Vilson F., Miller N.R., and **Bernstein, S.L**. (2011) Cellular Inflammation in Nonarteritic Anterior Ischemic Optic Neuropathy and Its Primate Model. Arch. Ophth. **129**, 1583-1591 PMCID: PMC3494739
9. **Bernstein, S.L**., Johnson, M.A. and Miller, N.R. (2011) Nonarteritic anterior ischemic optic neuropathy (NAION) and its experimental models. Progress in Retinal and Eye Research **30**, 167-187 PMCID: PMC3367439
10. Chen, CS*, Johnson, MA*, Flower, RA, Slater, BJ, Miller, NR, **Bernstein, SL**: Clinically relevant functional and cellular responses in a novel primate model of non-arteritic anterior ischemic optic neuropathy (pNAION). Invest Ophthalmol Vis Sci. 2008 Jul;49(7):2985-92. PMCID: PMC2754050
11. Zhang C., Guo Y., Slater B.J., Miller N.R., and **Bernstein S.L**. (2010) Axonal degeneration, regeneration and ganglion cell death in a rodent model of anterior ischemic optic neuropathy (rAION). Exp. Eye Res.91, 286-292 PMCID: PMC2907443

Additional recent publications of importance to the field (in chronological order)

12. Wyatt,M.K., Tsai,J-Y., Mishra,S., Campos,M., Jaworski,C., Fariss,R.N., Bernstein,S.L., and Wistow, G. (2013) Interaction of Complement Factor H and Fibulin3 in Age-Related Macular Degeneration PLoS One. 2013; 8(6): e68088. Published online 2013 June 28. PMCID: PMC3696004
13. **Bernstein S.L**., and Guo Y. (2011) Changes in Cholinergic Amacrine Cells after Rodent Anterior Ischemic Optic Neuropathy (rAION). Invest. Ophth. Vis. Sci. **52**, 904-910 PMCID: PMC3053113
14. Lin A.H., Cohen J.E., Wan Q., Niu K., Shrestha P., Bernstein S.L., and Abrams T.W. (2012) Serotonin stimulation of cAMP-dependent plasticity in Aplysia sensory neurons is mediated by calmodulin-sensitive adenylyl cyclase PNAS 2010 107 (35) 15607-15612
15. Zhang C, Guo Y, Miller N.R., and **Bernstein S. L.**. (2009) Optic Nerve Infarction and Post-Ischemic Inflammation in the Rodent Model of Anterior Ischemic Optic Neuropathy (rAION). Br. Res. 1264, 67-75 PMCID: PMC2783225
16. Slater, BJ, Guo, Y., Mehrabyan,Z., Hunter, A., and **Bernstein, S.L.** (2008). Rodent anterior ischemic optic neuropathy (rAION) induces regional retinal ganglion cell apoptosis with a unique temporal pattern. Invest. Ophth. Vis. Sci.**49**, 3671-3676 PMCID: PMC2692335
17. **Bernstein, S.L.**, Mehrabyan, Z., Guo, Y., Moianie, N. (2007) Estrogen is not neuroprotective in a rodent model of optic nerve stroke. Molecular Vision **13**, 1920-1925
18. **Bernstein, S.L**., Guo, Y., Slater, B.J., Puche, A., and Kelman, S.E. (2007) Analysis of retinal ganglion cell stress and loss following optic nerve stroke in double reporter transgenic mice. Invest. Ophth. Vis. Sci. **48,** 2304-2310

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Day, Adam

eRA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE: Graduate Student in Biochemical Engineering

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	START DATE MM/YYYY	END DATE MM/YYYY	FIELD OF STUDY
UMBC, Baltimore, MD	BS	01/2013	06/2016	Biochemical Engineering
National Cancer Institute, Frederick, MD	Other training	02/2008	03/2011	Research in Laboratory of Protein Dynamics and Signaling
UMBC, Baltimore, MD	Graduate Student	01/2017	present	Biochemical Engineering

A. Personal Statement

I am currently a PhD student in Biochemical Engineering at UMBC in Baltimore, MD. In 2016 I graduated magna cum laude with a BS in Biochemical Engineering from UMBC. I have three years of industrial experience working for Thermo Fisher Scientific (1.5 yrs), GlaxoSmithKline (0.5 yrs), Centocor (0.5 yrs), and Noramco of Delaware (0.5 yrs). I also have 3 years of laboratory research experience at the National Cancer Institute in the Laboratory of Protein Dynamics and Signaling, which resulted in 1 publication. My unique blend of academic, research, and industrial experience have allowed me to develop into a successful and unique researcher.

B. Positions and Honors

Positions and Employment

2004 - 2005 QC Lab Technician Co-op, Noramco of Delaware, Wilmington, DE
2005 - 2006 Process Engineering Co-op, Centocor, Malvern, PA
2006 - 2007 Process Engineering Co-op, GlaxoSmithKline, Conshohocken, PA
2012 - 2012 Associate Manufacturing Technician, Thermo Fisher Scientific, Frederick, MD

Other Experience and Professional Memberships

2016 - Member, Tau Beta Pi
2016 - Member, AiChE

Honors

2002 - 2003 Werner H. Kirsten Student Intern , National Cancer Institute Frederick

C. Contribution to Science

1. Performed research at NCI-Frederick in LPDS. Research efforts lead to publication in 2011

    a. Cohen MM, Amiott EA, Day AR, Leboucher GP, Pryce EN, Glickman MH, McCaffery JM, Shaw JM, Weissman AM. Sequential requirements for the GTPase domain of the mitofusin Fzo1 and the ubiquitin ligase SCFMdm30 in mitochondrial outer membrane fusion. J Cell Sci. 2011 May 1;124(Pt 9):1403-10. PubMed PMID: 21502136; PubMed Central PMCID: PMC3078809.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Ibarra, Bryan

eRA COMMONS USER NAME (credential, e.g., agency login): N/A

POSITION TITLE: Undergraduate majoring in Biomedical Engineering and Neuroscience

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	START DATE MM/YYYY	END DATE MM/YYYY	FIELD OF STUDY
University of Miami, Coral Gables, FL	BS	08/2014	05/2018	Biomedical Engineering
University of Miami, Coral Gables, FL	BS	08/2014	05/2018	Neuroscience

A. Personal Statement

I am currently a student at University of Miami majoring in Biomedical Engineering and Neuroscience. I am interested in the overlap of these two fields, specifically in neuroprosthetics. I have worked in labs focused on neural cell biology, neural signal analysis, and tissue engineering throughout my undergraduate career. I aspire to be a physician-scientist specialized in discovering treatments for patients with paralysis.

B. Positions and Honors

Positions and Employment

Other Experience and Professional Memberships

- President, Tau Beta Pi
- Member, Phi Beta Kappa

Honors

2017 The Leadership Alliance SR-EIP Program, Brown University

C. Contribution to Science

D. Additional Information: Research Support and/or Scholastic Performance

Feasibility Assessment: Screen Printing Retinal Models

TIMELINE

The overall project will take 36 months to completely validate.

Months 1-3: Organization, synthesis of materials, and expansion of necessary cells

Months 4-6: Validation of Bruch's membrane, optic nerve membrane, and retinal ECM. Printing of RPE cells

Months 7-18: Printing, reproducibility assessment and validation of structural and functional characteristics of outer retina

Months 18-36: Full validation of retinal model with structural and functional assessments including challenges with Bruch's membrane variation and 2-Aminophosphonobutyric acid (AP4).

SUPPORTING PRECEDENTS

Cells

We have isolated adult neural progenitor cells (aNPCs) from young and mature optic nerve in both the rodent and human. The latter cells will be used in this proposal. These cells are SOX2(+)/Nestin(+)/GFAP(+) (Fig 2), and can be distinguished from their surroundings by: 1) Their ability to be cultured continuously for >10 replications. 2) Their ability to give rise to astrocytes, oligodendrocytes and rarely, neurons. 3) Their ability to form neurospheres (Fig 3A and B). 4) Their age associated depletability.

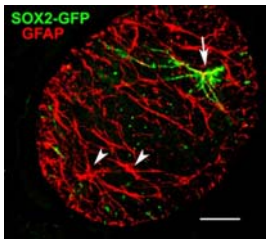

Fig 2. SOX2 ON-aNPC in rodent ON

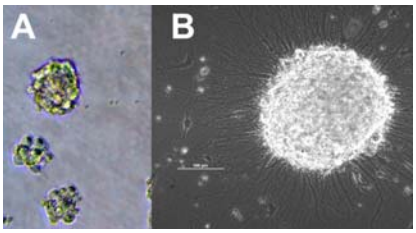

Fig 3. ON-aNPC neurospheres

Figure 2:SOX2-GFAP(+) ON-aNPCS expressed in a double mutant (ER2-SOX2-Cre X Td-Tomato-LoxP(GFP) animal). Both GFP(+)/GFAP(+) aNPCs and GFAP(+) astrocytes are seen.

Figure 3: aNPC neurospheres grown from rodent ON. Both early (A) neurospheres and neurospheres that were subsequently grown on a laminin coated plate (B) are shown. Scale bar in Fig 1: 50um. Scale bar in B: 100uM

Following aNPC growth and subculture, we can grow these cells on decellularized ON matrices (Fig 3A). The human cells grown on these matrices then 'stretch out', assuming a shape and expression more typical of cells *in vivo* (Fig 3B and C).

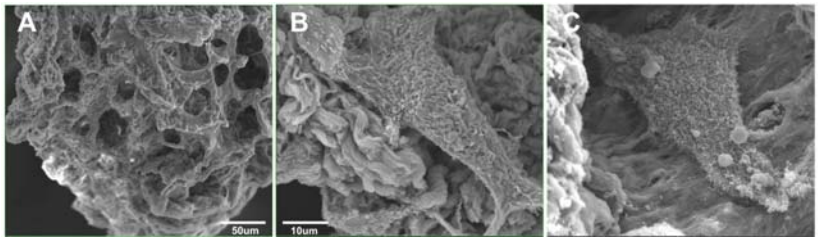

Figure 4: Scanning EM photos of: (A) decellularized ON. (B and C) human ON-aNPCs extending on a ON-laminar surface. The cells express microvillae and are SOX2(+)/GFAP(+)/Chx10(+) at this stage.

These cells provide a critical foundation to building retinal models and can be combined with human RPE, photoreceptor, bipolar, amacrine, and horizontal cells. However, the appropriate matrix is essential to successfully direct these cells into the appropriate cell types and structures.

Hydrogel matrix

We have used the PEG-polylysine hydrogel system either on its own or in the presence of laminin to support the organization and differentiation of a range of cell types from neural stem cells (Hynes et al., 2009; Royce Hynes, McGregor, Ford Rauch, & Lavik, 2007) and endothelial cells in vascular networks (Ford et al., 2006; Li, Ford, Lavik, & Madri, 2006; Rauch et al., 2008; Williams et al., 2012) to retinal progenitors (Hynes & Lavik, 2010; Lavik, Klassen, Warfvinge, Langer, & Young, 2005), retinal ganglion cells, and amacrine cells (Hertz et al., 2013) (Figure 5).

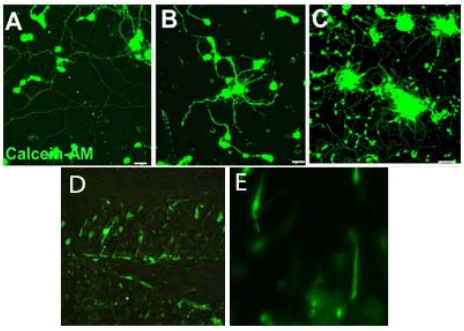

Figure 5: Growth of Retinal Ganglion Cells and Amacrine Cells in PLL-based gels. RGCs and ACs cells migrate into hydrogels and readily extend elaborate neurites in three dimensions. RGCs and ACs were seeded onto hydrogels and stained using calcein-AM (green) to label cell somas and neurites. Live confocal z-stack images were acquired. RGCs (A) and ACs (B) retained their stereotyped morphology as do cocultures (C). (D) and (E) Retinal progenitor cells orient in the polarized fashion in response to being in polymer scaffolds (Lavik et al., 2005).

These materials provide the foundation for supporting the survival, organization, and maturation of the retinal cells in this model system. By screen printing these materials, we are able to provide physical guidance cues through the permissive regions and non-permissive fully-PEG hydrogel regions as well as through the chemical guidance cues provided by the absorption of extracellular matrix molecules.

Figure 5E shows retinal progenitors lining up in one of our PLGA scaffolds. The cells follow the architectural cues, and in doing so, they take on morphologies that are consistent with retinal cells. The screenprinted hydrogel system will have similar architectural cues with columns of the PEG-polylysine matrix in a PEG-matrix. Figure 6 shows the PEG-polylysine gel (A, green) and the gel with absorbed fibronectin (B, rhodamine-antifibronectin antibody.) The extracellular matrix proteins absorb to the charged polylysine backbone of the gels. The absorption process is rapid and stable. The proteins do not release from the gel over weeks (Hynes et al., 2009). In contrast, no proteins absorb PEG hydrogels thus impeding cell attachment (Leach, Bivens, Collins, & Schmidt, 2004).

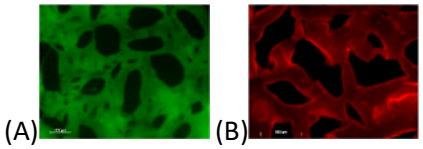

Figure 6: (A) PLL-based gel labeled with FITC which reacts with the charged free amines on lysine. (B) Anti-fibronectin immunostaining of PLL-based gel reacted with fibronectin. The fibronectin absorbs to the charged amines on the gels.

Screen printing matrices and cells

With mesh sizes of 100 um, we can obtain the following pattern (Figure 7) with the cross piece, the finest feature (90 um wide), connected.

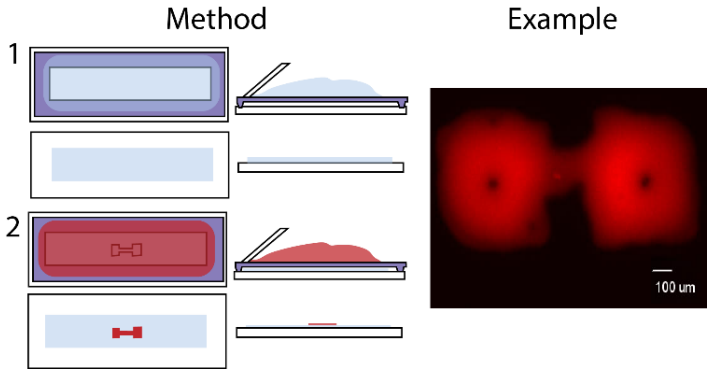

Figure 7: Dye-loaded hydrogel printed on hydrogel layer. The barbell structure is at the limits of the resolution available with a 100 um mesh screen. Details on the order of 100 microns are reproducibly printed with this size mesh. Finer meshes can lead to finer resolution features; however, we believe that this resolution is more than adequate to achieve the polarized orientations in the retina.

We have tested meshes with 100 micron pores to determine the viability of cells post printing. We have used iPS cells differentiated down a neural linage from our colleague, Michael Nestor. 84+/-2.6% of the cells are viable post-printing in the absence of any matrix which typically augments survival (Lozano et al., 2015). We have borrowed the idea for screen printing retinal models from the electronics industry which has used screen printing for decades to make cheap, highly reproducible structures with feature sizes on the order of tens of microns without specialized systems (Hyun, Lim, et al., 2015; Hyun, Secor, et al., 2015; Su et al., 2011). This approach is extremely well suited to building retinal structures and applies the knowledge gained by the electronics industry to biological systems.

SPECIAL RESOURCES

Imaging

We will use two methods to image the cells in the materials: standard fluorescence microscopy to look at detailed structures and a high throughput system, the acumen cellista which uses laser scanning technology to quantitatively assess fluorescent signals. This will allow us to rapidly image many as well as assess the final system for high throughput applications.

Electrode array system

The electrode array systems and LED stimulation systems for the in vitro variant of electroretinography are commercially available and validated with a number of in vitro and explant retinal models.

EXPERTISE FOR PROPOSAL

Building a retinal model with a screen printing approach requires an interdisciplinary team. We have both engineers and retinal biologists. Our complementary expertise is critical to the success of the project.

PROTECTIONS FOR HUMAN SUBJECTS

Human ON stem cells are obtained under an IRB exemption from the University of Maryland-Baltimore from adult human eyes which have been enucleated due to severe trauma, and without visual recovery potential. The eyes are enucleated after counseling by an unrelated Ophthalmologist, and the information from each donor is stripped from the samples, leaving only age of the donor.

References

Ahmado, A., Carr, A. J., Vugler, A. A., Semo, M., Gias, C., Lawrence, J. M., . . . Coffey, P. J. (2011). Induction of differentiation by pyruvate and DMEM in the human retinal pigment epithelium cell line ARPE-19. *Invest Ophthalmol Vis Sci, 52*(10), 7148-7159. doi:10.1167/iovs.10-6374

Al Gwairi, O., Thach, L., Zheng, W., Osman, N., & Little, P. J. (2016). Cellular and Molecular Pathology of Age-Related Macular Degeneration: Potential Role for Proteoglycans. *J Ophthalmol, 2016*, 2913612. doi:10.1155/2016/2913612

Barres, B. A., Silverstein, B. E., Corey, D. P., & Chun, L. L. (1988). Immunological, morphological, and electrophysiological variation among retinal ganglion cells purified by panning. *Neuron, 1*(9), 791-803.

Booij, J. C., Baas, D. C., Beisekeeva, J., Gorgels, T. G., & Bergen, A. A. (2010). The dynamic nature of Bruch's membrane. *Prog Retin Eye Res, 29*(1), 1-18. doi:10.1016/j.preteyeres.2009.08.003

Brandl, C., Zimmermann, S. J., Milenkovic, V. M., Rosendahl, S. M., Grassmann, F., Milenkovic, A., . . . Weber, B. H. (2014). In-depth characterisation of Retinal Pigment Epithelium (RPE) cells derived from human induced pluripotent stem cells (hiPSC). *Neuromolecular Med, 16*(3), 551-564. doi:10.1007/s12017-014-8308-8

Datta, P., Ayan, B., & Ozbolat, I. T. (2017). Bioprinting for vascular and vascularized tissue biofabrication. *Acta Biomater, 51*, 1-20. doi:10.1016/j.actbio.2017.01.035

Dubbin, K., Hori, Y., Lewis, K. K., & Heilshorn, S. C. (2016). Dual-Stage Crosslinking of a Gel-Phase Bioink Improves Cell Viability and Homogeneity for 3D Bioprinting. *Adv Healthc Mater*. doi:10.1002/adhm.201600636

Fernandez-Godino, R., Pierce, E. A., & Garland, D. L. (2016). Extracellular Matrix Alterations and Deposit Formation in AMD. *Adv Exp Med Biol, 854*, 53-58. doi:10.1007/978-3-319-17121-0_8

Ford, M. C., Bertram, J. P., Hynes, S. R., Michaud, M., Li, Q., Young, M., . . . Lavik, E. B. (2006). A macroporous hydrogel for the coculture of neural progenitor and endothelial cells to form functional vascular networks in vivo. *Proc Natl Acad Sci U S A, 103*(8), 2512-2517. doi:10.1073/pnas.0506020102

Goldberg, H. D., Brown, R. B., Liu, D. P., & Meyerhoff, M. E. (1994). Screen printing: a technology for the batch fabrication of integrated chemical-sensor arrays. *Sensors and Actuators B: Chemical, 21*(3), 171-183.

Hertz, J., Robinson, R., Valenzuela, D. A., Lavik, E. B., & Goldberg, J. L. (2013). A tunable synthetic hydrogel system for culture of retinal ganglion cells and amacrine cells. *Acta Biomater, 9*(8), 7622-7629. doi:10.1016/j.actbio.2013.04.048

Hollyfield, J. G., Bonilha, V. L., Rayborn, M. E., Yang, X., Shadrach, K. G., Lu, L., . . . Perez, V. L. (2008). Oxidative damage-induced inflammation initiates age-related macular degeneration. *Nat Med, 14*(2), 194-198.

Hynes, S. R., & Lavik, E. B. (2010). A tissue-engineered approach towards retinal repair: scaffolds for cell transplantation to the subretinal space. *Graefes Arch Clin Exp Ophthalmol, 248*(6), 763-778. doi:10.1007/s00417-009-1263-7

Hynes, S. R., Rauch, M. F., Bertram, J. P., & Lavik, E. B. (2009). A library of tunable poly(ethylene glycol)/poly(L-lysine) hydrogels to investigate the material cues that influence neural stem cell differentiation. *J Biomed Mater Res A, 89*(2), 499-509. doi:10.1002/jbm.a.31987

Hyun, W. J., Lim, S., Ahn, B. Y., Lewis, J. A., Frisbie, C. D., & Francis, L. F. (2015). Screen Printing of Highly Loaded Silver Inks on Plastic Substrates Using Silicon Stencils. *ACS Appl Mater Interfaces, 7*(23), 12619-12624. doi:10.1021/acsami.5b02487

Hyun, W. J., Secor, E. B., Hersam, M. C., Frisbie, C. D., & Francis, L. F. (2015). High-resolution patterning of graphene by screen printing with a silicon stencil for highly flexible printed electronics. *Adv Mater, 27*(1), 109-115. doi:10.1002/adma.201404133

Jin, R., Moreira Teixeira, L. S., Krouwels, A., Dijkstra, P. J., van Blitterswijk, C. A., Karperien, M., & Feijen, J. (2010). Synthesis and characterization of hyaluronic acid-poly(ethylene glycol) hydrogels via Michael addition: An injectable biomaterial for cartilage repair. *Acta Biomater, 6*(6), 1968-1977. doi:10.1016/j.actbio.2009.12.024

Jung, J. P., Bhuiyan, D. B., & Ogle, B. M. (2016). Solid organ fabrication: comparison of decellularization to 3D bioprinting. *Biomater Res, 20*(1), 27. doi:10.1186/s40824-016-0074-2

Kaluzny, J., Purta, P., Poskin, Z., Rogers, J. D., & Fawzi, A. A. (2016). Ex Vivo Confocal Spectroscopy of Autofluorescence in Age-Related Macular Degeneration. *PLoS One, 11*(9), e0162869. doi:10.1371/journal.pone.0162869

Lavik, E. B., Klassen, H., Warfvinge, K., Langer, R., & Young, M. J. (2005). Fabrication of degradable polymer scaffolds to direct the integration and differentiation of retinal progenitors. *Biomaterials, 26*(16), 3187-3196. doi:10.1016/j.biomaterials.2004.08.022

Leach, J., Bivens, K., Collins, C., & Schmidt, C. (2004). Development of photocrosslinkable hyaluronic acid-polyethylene glycol-peptide composite hydrogels for soft tissue engineering. *Journal of Biomedical Materials Research Part A, 70*(1), 74-82. doi:10.1002/jbm.a.30063

Li, Q., Ford, M. C., Lavik, E. B., & Madri, J. A. (2006). Modeling the neurovascular niche: VEGF- and BDNF-mediated cross-talk between neural stem cells and endothelial cells: an in vitro study. *J Neurosci Res, 84*(8), 1656-1668. doi:10.1002/jnr.21087

Lozano, R., Stevens, L., Thompson, B. C., Gilmore, K. J., Gorkin, R., 3rd, Stewart, E. M., . . . Wallace, G. G. (2015). 3D printing of layered brain-like structures using peptide modified gellan gum substrates. *Biomaterials, 67*, 264-273. doi:10.1016/j.biomaterials.2015.07.022

Meyer-Franke, A., Kaplan, M. R., Pfrieger, F. W., & Barres, B. A. (1995). Characterization of the signaling interactions that promote the survival and growth of developing retinal ganglion cells in culture. *Neuron, 15*(4), 805-819.

Nah, J. W., Yu, L., Han, S. O., Ahn, C. H., & Kim, S. W. (2002). Artery wall binding peptide-poly(ethylene glycol)-grafted-poly(L-lysine)-based gene delivery to artery wall cells. *J Control Release, 78*(1-3), 273-284.

Ozbolat, I. T., & Hospodiuk, M. (2016). Current advances and future perspectives in extrusion-based bioprinting. *Biomaterials, 76*, 321-343. doi:10.1016/j.biomaterials.2015.10.076

Peng, Q., Pei, K., Han, B., Li, R., Zhou, G., Liu, J. M., . . . Gao, J. (2016). Inexpensive transparent nanoelectrode for crystalline silicon solar cells. *Nanoscale Res Lett, 11*(1), 312. doi:10.1186/s11671-016-1533-3

Rauch, M. F., Michaud, M., Xu, H., Madri, J. A., & Lavik, E. B. (2008). Co-culture of primary neural progenitor and endothelial cells in a macroporous gel promotes stable vascular networks in vivo. *J Biomater Sci Polym Ed, 19*(11), 1469-1485. doi:10.1163/156856208786140409

Ravnic, D. J., Leberfinger, A. N., Koduru, S. V., Hospodiuk, M., Moncal, K. K., Datta, P., . . . Ozbolat, I. T. (2017). Transplantation of Bioprinted Tissues and Organs: Technical and Clinical Challenges and Future Perspectives. *Ann Surg, 266*(1), 48-58. doi:10.1097/sla.0000000000002141

Royce Hynes, S., McGregor, L. M., Ford Rauch, M., & Lavik, E. B. (2007). Photopolymerized poly(ethylene glycol)/poly(L-lysine) hydrogels for the delivery of neural progenitor cells. *J Biomater Sci Polym Ed, 18*(8), 1017-1030. doi:10.1163/156856207781494368

Schaefer, K. A., Toral, M. A., Velez, G., Cox, A. J., Baker, S. A., Borcherding, N. C., . . . Mahajan, V. B. (2016). Calpain-5 Expression in the Retina Localizes to Photoreceptor Synapses. *Invest Ophthalmol Vis Sci, 57*(6), 2509-2521. doi:10.1167/iovs.15-18680

Shadforth, A. M., Suzuki, S., Theodoropoulos, C., Richardson, N. A., Chirila, T. V., & Harkin, D. G. (2015). A Bruch's membrane substitute fabricated from silk fibroin supports the function of retinal pigment epithelial cells in vitro. *J Tissue Eng Regen Med*. doi:10.1002/term.2089

Stett, A., Egert, U., Guenther, E., Hofmann, F., Meyer, T., Nisch, W., & Haemmerle, H. (2003). Biological application of microelectrode arrays in drug discovery and basic research. *Anal Bioanal Chem, 377*(3), 486-495. doi:10.1007/s00216-003-2149-x

Su, W., Li, P., Yao, L., Yang, F., Liang, L., & Chen, J. (2011). Direct patterning of copper on polyimide by site-selective surface modification via a screen-printing process. *Chemphyschem, 12*(6), 1143-1147. doi:10.1002/cphc.201100040

Suikkola, J., Bjorninen, T., Mosallaei, M., Kankkunen, T., Iso-Ketola, P., Ukkonen, L., . . . Mantysalo, M. (2016). Screen-Printing Fabrication and Characterization of Stretchable Electronics. *Sci Rep, 6*, 25784. doi:10.1038/srep25784

Williams, C., Rauch, M. F., Michaud, M., Robinson, R., Xu, H., Madri, J., & Lavik, E. (2012). Short term interactions with long term consequences: modulation of chimeric vessels by neural progenitors. *PLoS One, 7*(12), e53208. doi:10.1371/journal.pone.0053208

Yue, Z., Liu, X., Coates, P. T., & Wallace, G. G. (2016). Advances in printing biomaterials and living cells: implications for islet cell transplantation. *Curr Opin Organ Transplant, 21*(5), 467-475. doi:10.1097/mot.0000000000000346

Zhou, W., Stukel, J. M., Cebull, H. L., & Willits, R. K. (2016). Tuning the Mechanical Properties of Poly(Ethylene Glycol) Microgel-Based Scaffolds to Increase 3D Schwann Cell Proliferation. *Macromol Biosci, 16*(4), 535-544. doi:10.1002/mabi.201500336

Zustiak, S. P., & Leach, J. B. (2010). Hydrolytically degradable poly(ethylene glycol) hydrogel scaffolds with tunable degradation and mechanical properties. *Biomacromolecules, 11*(5), 1348-1357. doi:10.1021/bm100137q